ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

AGRICULTURAL BIOLOGY

Since January, 1966

PLANT BIOLOGY

Vol. 58, Issue 1
January-February

2023 Moscow

A peer-reviewed academic journal for delivering current original research results and reviews on classic and modern biology of agricultural plants, animals and microorganisms
Covered in Scopus, Web of Science (BIOSIS Previews, Biological Abstracts, CAB Abstracts, Russian Science Citation Index), Agris

Science editors: E.V. Karaseva, L.M. Fedorova

Publisher: Agricultural Biology Editorial Office NPO

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For citation: Agricultural Biology,
Сельскохозяйственная биология, Sel'skokhozyaistvennaya biologiya

ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)
ISSN 2412-0324 (English ed. Online)

SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA
[AGRICULTURAL BIOLOGY], 2023, Vol. 58, № 1

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2023, V. 58, Iss. 1, pp. 3-22
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ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

, Reviews, challenges

UDC 631.811.982:581.1:632

doi: 10.15389/agrobiology.2023.1.3eng
doi: 10.15389/agrobiology.2023.1.3rus

PROSPECTS FOR THE APPLICATION OF JASMONATES, SALICYLATES, AND ABSCISIC ACID IN AGRICULTURE TO INCREASE PLANT STRESS RESISTANCE

(review)

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The authors declare no conflict of interests
Acknowledgements:
Supported financially from the Russian Science Foundation, project № 22-24-00489.
Final revision received July 31, 2022
Accepted August 26, 2022

Abstract

Nowadays, the search for new effective methods and approaches based on using natural bioactive compounds that control plant growth, development, and plant productivity with minimal impact to the environment and human health is still in great demand. One of the directions developing during the last decades contributing to the "greening" of agricultural production is the application agrochemicals based on phytohormones with protective functions, such as abscisic acid, salicylic acid, and jasmonates. The use of these phytohormones is very promising since it can significantly increase plant tolerance to unfavorable factors of biotic and abiotic nature. This review summarizes the current information on the biological functions of abscisic acid, jasmonates, and salicylates, presents the examples demonstrating crop species treatment with the agrochemicals based on these phytohormones, and discusses the promising directions for the phytohormones application in agriculture. Abscisic acid, jasmonates, and salicylates are often referred to as stress hormones because they regulate the plant adaptive responses to adverse environmental conditions. Abscisic acid is a regulator of plant growth and development throughout ontogenesis, as well as tolerance to abiotic and biotic stress factors (J. Li et al., 2017), plays a role in the stomata closure, regulating the ion flow in the guard cells, controls all stages of seed maturation (K. Chen et al., 2020). Abscisic acid can play positive and negative roles in plant protection against pathogens (L. Lievens et al., 2017; K. Xie et al., 2018) and influence the symbiotic relationships with fungi and bacteria (A. Tsyganova, V. Tsyganov, 2015). Salicylic acid controls plant tolerance to pathogens (A. Vlot et al., 2009; P. Ding, Y. Ding, 2020), plays a role in the development of hypersensitive response, death of infected cells (D. Klessig and J. Malamy, 1994; M. Alvarez, 2000), and formation of tolerance in unaffected plant parts (systemic acquired resistance) (M. Bürger, J. Chory, 2019). Salicylic acid may also be involved in the enhancement of plant tolerance to salt and low temperature stress (E. Horvath et al., 2015; Yu. Kolupaev, Yu. Karpets, 2021; W. Wang et al., 2018) and maintenance of the root zone microbiome (S. Lebeis et al., 2015). The range of regulatory effects of jasmonates is broad, but their functions are primarily associated with the regulation of mechanisms that determine plant tolerance to necrotrophic pathogens and insects, including root pests (C. Rohwer, J. Erwin, 2008; S. Johnson et al., 2018). Jasmonates also control plant tolerance to low temperature, salt stress, flooding, drought, ozone, heavy metals, and ultraviolet radiation (T. Savchenko et al., 2014; D. Pandita, 2022; T. Savchenko et al., 2019; K. Kazan, 2015; H. Kim et al., 2021). The high biological activity of abscisic acid, salicylates and jasmonates determines the significant potential of their application in agriculture to increase plant stress tolerance. At the same time, according to published data, the increase in plant tolerance mediated by the mentioned phytohormones is often accompanied by the suppression of growth-related processes, which can adversely affect crop

yields and product quality. To assess the prospects for the practical use of agrochemicals based on abscisic acid, jasmonates, and salicylic acid, a comprehensive analysis of the available data on the physiological effects caused by these substances is necessary due to their spectrum of actions, dependent on species/variety specificity, phase of plant development,-susceptibility of the target tissue, chemicals concentration, duration of treatment and conditions of application.

Keywords: phytohormones, abscisic acid, jasmonic acid, salicylic acid, physiological effects, plant tolerance, abiotic stress, biotic stressors, exogenous treatment, adaptive response

Phytohormones regulate plant growth, ontogeny, metabolism and adaptive responses to changing environmental conditions. Since the beginning of the 21st century, researchers have made significant progress in understanding the biological functions of plant hormones, identifying regulatory mechanisms and signal transduction pathways that they control to form a background for their use in agricultural practice. Auxins, ethylene, gibberellins, abscisic acid, cytokinins, the so-called classical plant hormones have been well known since the mid-20th century. The potential of jasmonates, salicylic acid, brassinosteroids and strigolactones, the compounds with regulatory functions proven relatively recently is currently being actively studied. The number of new regulatory molecules is growing, and, in addition to the classes of compounds mentioned, hormone-like properties have been discovered in polyamines, karrikins, triacontanol, turgorins, and peptide hormones [1-3].

The prospects for using phytohormones in agriculture as plant growth regulators and inducers of protective responses are beyond doubt [4-6). Preparations based on cytokinins, auxins, gibberellins, brassinosteroids and their functional analogues for the treatment of fruits during the post-harvest period [7], seeds and vegetative tissues [8-10], have successfully entered into practice. The potential of phytohormones with pronounced protective properties, such as abscisic acid, jasmonates and salicylates, has not yet been discovered. In Russia, preparations based on these phytohormones have not yet been used. Wider application is hampered not only by the difficulties of industrial scale roduction of these compounds but also by the lack of necessary approaches and practical recommendations for various crops.

Here, we analyze current data on the effect of abscisic acid, jasmonates and salicylates on various crops, and also to outline possible prospects for the practical use of each of the phytohormones under consideration in widespread agricultural practice.

Abscisic acid. Abscisic acid (ABA) is involved in the regulation of plant growth and development throughout ontogenesis and determines resistance to abiotic and biotic stress factors [11]. ABA regulates ion fluxes in stomatal guard cells. ABA-mediated stomatal closure can occur in response to drought, low humidity, high CO_2 concentrations, pathogen attack, darkness, etc. Stomata allow gas exchange and transpiration, and can also allow pathogens to enter, so regulating the opening and closing of stomata is important in ensuring plant resistance to adverse environmental influences [12].

ABA is involved in the regulation of seed maturation [12]. Early on, ABA slows down the cell cycle at the G1/S transition stage [13, 14], which inhibits embryonic growth through cell division and activates growth through cell elongation. During the early stages of seed development, ABA accumulates through transport from the mother plant [15]. Later, ABA is synthesized in the cells of the embryo itself and regulates the activity of the network of LAFL transcription factors LEC1/ABSCISIC ACID (ABA)-INSENSITIVE3 (ABI3), FUSCA3 (FUS3) and LEAFY COTYLEDON2 (LEC2) which control seed maturation. Seed desiccation and nutrient accumulation are also controlled by ABA [12]. ABA is a key regulator of seed dormancy, since in mutants with reduced ABA content, seeds

germinate prematurely while still on the mother plant [15].

During evolution, plants have acquired complex mechanisms that ensure seed germination only under optimal environmental conditions. The ratio of ABA and gibberellic acid (GA) is crucial in maintaining seed dormancy which is regulated by both endogenous factors associated with plant development and external influences. During germination, ABA catabolism and gibberellin synthesis are enhanced, and GC signaling is activated [11]. A change in the ABA:GA ratio is achieved primarily through changes in the expression of the *RGL2* gene, which encodes RGA-like 2 protein (Repressor of GA). Exogenous ABA is able to activate the expression of *RGL2*, and in the seeds of mutant plants carrying a nonfunctional *rgl2* variant, the ABA content is reduced after imbibition, which leads to accelerated dormancy release and germination. With a high content of gibberellins, DELLA proteins, key negative regulators of the gibberellin signal, are destroyed. This leads to a decrease in the activity of the regulatory module, which, in addition to DELLA, includes the ABI3 and ABI5 proteins (ABA-dependent transcription factors, the main negative regulators of seed germination). As a result, the expression of gibberellin-dependent genes is induced and accelerated germination occurs. It has been shown that during cold stratification, the expression of genes of the *CYP707A* family, involved in ABA catabolism, and the *AtGA3OX1* gene, involved in the biosynthesis of gibberellins, increases [12]. When exposed to high temperature, increased activity of a regulatory module including DELLA, ABI3, and ABI5 inhibits germination [12]. ABA, produced in the tissues of the mother plant, plays an important role in the development of the embryo and affects plant yield [16]. When unfavorable conditions occur, ABA causes growth arrest to protect the seedling [12].

The action of ABA inhibits cell division and elongation, regulates the transition from cell proliferation to differentiation, the development of lateral roots, and the formation of the suberin barrier in roots subject to water stress, providing control of water and nutrient flows [17]. Under normal conditions, ABA suppresses the emergence of new leaves [18] and plays a critical role in accelerating leaf senescence. This is necessary for the efficient distribution of resources from senescent leaves to the floral meristem and seeds. ABA serves as an inhibitor in the regulation of floral meristem activity and flowering time [12]. The participation of ABA in the development of male and female gametophytes and the flower as a whole is discussed in detail in the work of Y. Zhao et al. [19].

With transgenic *Arabidopsis* plants in the mesophilic leaves of which ABA signaling is constitutively suppressed, it was shown that ABA does not directly affect photosynthesis, but the presence of ABA is necessary to achieve maximum plant productivity. Under optimal conditions, transgenic plants with impaired ABA signaling were characterized by more vigorous growth at the initial stages of development, earlier flowering, smaller flowers, delayed chlorophyll degradation and fewer seeds compared to wild-type plants, but no such differences were observed under drought conditions [20].

ABA accumulates rapidly in plants in response to a variety of stress factors. When favorable conditions return, the ABA content decreases due to glycosylation or oxidation to phaseic acid, which is further converted into dihydrophaseic acid. When a plant is exposed to a stress factor with the participation of ABA, the stomata close, the expression of aquaporin genes is inhibited, but the expression of genes encoding chaperone proteins, hydrophilic LEA proteins (late embryogenesis abundant, dehydrins) and antifreeze proteins, enzymes for the synthesis of wax and suberin are activated, and accumulate sugars and proline, the antioxidant system is activated, and other protective changes occur [11]. The prevailing view in the scientific community is that ABA is a growth inhibitory hormone, but recent

studies show that nanomolar concentrations of exogenous ABA can stimulate growth, including a positive effect on hypocotyl growth in the dark [21].

The functions of ABA in protecting plants from pathogens are carried out in interaction with other hormones: salicylic acid (SA), jasmonic acid (JA) and ethylene. ABA can cause stomatal closure to block pathogen entry and stimulate callose deposition in plant cells, limiting pathogen spread. Virulence factors of some pathogens are aimed at suppressing ABA signaling in plants, although in other cases, on the contrary, ABA produced by the pathogen acts as an effector that suppresses defense responses [22]. Interestingly, ABA can play both positive and negative roles in plant resistance to viruses [23]. The positive effect of ABA on the symbiotic relationships of plants with fungi and bacteria is the formation of arbuscular mycorrhiza, while the negative effect is the establishment of rhizobial symbiosis [24].

The use of exogenous ABA for pre-sowing seed treatment and foliar treatment of plants increases the stress resistance of grain crops, which leads to an increase in yield [9]. Based on ABA, Valent BioSciences (USA) has developed the BioNik™ drug which is used to delay the development of plants of inbred lines of pollen donors in order to synchronize and extend the period of cross-pollination when growing corn for grain (https://www.valentbiosciences.com).

Exogenous treatment of soybean plants with abscisic acid over several seasons of field and greenhouse trials increased dry mass of aerial parts, root length density, leaf area, number of seeds per pod, and seed oil content [25]. Due to this and due to the distribution of metabolic flows from the vegetative parts of the plant to the seeds, ABA promotes an increase in soybean yields [25].

The use of ABA on sunflower under conditions of sufficient water supply negatively affects plants, while spraying under drought conditions can mitigate the negative effects of stress by increasing the leaf blade area, flowerhead diameter, number of seeds per head, yield, 1000-seed weight and oil yield [26, 27]. Spraying ABA during the budding stage is more effective than spraying during the flowering stage, while the treatment efficiency was different for different hybrids.

The use of the drug ProTone™ (20% ABA, Valent BioSciences) contributed to 100% leaf fall from apple trees in early autumn, without affecting the shoots of axillary buds [28], which indicates the possibility of using this drug to prepare the plant for harvesting and wintering. Exogenous ABA protected apple trees during drought by stimulating stomatal closure [29). Spraying the crown of cherry trees or directly treating fruits enhanced the color of drupes in various varieties [30]. The use of ABA on citrus trees improved the color of fruits, increased resistance to cold, and reduced the content of organic acids in fruits. It was noted that the observed effect was achieved only by foliar treatment while root treatment did not have any effect [31, 32].

The use of ABA on grapes has been well studied. ABA stimulates the ripening of berries, enhances their color by increasing the content of anthocyanins and phenolic compounds, and reduces the content of organic acids [33]. This is due to the fact that ABA controls the biosynthesis of phenolic compounds and anthocyanins [34-36]. The ability of ABA to control the timing of grape berry ripening depends on the concentration of the sprayed solution and also on the target organ, since different tissues demonstrate unequal absorption rates due to the permeability of the cuticle. Cabernet Sauvignon berries absorbed ABA less readily than leaves, but in both cases, ABA treatment accelerated the onset of berry coloring. A cool and wet growing season enhances the effect of exogenous ABA on fruit quality. The bunches treated with ABA had a lower berry weight and a higher dry skin weight which is acceptable for winemaking. Exogenous application of ABA can be an alternative agronomic technique to accelerate berry ripening

and improve their quality in cool years, in humid climates and in regions where the likelihood of early frosts is high [33].

In a recent study, J. Li et al. [37] showed a relationship between exogenous exposure to ABA and the content of endogenous phytohormones and metabolites that determine the quality of Ruidu Hongyu grape berries. Treatment with ABA significantly improved the appearance of berries and the content of a number of metabolites (sugars, anthocyanins, polyphenols, soluble sugars, ascorbic acid) by increasing the expression of genes involved in the biosynthesis of these substances. In addition, an increase occurred in the content of endogenous ABA, auxin and cytokinin and the transcription of genes associated with ABA biosynthesis and signaling in fruits.

ABA-based ProTone™ (200 to 400 g/ha) is used in many countries to improve the color of red table grapes. The action of the drug is based on increasing the activity of UDP-glucose flavonoid 3-0-glucosyltransferase (UFGT). The effect of ProTone™ is similar to that of 2-chloroethylphosphonic acid (Ethephon), a precursor of ethylene, but ProTone™ does not lead to softening of fruit tissue and is more technologically advanced because it is not volatile, unlike ethylene (https://www.valentbiosciences.com). The mechanisms by which ABA regulates fruit ripening are discussed in detail in a review article by X. Kou et al. [38].

ABA can find application in vegetable growing. It was shown that exogenous ABA treatment of red and green leaf lettuce significantly reduced yield, but induced the accumulation of chlorophyll b and an increase in the content of total carotenoids in the leaves, while the content of phenols and anthocyanins in red leaf lettuce significantly increased [39]. Exogenous ABA treatment increased carotenoid accumulation in tomatoes [40].

Salicylic acid. Salicylic acid (SA) provides plant resistance to pathogens [41, 42]. During infection, SA synthesis plays a key role in the development of a hypersensitivity reaction, local death of plant cells together with the pathogen [43, 44], as well as the formation of resistance (systemic acquired resistance) in unaffected parts of the plant [45].

The most compelling evidence of the protective role of SA was obtained by analyzing *Arabidopsis thaliana* (L.) Heynh. plants which are unable to accumulate it due to the expression of the bacterial gene *NahG*, which encodes the enzyme salicylate hydroxylase which converts SA into catechol. After infection, these plants could not develop systemic acquired resistance because they did not express PR (pathogenesis-related) genes and were vulnerable to attack by the pathogen. Treatment with a synthetic analogue of SA restored plant resistance and expression of PR genes [46, 47].

The main molecules through which the SA signal is transmitted are the NPR1 and NPR3/NPR4 proteins (non-expressor of PR proteins) and the SABP group of proteins (salicylic acid-binding proteins) [48]. Signal transmission into the nucleus occurs through NPR proteins which, after the action of SA, enter the nucleus and activate the expression of a large group of genes encoding PR proteins, among which are genes encoding chitinases (PR-3) and β-1,3-glucanases (PR-2), proteinase inhibitors (PR-6), cysteine-rich proteins, similar thaumatin (PR-5), as well as a group of proteins grouped in the PR-1 family, which inhibit fungal growth in an in vitro system [49]. The role of other SA-regulated proteins is not yet entirely clear, but their expression is associated with increased resistance to a large number of bacterial, fungal and viral infections. It should be noted that while NPR1 positively regulates the expression of PR genes, NPR3 and NPR4 (paralogues of NPR1) function more as transcriptional repressors of salicylate-activated genes at low SA content in the cell [50]. SABP proteins do not transmit a signal to the nucleus, but change their activity upon SA binding. Among the SABP

proteins, in particular, catalases (SABP1, CAT2) and phosphatase 2A are distinguished, which negatively regulate the PIN2 protein associated with auxin transport [51, 52].

Treatment with salicylates is often used to make plants resistant to various infections [53]. For example, treatment with SA increased resistance to *Fusarium oxysporum* [54] and yellow leaf curl virus [55] in tomatoes, to *Magnaporthe grisea* and *Xanthomonas oryzae* [56, 57] in rice, and to *Xanthomonas axonopodis* [58] in citrus plants. However, it should be taken into account that SA has an antagonistic relationship with jasmonates and often inhibits jasmonate-regulated responses to necrotrophic pathogens [59-61]. Thus, exogenous treatment with SA suppresses plant resistance to necrotrophic infections for which jasmonates are responsible. SA is important for the resistance to *Botrytis cinerea*. S. Ferrari et al. [62] showed that, along with ethylene and FA, the activity of SA signaling pathways is required for the formation of local resistance to *B. cinerea* in *Arabidopsis*. Treatment of tomatoes with SA resulted in the accumulation of reactive oxygen species in tissues and increased resistance to pathogens of the genus *Botrytis* [63].

SA may be involved in the formation of plant resistance to abiotic stresses. Treatment with SA contributed to an increase in the resistance of tomatoes to salt stress [64, 65] and frost resistance of wheat [66]. There are known examples of the participation of SA in the regulation of plant growth and development [67] and in the process of microbiome formation in the root zone [68].

One of the effects associated with the use of SA is inhibition of plant growth. Like other protective hormones, SA regulates the distribution of resources between processes that ensure plant growth and protection. Exogenous SA can have different effects on plant growth depending on the dose, duration of treatment, species, and stage of plant development [67]. If the use of small doses stimulates seed germination, then in high concentrations SA almost always has a negative effect. For example, treatment with a 1 mM SA solution significantly inhibited the growth and development of Arabidopsis seedlings [69]. Disruption in SA hydroxylation resulted in a pronounced dwarf phenotype in *A. thaliana* [48, 70, 71].

A special physiological effect of SA was discovered when studying thermogenesis in aroids. During flowering of *Sauromatum guttatum* (Wall.) Schott, two periods of thermogenesis are noted (increase in temperature in the flower by 10-12 °C), and shortly before this there is an almost 100-fold increase in the endogenous content of SA [72]. Exogenous treatment with SA or its analogues is capable of stimulating thermogenesis, while only two substances (aspirin and 2,6-dihydrobenzoic acid) which are most similar to SA, increased the temperature in flowers, while other analyzed SA analogues (31 compounds) did not have such an effect possessed. The observed increase in temperature is associated with activation of mitochondrial alternative oxidase [73].

In the 1970s, it was suggested that SA might be a flowering inducer because exogenous treatments stimulated flowering in both short- and long-day plants [74]. The participation of SA in the regulation of flowering is confirmed by the following facts: mutant Arabidopsis plants with SA deficiency and transgenic *NahG* forms expressing the salicylate hydroxylase gene are significantly delayed in flowering under short-day conditions [75]; SA synthesis and accumulation are required for the transition to far-ultraviolet (UV-C, wavelength 200-290 nm)-activated flowering [75]; plants accumulating SA are characterized by an early flowering phenotype [48, 76].

There is evidence of the involvement of SA in the regulation of the aging process of plants. Thus, during *Arabidopsis* aging, the amount of SC in tissues increased. In addition, in plants with reduced SA content (*npr1* mutant and plants

overexpressing *NahG*), and the number of transcripts of a number of genes associated with aging decreased [77].

SA treatment can improve crop yields. For example, treatment of tomato leaves with SA solution (> 0.125 mM) for 2 weeks increased yield (number and size of fruits) and improved consumer qualities (increased density of fruit pulp, increased content of phenols, lycopene and vitamin C) [78]. An effective way to increase stress resistance of agricultural crops is treatment with SA at the stage of seeds and early seedlings. Soaking tomato and bean seeds in SA solution or watering the soil during sowing increased the survival of seedlings under drought conditions and during high and low temperature stress [79]. Pretreatment of lupine seedlings with SA increased plant resistance to high temperatures [80]. Treatment of leaves of adult tomato plants with SA stimulated growth under salinity conditions, increased root mass, proline content and soluble carbohydrates in leaves, significantly increasing salt tolerance [81]. Salicylic acid helps keep cut flowers fresh [82].

Jasmonates. Modern scientific literature has accumulated a significant amount of experimental data on the physiological effects caused by endogenously produced and exogenously applied jasmonates to plants [83-86]. In higher plants, jasmonates are represented by 12-oxo-phytodienoic acid (12-OPDA), jasmonic acid (JA) and its derivatives, including methyl jasmonate (MeJA) and a conjugate of jasmonate with isoleucine which is responsible for the regulation of most jasmonate-dependent processes. It was found that 12-OPDC which serves as the final product of the plastid stage of biosynthesis, FA and its derivatives exhibit biological activity, while their functions overlap only partially [87, 88). The question of the functional specificity of certain jasmonates is of particular interest. Thus, there are known genes whose expression is regulated by 12-OPDK, but not by FA or MeFA, and the 12-OPDK signal can be transmitted through components of the FA signaling pathway or through other signaling pathways [89-92].

The regulatory effects of jasmonates are varied, but primarily the functions of jasmonates are associated with the regulation of mechanisms that determine plant resistance to necrotrophic pathogens and insects, including root pests [93, 94]. Plants lacking jasmonates are very sensitive to the action of these biotic environmental factors. Extensive evidence suggests a role for these substances in regulating resistance to biotrophic pathogens [95]. In response to mechanical damage and disruption of tissue integrity, jasmonates activate a complex of responses, the so-called wound responses, associated with changes in the expression of many genes [96, 97]. The protective responses induced by jasmonates include the biosynthesis of secondary metabolites, toxic compounds, as well as substances or enzymes that reduce the nutritional value of plant tissues, such as inhibitors of proteinases, deaminases and polyphenol oxidases [98-101]. An important aspect of FA participation in plant defense responses to insect attacks is the regulation of circadian genes, which allows synchronizing the rhythms of defense processes with insect behavior [102]. In response to the presence of pathogens, it is with the participation of jasmonates that the biosynthesis of protective secondary metabolites with antimicrobial and antioxidant properties (phytoalexins, phenylpropanoids, terpenoids, polyamines, and alkaloids) is initiated [103]. Jasmonates regulate the accumulation of free amino acids, which have protective properties (104). There is evidence that these hormones have a direct effect on the pathogens themselves [93]. Jasmonates help the plant fight competitors. For example, MeFA activates the biosynthesis of sorghum, a compound with pronounced herbicidal activity, in sorghum roots [105].

Jasmonates are involved in the regulation of indirect defense responses associated with the release of volatile compounds that can attract natural enemies

that attack insects [106-108]. The response of plants to insect pest attacks depends largely on the type of the damage, the insect feeding, and the type of pest mouthparts [103, 109, 110]. Volatile compounds released may also serve as an alarm signal to neighboring plants, allowing coordination of defense responses at the population level [111, 112].

Regulation of adaptive responses under conditions of biotic stress occurs as a result of the coordinated action of jasmonates and other phytohormones, including salicylic acid, ethylene, and ABA.

The role of jasmonates in regulating plant adaptation to abiotic stresses is also well known [113-118]. Jasmonates control resistance to low temperature and salt stress, flooding, drought, ozone, heavy metals and ultraviolet radiation. They serve as the main regulators of the most important signaling pathway that controls plant frost resistance — (ICE)-C-repeat Binding Factor/DRE Binding factor1 (CBF/DREB1) [119]. Data on the role of jasmonates in the formation of plant resistance to elevated temperatures are very contradictory. Most likely, jasmonates play a negative role under high temperature conditions, and increased catabolism of active forms of jasmonates under these conditions is an important adaptive mechanism [120]. The importance of FA and MeFA in plant protection under drought conditions has been demonstrated for many crops [117, 118, 121, 122]. The participation of 12-OPDC in the formation of drought resistance in *Arabidopsis* plants was also determined [121, 124]. Numerous studies indicate the protective effects of jasmonates under salinity conditions [116, 117, 125, 126]. Coronatine, a phytotoxin from *Pseudomonas syringae* (a functional analogue of jasmonates), significantly increases the resistance of maize to water deficiency and osmotic stress caused by polyethylene glycol by stimulating the formation of ROS and activating the antioxidant system [127].

The signaling and protective functions of jasmonates under biotic and abiotic stress conditions are in many cases associated with both oxidative stress and the antioxidant system [128]. Jasmonates regulate the formation of ROS, primarily $O_2^{\bullet -}$ (superoxide anion radical) and $HO^{\bullet}$ (hydroxyl radical). At the same time, treatment with jasmonates stimulates the activity of antioxidant enzymes [129].

In addition to adaptive processes under stress conditions, jasmonates regulate plant growth, development [95, 130-132] and flower formation [133], control fertility [87, 134] and flowering time [135], influence photosynthesis [136 and seed germination [137]. They inhibit root and shoot growth [96], but very low concentrations of these phytohormones can enhance stem growth, as it has been shown in grapes and morning glory (*Pharbitis nil*) [138, 139].

The high biological activity of jasmonates certainly determines the significant potential for their use in agriculture [140]. Not only jasmonates are used, but also their functional analogues, such as coronatine [83] and prohydrojasmone [141]. MeFA can be used as a volatile compound in closed containers/rooms, as well as in aerosols, in the form of diluted solutions. There are examples of the use of jasmonates to regulate flowering time, slow down plant growth, change their morphology, accumulate secondary metabolites and, of course, protect against insects and pathogens [140, 142]. Stimulation of the formation of storage organs, tubers, and bulbs has been demonstrated in many crops, including potatoes, *Dioscorea polystachya*, and orchids [143-146]. Exogenous treatment with jasmonate has been shown to inhibit unwanted sprouting of potato tubers and also prevent color change during processing or cooking [147]. Recent studies indicate that jasmonates regulate the distribution of metabolic and energy resources between processes leading to growth and biomass accumulation and processes associated with the synthesis of protective metabolites [148]. That is, by influencing the activity of the jasmonate system, it is possible to control central metabolism, stability,

and, consequently, plant productivity and crop quality. It is important that the effects of growth suppression are short-lived. This means that correct short-term use of these hormones should not affect plant growth and productivity, making their widespread use possible in practice [149].

Jasmonates can be used in fields to protect plants from abiotic and biotic stress factors during growth, crop ripening and after harvest without additional use of chemicals. In addition, jasmonates can improve the quality and phytochemical composition of food crops, make fruits more vibrant, aromatic, sweet, tasty, resistant to cracking, accelerate their ripening and increase their content of secondary metabolites (especially phenolic compounds), antioxidants and vitamins [93, 141, 150-152], slow down the deterioration and softening of tissues of berries and fruits [153-155], increase the ability to trap free radicals [153, 154], preserve the bright color of cut flowers [156]. Unlike many chemicals used in crop production, jasmonates are considered completely safe compounds, and there are no restrictions on their use as plant growth regulators [150].

Effects from treating various crops with jasmonates, salicylates and abscisic acid

Crop	Concentration	Stage of ontogenesis/organs	Effects	References
		Abscisic acid		
Triticum aestivum L., *Oryza sativa* L., *Sorghum bicolor* (L.) Moench, *Zea mays* L.	1 μM-1 mM	Seeds, seed germination, flowering	Regulation of growth and metabolic processes; stimulation of antioxidant protection, biosynthesis of stress proteins and secondary metabolites; increasing stress resistance and productivity	[9]
Glycine max (L.) Merr.	300 mg/l	7 leaves	Improving the distribution of metabolic flows; an increase in the dry mass of the above-ground parts, root density, leaf area, number of seeds in the bean and oil concentration, but not protein in the seeds; increase in soybean yield	[25]
Helianthus annuus L.	0.5-10 μM	Budding (preferred), flowering	Mitigation of the negative consequences of stress; increase in leaf area, basket diameter, number of achenes per basket, yield, weight of 1000 achenes, oil yield. Under sufficient moisture, negative effects occure	[26, 27]
Malus domestica Borkh.	20% ProTone™ (Valent BioSciences, USA)	Crown	Fall of leaves (without affecting the shoots of axillary buds)	[28]
Prunus avium (L.) L.	400 mg/l	Crown, fruits	Enhanced coloration of drupes	[30]
Citrus × *paradise* Macfad, *Citrus reticulate* Blanco	500 μM and 1 mM (crown), 1 nM-1 mM (roots)	Crown, roots (no effect)	Increased cold resistance; improving the color of fruits and reducing the content of organic acids in them	[31, 32]
Vitis vinifera L.	300 and 500 mg/l, 10 or 20% ProTone™ (Valent BioSciences, USA) at 200-400 g/ha	Vines, leaves only or bunches only	Acceleration of the beginning of berry ripening and increased color intensity; a decrease in the weight of berries with an increase in the dry weight of the skin; increased content of sugar, phenols, anthocyanins; decrease in transpiration rate	[33, 37, 157]
Lactuca sativa L.	150 and 300 μM	Leaves	Decrease in yield; an increase in the content of phenolic compounds and anthocyanins in red leaf lettuce, but not in green; inducing the accumulation of chlorophyll b and total carotenoids	[39]

Continued Table

Solanum lycopersicum L.	500 mg/l (foliar treatment) and 50 mg/l (root treatment)	Leaves, roots	Foliar application increases the content of carotenoids and chlorophylls in leaves and fruits, and root application reduces it; foliar and root treatment increases the sugar content in fruits and reduces the content of organic acids in them	[40]
Zea mays L.	25% BioNik™ (Valent BioSciences, USA)	Seeds	Delay in germination of male inbred lines to synchronize the pollination period with female flowers	[157]
		Salicylates		
Solanum lycopersicum L.	0.2 mM	Root feeding and leaf treatment	Resistance to *Fusarium oxysporum*	[54]
Solanum lycopersicum L.	2 mM	Spraying leaves	Resistance to tomato yellow leaf curl virus	[55]
Oryza sativa L.	0.05-8 mM	In a hydroponic solution and spraying leaves	Resistance to *Magnaporthe grisea* and *Xanthomonas oryzae*	[56, 57]
Solanum lycopersicum L.	0.1 μM and 0.1 mM	In nutritional solution	Salt stress tolerance	[64]
Triticum aestivum L.	10-1000 μM (100 μM is optimal concentration)	Leaves	Increased frost resistance	[66]
Solanum lycopersicum L.	0,.025 mM-0.125 mM	Leaves	Increased yield (number of fruits and their size) and consumer qualities (increased density, increased content of phenols, lycopene and vitamin C)	[78]
Phaseolus vulgaris L., *Lycopersicon esculentum* L.	0.1-0.5 mM	Seeds	Increased survival under drought, high and low temperature stress	[79]
Lupinus angustifolius L.	0.5 mM	Sprouts	Resistance to elevated temperatures	[80]
Solanum lycopersicum L.	100 mg/l	Roots and leaves	Stimulation of plant growth under salinity conditions	[81]
Rosa hybrida E.H.L. Krause, *Lilium asiaticum*, *Gerbera jamesonii* Bolus ex Hooker f.	100-300 mg/l	Cut flowers in a vase	Cut flowers stay fresh longer	[82]
		Jasmonic acid and jasmonates		
Garden and vegetable crops, cereals, legumes	Jasmonates, 10^{-7}-10^{-3} M	Various	Formation of storage organs, degradation of chlorophyll and leaf fall, reduction of transpiration, synthesis of secondary metabolites, protection from pests and pathogens	[93]
Microlaena stipoides (Labill.) R.Br.	MeJA, 10 μg/ml	Leaves	Protection from the root pest *Dermolepida albohirtum*	[94]
Larix olgensis A. Henry	Cis-jasmone, MeJA, JA, 0.01-1 mM	Sprouts	Induction of defense mechanisms due to the accumulation of free amino acids	[104]
Sorghum bicolor L.	MeJA, 0.5-500 μM	Seed soaking and sprout treatment	Biosynthesis of the natural herbicide sorgaleon	[105]
Oryza sativa L.	JA, 30 μM	Hydroponics	Increased salt tolerance	[126]
Zea mays L.	Coronatine, 0.0001-0.1 μM	Immerse the stems in the solution for 12 hours	Increased resistance to drought and osmotic stress	[127]
Solanum tuberosum L.	MeJA, JA, 0.1-0 μM	Stem segments	Stimulation of tuber formation	[144]
Solanum tuberosum L.	MeJA, 0,001 mM-0.1 mM	Potato tubers	Suppression of tuber germination and darkening	[147]
Prunus mume Sieb.	Prohydrojasmone, 0.4 mM	Fruit dipping in the solution	Increased aroma and resistance to *Colletotrichum gloeosporioides*	[152]
Malus domestica Borkh, *Vitis vinifera* L.	Prohydrojasmone, ~ 1 l/ha	Treating fruits on the plant	Enhance color, synthesis of anthocyanins, increase resistance to low temperatures, protection from pests	[158]

Note. MeJA — methyl jasmonate, JA — jasmonic acid.

The phytohormones that regulate plant stress responses can be a promising

alternative to modern plant protection products used in agriculture. The table shows examples of treating various plants with phytohormones and a description of the effects caused by the treatment.

Thus, the modern literature provides a significant amount of information on the effects of abscisic acid, jasmonates and salicylates on various crops, but most of the data is based on the results of lab tests, and there is an obvious lack of information on the physiological effects caused by these substances in field conditions. The widespread use of these compounds is largely limited by the possibility of their production, since the production of some phytohormones and their functional analogues on an industrial scale still remains a difficult task. If the cost of producing drugs based on salicylates is economically feasible, then the production of jasmonates, and especially abscisic acid, requires the use of expensive processes. Chemical stability of such compounds is an important aspect. It should be remembered that plant hormones are low-molecular substances (≤ 500 Da), except for polypeptide hormones, which serve as derivatives of basic biochemical compounds of plants, namely amino acids, carotenoids, terpenoids, phytosterols and fatty acids. Therefore, the most promising way to produce phytohormones seems to be the reconstruction of biosynthetic pathways in a living cell and the creation of bioproducers. Most likely, it is the successful development of biotechnologies with the use of bioproducers that will determine the scale of production and introduction of new drugs based on plant hormones in agriculture in the near future.

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REFERENCES

1. Meng Y., Shuai H., Luo X., Chen F., Zhou W., Yang W., Shu K. Karrikins: regulators involved in phytohormone signaling networks during seed germination and seedling development. *Frontiers in Plant Science*, 2017, 7: 02021 (doi: 10.3389/fpls.2016.02021).
2. Banerjee A., Roychoudhury A. Chapter 18 — Roles of turgorins and systemins in promoting agriculture. In: *Emerging plant growth regulators in agriculture*. T. Aftab, M. Naeem (eds.). Academic Press, 2022: 415-422 (doi: 10.1016/B978-0-323-91005-7.00003-5).
3. Handa A.K., Fatima T., Mattoo A.K. Polyamines: bio-molecules with diverse functions in plant and human health and disease. *Frontiers in Chemistry*, 2018, 6: 10 (doi: 10.3389/fchem.2018.00010).
4. Koprna R., De Diego N., Dundálková L., Spíchal L. Use of cytokinins as agrochemicals. *Bioorganic and Medicinal Chemistry*, 2016, 24(3): 484-92 (doi: 10.1016/j.bmc.2015.12.022).
5. Skůpa P., Opatrný Z., Petrášek J. Auxin biology: applications and the mechanisms behind. In: *Applied plant cell biology: cellular tools and approaches, vol. 22.* P. Nick, Z. Opatrny (eds.). Berlin, Springer, 2014 (doi: 10.1007/978-3-642-41787-0_3).
6. Rademacher W. Chemical regulators of gibberellin status and their application in plant production. In: *Annual plant reviews.* J.A. Roberts (eds.). Wiley-Blackwell, Hoboken, 2017: 359-403 (doi: 10.1002/9781119312994.apr0541).
7. Ghosh S., Halder S. Effect of different kinds of gibberellin on temperate fruit crops: a review. *Pharma Innovation*, 2018, 7(3): 315-319.
8. Bagale P., Pandey S., Regmi P., Bhusal S. Role of plant growth regulator “gibberellins” in vegetable production: an overview. *International Journal of Horticultural Science and Technology*, 2022, 9(3): 291-299 (doi: 10.22059/ijhst.2021.329114.495).
9. Kosakivska I.V., Vedenicheva N.P., Babenko L.M., Voytenko L.V., Romanenko K.O., Vasyuk V.A. Exogenous phytohormones in the regulation of growth and development of cereals under abiotic stresses. *Molecular Biology Reports*, 2022, 49(1): 617-628 (doi: 10.1007/s11033-021-06802-2).
10. Coll Y., Coll F., Amorys A., Pujol M. Brassinosteroids roles and applications: an up-date. *Biologia*, 2015, 70(6): 726-732 (doi: 10.1515/biolog-2015-0085).
11. Li J., Wu Y., Xie Q., Gong Z. Abscisic acid. In: *Hormone metabolism and signaling in plants* /S.M. Smith (eds.). Academic, Elsevier, New York, 2017: 161-202.
12. Chen K., Li G.-J., Bressan R.A., Song C.-P., Zhu J.-K., Zhao Y. Abscisic acid dynamics, signaling, and functions in plants. *Journal of Integrative Plant Biology*, 2020, 62(1): 25-54 (doi: 10.1111/jipb.12899).
13. Levi M., Brusa P., Chiatante D., Sparvoli E. Cell cycle reactivation in cultured pea embryo axes. Effect of abscisic acid. *In Vitro Cellular & Developmental Biology — Plant*, 1993, 29(2): 47-50 (doi: 10.1007/BF02632250).
14. Liu Y., Bergervoet J.H.W., De Vos C.H.R., Hilhorst H.W.M., Kraak H.L., Karssen C.M., Bino R.J.. Nuclear replication activities during imbibition of abscisic acid and gibberellin-deficient tomato

(*Lycopersicon esculentum* Mill.) seeds. *Planta*, 1994, 194(3): 368-373 (doi: 10.1007/BF00197537).
15. Finkelstein R. Abscisic acid synthesis and response. *The Arabidopsis Book*, 2013, 11: e0166 (doi: 10.1199/tab.0166).
16. Frey A., Godin B., Bonnet M., Sotta B., Marion-Poll A. Maternal synthesis of abscisic acid controls seed development and yield in *Nicotiana plumbaginifolia*. *Planta*, 2004, 218(6): 958-964 (doi: 10.1007/s00425-003-1180-7).
17. Hewage K.A.H., Yang J.-F., Wang D., Hao G.-F., Yang G.-F., Zhu J.-K. Chemical manipulation of abscisic acid signaling: a new approach to abiotic and biotic stress management in agriculture. *Advanced Science,* 2020, 7(18): 2001265 (doi: 10.1002/advs.202001265).
18. Yoshida T., Obata T., Feil R., Lunn J.E., Fujita Y., Yamaguchi-Shinozaki K., Fernie A.R. The role of abscisic acid signaling in maintaining the metabolic balance required for arabidopsis growth under nonstress conditions. *The Plant Cell*, 2019, 31(1): 84-105 (doi: 10.1105/tpc.18.00766).
19. Zhao Y., Zhang Z., Gao J., Wang P., Hu T., Wang Z., Hou Y.-J., Wan Y., Liu W., Xie S., Lu T., Xue L.,Liu Y., Macho A.P., Tao W.A., Bressan R.A., Zhu J.-K. Arabidopsis duodecuple mutant of PYL ABA receptors reveals PYL repression of ABA-independent SnRK2 activity. *Cell Reports*, 2018, 23(11): 3340-3351 (doi: 10.1016/j.celrep.2018.05.044).
20. Negin B., Yaaran A., Kelly G., Zait Y., Moshelion M. Mesophyll Abscisic Acid restrains early growth and flowering but does not directly suppress photosynthesis. *Plant Physiology*, 2019, 180(2): 910-925 (doi: 10.1104/pp.18.01334).
21. Humplík J.F., Bergougnoux V., Van Volkenburgh E. To stimulate or inhibit? That is the question for the function of abscisic acid. *Trends in Plant Science*, 2017, 22(10): 830-841 (doi: 10.1016/j.tplants.2017.07.009).
22. Lievens L., Pollier J., Goossens A., Beyaert R., Staal J. Abscisic acid as pathogen effector and immune regulator. *Frontiers in Plant Science,* 2017, 8: 587 (doi: 10.3389/fpls.2017.00587).
23. Xie K., Li L., Zhang H., Wang R., Tan X., He Y., Hong G., Li J., Ming F., Yao X., Yan F., Sun Z., Chen J. Abscisic acid negatively modulates plant defence against rice black-streaked dwarf virus infection by suppressing the jasmonate pathway and regulating reactive oxygen species levels in rice: a negative role of ABA in rice response to RBSDV. *Plant, Cell and Environment*, 2018, 41(10): 2504-2514 (doi: 10.1111/pce.13372).
24. Tsyganova A.V., Tsyganov V.E. Negative hormonal regulation of symbiotic nodule development. i. ethylene (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2015, 50(3): 267-277 (doi: 10.15389/agrobiology.2015.3.267eng).
25. Travaglia C., Reinoso H., Bottini R. Application of abscisic acid promotes yield in field-cultured soybean by enhancing production of carbohydrates and their allocation in seed. *Crop and Pasture Science*, 2009, 60(12): 1131-1136 (doi: 10.1071/CP08396).
26. Hussain S., Ma B.L., Saleem M.F., Anjum S.A., Saeed A., Iqbal J. Abscisic acid spray on sunflower acts differently under drought and irrigation conditions. *Agronomy Journal*, 2012, 104(3): 561-568 (doi: 10.2134/agronj2011.0315).
27. Hussain S., Saleem M.F., Iqbal J., Ibrahim M.M., Atta S., Ahmed T., Rehmani M.I.A. Exogenous application of abscisic acid may improve the growth and yield of sunflower hybrids under drought. *Pakistan Journal of Agricultural Sciences*, 2014, 51(1): 49-58.
28. Esperança C.D.F., Petri J.L., Rossi A.D., Couto M., Sezerino A.A., Gabardo G.C. Induction of senescence and foliar abscission in apple trees with the use of abscisic acid. *Journal of Experimental Agriculture International*, 2019, 35(5): 1-10 (doi: 10.9734/JEAI/2019/v35i530217).
29. Jarolmasjed S., Sankaran S., Kalcsits L., Khot L.R. Proximal hyperspectral sensing of stomatal conductance to monitor the efficacy of exogenous abscisic acid applications in apple trees. *Crop Protection*, 2018, 109: 42-50 (doi: 10.1016/j.cropro.2018.02.022).
30. Time A., Ponce C., Kuhn N., Arellano M., Sagredo B., Donoso J.M., Meisel L.A. Canopy spraying of abscisic acid to improve fruit quality of different sweet cherry cultivars. *Agronomy*, 2021, 11(10): 1947 (doi: 10.3390/agronomy11101947).
31. Melgoza F.J., Kusakabe A., Nelson S.D., Melgar J.C. Exogenous applications of abscisic acid increase freeze tolerance in citrus trees. *International Journal of Fruit Science*, 2014, 14(4): 376-387 (doi: 10.1080/15538362.2014.899138).
32. Wang X., Yin W., Wu J., Chai L., Yi H. Effects of exogenous abscisic acid on the expression of citrus fruit ripening-related genes and fruit ripening. *Scientia Horticulturae*, 2016, 201: 175-183 (doi: 10.1016/j.scienta.2015.12.024).
33. Balint G., Reynolds A.G. Impact of exogenous abscisic acid on vine physiology and grape composition of Cabernet Sauvignon. *American Journal of Enology and Viticulture*, 2013, 64(1): 74-87 (doi: 10.5344/ajev.2012.12075).
34. Gagné S., Esteve K., Deytieux-Belleau C., Saucier C., Geny L. Influence of abscisic acid in triggering "véraison" in grape berry skins of *Vitis vinifera* L. cv. Cabernet-Sauvignon. *OENO One*, 2006, 40(1): 7 (doi: 10.20870/oeno-one.2006.40.1.882).
35. Gambetta G.A., Matthews M.A., Shaghasi T.H., McElrone A.J., Castellarin S.D. Sugar and abscisic acid signaling orthologs are activated at the onset of ripening in grape. *Planta*, 2010, 232(1): 219-234 (doi: 10.1007/s00425-010-1165-2).

36. Koyama K., Sadamatsu K., Goto-Yamamoto N. Abscisic acid stimulated ripening and gene expression in berry skins of the Cabernet Sauvignon grape. *Functional and Integrative Genomics*, 2010, 10(3): 367-381 (doi: 10.1007/s10142-009-0145-8).
37. Li J., Liu B., Li X., Li D., Han J., Zhang Y., Ma C., Xu W., Wang L., Jiu S., Zhang C., Wang S. Exogenous abscisic acid mediates berry quality improvement by altered endogenous plant hormones level in "Ruiduhongyu" grapevine. *Frontiers in Plant Science*, 2021, 12: 739964 (doi: 10.3389/fpls.2021.739964).
38. Kou X., Yang S., Chai L., Wu C., Zhou J., Liu Y., Xue Z. Abscisic acid and fruit ripening: Multifaceted analysis of the effect of abscisic acid on fleshy fruit ripening. *Scientia Horticulturae*, 2021, 281: 109999 (doi: 10.1016/j.scienta.2021.109999).
39. Li Z., Zhao X., Sandhu A.K., Gu L. Effects of exogenous abscisic acid on yield, antioxidant capacities, and phytochemical contents of greenhouse grown lettuces. *Journal of Agricultural and Food Chemistry*, 2010, 58(10): 6503-6509 (doi: 10.1021/jf1006962).
40. Barickman T.C., Kopsell D.A., Sams C.E. Abscisic acid impacts tomato carotenoids, soluble sugars, and organic acids. *HortScience*, 2016, 51(4): 370-376 (doi: 10.21273/HORTSCI.51.4.370).
41. Vlot A.C., Dempsey D.A., Klessig D.F. Salicylic acid, a multifaceted hormone to combat disease. *Annual Review of Phytopathology*, 2009, 47: 177-206 (doi: 10.1146/annurev.phyto.050908.135202).
42. Ding P., Ding Y. Stories of salicylic acid: a plant defense hormone. *Trends in Plant Science*, 2020, 25(6): 549-565 (doi: 10.1016/j.tplants.2020.01.004).
43. Klessig D.F., Malamy J. The salicylic acid signal in plants. *Plant Molecular Biology*, 1994, 26(5): 1439-1458 (doi: 10.1007/BF00016484).
44. Alvarez M.E. Salicylic acid in the machinery of hypersensitive cell death and disease resistance. *Plant Molecular Biology*, 2000, 44(3): 429-442 (doi: 10.1023/a:1026561029533).
45. Bürger M., Chory J. Stressed out about hormones: how plants orchestrate immunity. *Cell Host & Microbe*, 2019, 26(2): 163-172 (doi: 10.1016/j.chom.2019.07.006).
46. Delaney T.P., Uknes S., Vernooij B., Friedrich L., Weymann K., Negrotto D., Gaffney T., Gut-Rella M., Kessmann H., Ward E., Ryals J. A central role of salicylic acid in plant disease resistance. *Science*, 1994, 266(5188): 1247-1250 (doi: 10.1126/science.266.5188.1247).
47. Vernooij B., Friedrich L., Goy P.A., Staub T., Kessmann H., Ryals J. 2, 6-Dichloroisonicotinic acid-induced resistance to pathogens without the accumulation of salicylic acid. *Molecular plant-Microbe Interactions*, 1995, 8(2): 228-234 (doi: 10.1094/MPMI-8-0228).
48. Peng Y., Yang J., Li X., Zhang Y. Salicylic acid: biosynthesis and signaling. *Annual Review of Plant Biology*, 2021, 72: 761-791 (doi: 10.1146/annurev-arplant-081320-092855).
49. Sinha M., Singh R.P., Kushwaha G.S., Iqbal N., Singh A., Kaushik S., Kaur P., Sharma S., Singh T.P. Current overview of allergens of plant pathogenesis related protein families. *The Scientific World Journal*, 2014, 2014: 543195 (doi: 10.1155/2014/543195).
50. Zhang Y., Cheng Y.T., Qu N., Zhao Q., Bi D., Li X. Negative regulation of defense responses in *Arabidopsis* by two NPR1 paralogs. *The Plant Journal*, 2006, 48(5): 647-656 (doi: 10.1111/j.1365-313X.2006.02903.x).
51. Chen Z., Silva H., Klessig D.F. Active oxygen species in the induction of plant systemic acquired resistance by salicylic acid. *Science*, 1993, 262(5141): 1883-1886 (doi: 10.1126/science.8266079).
52. Tan S., Abas M., Verstraeten I., Glanc M., Molnár G., Hajný J., Lasák P., Petřík I., Russinova E., Petrášek J., Novák O., Pospíšil J., Friml J. Salicylic acid targets protein phosphatase 2A to attenuate growth in plants. *Current Biology*, 2020, 30(3): 381-395 e8 (doi: 10.1016/j.cub.2019.11.058).
53. Koo Y.M., Heo A.Y., Choi H.W. Salicylic acid as a safe plant protector and growth regulator. *The Plant Pathology Journal*, 2020, 36(1): 1-10 (doi: 10.5423/PPJ.RW.12.2019.0295).
54. Mandal S., Mallick N., Mitra A. Salicylic acid-induced resistance to *Fusarium oxysporum* f. sp. lycopersici in tomato. *Plant Physiology and Biochemistry*, 2009, 47(7): 642-649 (doi: 10.1016/j.plaphy.2009.03.001).
55. Li T., Huang Y., Xu Z.-S., Wang F., Xiong A.-S. Salicylic acid-induced differential resistance to the Tomato yellow leaf curl virus among resistant and susceptible tomato cultivars. *BMC Plant Biology*, 2019, 19(1): 173 (doi: 10.1186/s12870-019-1784-0).
56. Daw B.D., Zhang L.H., Wang Z.Z. Salicylic acid enhances antifungal resistance to *Magnaporthe grisea* in rice plants. *Australasian Plant Pathology*, 2008, 37(6): 637-644 (doi: 10.1071/AP08054).
57. Mohan Babu R., Sajeena A., Vijaya Samundeeswari A., Sreedhar A., Vidhyasekaran P., Seetharaman K., Reddy M.S. Induction of systemic resistance to Xanthomonas oryzae pv. oryzae by salicylic acid in *Oryza sativa* (L.). *Journal of Plant Diseases and Protection*, 2003, 110(5): 419-431 (doi: 10.1007/BF03356119).
58. Wang Y., Liu J.H. Exogenous treatment with salicylic acid attenuates occurrence of citrus canker in susceptible navel orange (*Citrus sinensis* Osbeck). *Journal of Plant Physiology*, 2012, 169(12): 1143-1149 (doi: 10.1016/j.jplph.2012.03.018).
59. Glazebrook J. Contrasting mechanisms of defense against biotrophic and necrotrophic pathogens. *Annual Review of Phytopathology*, 2005, 43: 205-227 (doi: 10.1146/annurev.phyto.43.040204.135923).
60. El Oirdi M., El Rahman T.A., Rigano L., El Hadrami A., Rodriguez M.C., Daayf F., Vojnov A., Bouarab K. *Botrytis cinerea* manipulates the antagonistic effects between immune pathways to

promote disease development in tomato. *The Plant Cell*, 2011, 23(6): 2405-2421 (doi: 10.1105/tpc.111.083394).
61. Khanam N.N., Ueno M., Kihara J., Honda Y., Arase S. Suppression of red light-induced resistance in broad beans to *Botrytis cinerea* by salicylic acid. *Physiological and Molecular Plant Pathology*, 2005, 66(1-2): 20-29 (doi: 10.1016/j.pmpp.2005.03.006).
62. Ferrari S., Plotnikova J.M., De Lorenzo G., Ausubel F.M. *Arabidopsis* local resistance to *Botrytis cinerea* involves salicylic acid and camalexin and requires EDS4 and PAD2, but not *SID2*, *EDS5* or *PAD4*. *The Plant Journal*, 2003, 35(2): 193-205 (doi: 10.1046/j.1365-313x.2003.01794.x).
63. Li L., Zou Y. Induction of disease resistance by salicylic acid and calcium ion against *Botrytis cinerea* in tomato (*Lycopersicon esculentum*). *Emirates Journal of Food and Agriculture*, 2016, 29(1): 78-82 (doi: 10.9755/ejfa.2016-10-1515).
64. Horvath E., Csiszar J., Galle A., Poor P., Szepesi A., Tari I. Hardening with salicylic acid induces concentration-dependent changes in abscisic acid biosynthesis of tomato under salt stress. *Journal of Plant Physiology*, 2015, 183: 54-63 (doi: 10.1016/j.jplph.2015.05.010).
65. Kolupaev Yu.E., Karpets' Yu.V. *Vestnik Tomskogo gosudarstvennogo universiteta. Biologiya*, 2021, 55 (doi: 10.17223/19988591/55/8) (in Russ.).
66. Wang W., Wang X., Huang M., Cai J., Zhou Q., Dai T., Cao W., Jiang D. Hydrogen peroxide and abscisic acid mediate salicylic acid-induced freezing tolerance in wheat. *Frontiers in Plant Science*, 2018, 9: 1137 (doi: 10.3389/fpls.2018.01137).
67. Rivas-San Vicente M., Plasencia J. Salicylic acid beyond defence: its role in plant growth and development. *Journal of Experimental Botany*, 2011, 62(10): 3321-3338 (doi: 10.1093/jxb/err031).
68. Lebeis S.L., Paredes S.H., Lundberg D.S., Breakfield N., Gehring J., McDonald M., Malfatti S., Glavina del Rio T., Jones C.D., Tringe S.G., Dangl J.L. Salicylic acid modulates colonization of the root microbiome by specific bacterial taxa. *Science*, 2015, 349(6250): 860-864 (doi: 10.1126/science.aaa8764).
69. Rajjou L., Belghazi M., Huguet R., Robin C., Moreau A., Job C., Job D. Proteomic investigation of the effect of salicylic acid on *Arabidopsis* seed germination and establishment of early defense mechanisms. *Plant Physiology*, 2006, 141(3): 910-923 (doi: 10.1104/pp.106.082057).
70. Zeilmaker T., Ludwig N.R., Elberse J., Seidl M.F., Berke L., Van Doorn A., Schuurink R.C., Snel B., Van den Ackerveken G. DOWNY MILDEW RESISTANT 6 and DMR6-LIKE OXYGENASE 1 are partially redundant but distinct suppressors of immunity in *Arabidopsis*. *The Plant Journal*, 2015, 81(2): 210-222 (doi: 10.1111/tpj.12719).
71. Zhang Y., Zhao L., Zhao J., Li Y., Wang J., Guo R., Gan S., Liu C.-J., Zhang K. *S5H/DMR6* encodes a salicylic acid 5-Hydroxylase that fine-tunes salicylic acid homeostasis. *Plant Physiology*, 2017, 175(3): 1082-1093 (doi: 10.1104/pp.17.00695).
72. Raskin I., Turner I.M., Melander W.R. Regulation of heat production in the inflorescences of an *Arum* lily by endogenous salicylic acid. *Proceedings of the National Academy of Sciences*, 1989, 86(7): 2214-2218 (doi: 10.1073/pnas.86.7.2214).
73. Rhoads D.M., McIntosh L. Salicylic acid regulation of respiration in higher plants: alternative oxidase expression. *The Plant Cell*, 1992, 4(9): 1131-1139 (doi: 10.1105/tpc.4.9.1131).
74. Cleland C.F., Tanaka O. Effect of daylength on the ability of salicylic acid to induce flowering in the long-day plant *Lemna gibba* G3 and the short-day plant *Lemna paucicostata* 6746. *Plant Physiology*, 1979, 64(3): 421-424 (doi: 10.1104/pp.64.3.421).
75. Martinez C., Pons E., Prats G., Leon J. Salicylic acid regulates flowering time and links defence responses and reproductive development. *The Plant Journal*, 2004, 37(2): 209-217 (doi: 10.1046/j.1365-313x.2003.01954.x).
76. Jin J.B., Jin Y.H., Lee J., Miura K., Yoo C.Y., Kim W.-Y., Van Oosten M., Hyun Y., Somers D.E., Lee I., Yun D.-J., Bressan R.A., Hasegawa P.M. The SUMO E3 ligase, *AtSIZ1*, regulates flowering by controlling a salicylic acid-mediated floral promotion pathway and through affects on *FLC* chromatin structure. *The Plant Journal*, 2008, 53(3): 530-540 (doi: 10.1111/j.1365-313X.2007.03359.x).
77. Morris K., A.-H.-Mackerness S., Page T., John C.F., Murphy A.M., Carr J.P., Buchanan-Wollaston V. Salicylic acid has a role in regulating gene expression during leaf senescence. *The Plant Journal*, 2000, 23(5): 677-685 (doi: 10.1046/j.1365-313x.2000.00836.x).
78. Sariñana-Aldaco O., Sánchez-Chávez E., Troyo-Diéguez E., Tapia-Vargas L.M., Díaz-Pérez J.C., Preciado-Rangel P. Foliar aspersion of salicylic acid improves nutraceutical quality and fruit yield in tomato. *Agriculture*, 2020, 10(10): 482 (doi: 10.3390/agriculture10100482).
79. Senaratna T., Touchell D., Bunn E., Dixon K. Acetyl salicylic acid (Aspirin) and salicylic acid induce multiple stress tolerance in bean and tomato plants. *Plant Growth Regulation*, 2000, 30(2): 157-161 (doi: 10.1023/A:1006386800974).
80. Butsanets P.A., Shugaev A.G. *Mezhdunarodnyy nauchno-issledovatel'skiy zhurnal*, 2021, 12(114): 63-66 (doi: 10.23670/IRJ.2021.114.12.043) (in Russ.).
81. Souri M.K., Tohidloo G. Effectiveness of different methods of salicylic acid application on growth characteristics of tomato seedlings under salinity. *Chemical and Biological Technologies in Agriculture*, 2019, 6(1): 26 (doi: 10.1186/s40538-019-0169-9).
82. Bayat H., Aminifard M.H. Salicylic acid treatment extends the vase life of five commercial cut

flowers. *Electronic Journal of Biology*, 2017, 13(1): 67-72.
83. Ahmad P., Rasool S., Gul A., Sheikh S.A., Akram N.A., Ashraf M., Kazi A.M., Gucel S. Jasmonates: multifunctional roles in stress tolerance. *Frontiers in Plant Science*, 2016, 7: 813 (doi: 10.3389/fpls.2016.00813).
84. Huang H., Liu B., Liu L., Song S. Jasmonate action in plant growth and development. *Journal of Experimental Botany*, 2017, 68(6): 1349-1359 (doi: 10.1093/jxb/erw495).
85. Wasternack C., Hause B. Jasmonates: biosynthesis, perception, signal transduction and action in plant stress response, growth and development. An update to the 2007 review in Annals of Botany. *Annals of Botany*, 2013, 111(6): 1021-1058 (doi: 10.1093/aob/mct067).
86. Tran L.-S.P., Pal S. *Phytohormones: a window to metabolism, signaling and biotechnological applications*. New York, Springer-Verla, 2014.
87. Stintzi A., Browse J. The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proceedings of the National Academy of Sciences*, 2000, 97(19): 10625-10630 (doi: 10.1073/pnas.190264497).
88. Schaller A., Stintzi A. Enzymes in jasmonate biosynthesis — structure, function, regulation. *Phytochemistry*, 2009, 70(13-14): 1532-1538 (doi: 10.1016/j.phytochem.2009.07.032).
89. Stintzi A., Weber H., Reymond P., Browse J., Farmer E.E. Plant defense in the absence of jasmonic acid: the role of cyclopentenones. *Proceedings of the National Academy of Sciences*, 2001, 98(22): 12837-12842 (doi: 10.1073/pnas.211311098).
90. Taki N., Sasaki-Sekimoto Y., Obayashi T., Kikuta A., Kobayashi K., Ainai T., Yagi K., Sakurai N., Suzuki H., Masuda T., Takamiya K., Shibata D., Kobayashi Y., Ohta H. 12-oxo-phytodienoic acid triggers expression of a distinct set of genes and plays a role in wound-induced gene expression in *Arabidopsis*. *Plant Physiology*, 2005, 139(3): 1268-1283 (doi: 10.1104/pp.105.067058).
91. Ribot C., Zimmerli C., Farmer E.E., Reymond P., Poirier Y. Induction of the Arabidopsis PHO1;H10 gene by 12-oxo-phytodienoic acid but not jasmonic acid via a CORONATINE INSENSITIVE1-dependent pathway. *Plant Physiology*, 2008, 147(2): 696-706 (doi: 10.1104/pp.108.119321).
92. Arnold M.D., Gruber C., Flokova K., Miersch O., Strnad M., Novak O., Wasternack C., Hause B. The recently identified isoleucine conjugate of cis-12-Oxo-Phytodienoic acid is partially active in cis-12-oxo-phytodienoic acid-specific gene expression of *Arabidopsis thaliana*. *PLoS ONE*, 2016, 11(9): e0162829 (doi: 10.1371/journal.pone.0162829).
93. Rohwer C.L., Erwin J.E. Horticultural applications of jasmonates. *The Journal of Horticultural Science and Biotechnology*, 2008, 83(3): 283-304 (doi: 10.1080/14620316.2008.11512381).
94. Johnson S.N., Glauser G., Hiltpold I., Moore B.D., Ryalls J.M.W. Root herbivore performance suppressed when feeding on a jasmonate-induced pasture grass. *Ecological Entomology*, 2018, 43(4): 547-550 (doi: 10.1111/een.12527).
95. Trang Nguyen H., Thi Mai To H., Lebrun M., Bellafiore S., Champion A. Jasmonates-the master regulator of rice development, adaptation and defense. *Plants*, 2019, 8(9): 339 (doi: 10.3390/plants8090339).
96. Vega-Muñoz I., Duran-Flores D., Fernández-Fernández Á.D., Heyman J., Ritter A., Stael S. Breaking bad news: dynamic molecular mechanisms of wound response in plants. *Frontiers in Plant Science*, 2020, 11: 610445 (doi: 10.3389/fpls.2020.610445).
97. Pieterse C.M., Van der Does D., Zamioudis C., Leon-Reyes A., Van Wees S.C. Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, 2012, 28: 489-521 (doi: 10.1146/annurev-cellbio-092910-154055).
98. Felton G.W., Donato K.K., Broadway R.M., Duffey S.S. Impact of oxidized plant phenolics on the nutritional quality of dietar protein to a noctuid herbivore, *Spodoptera exigua*. *Journal of Insect Physiology*, 1992, 38(4): 277-285 (doi: 10.1016/0022-1910(92)90128-Z).
99. Fidantsef A.L., Stout M.J., Thaler J.S., Duffey S.S., Bostock R.M. Signal interactions in pathogen and insect attack: expression of lipoxygenase, proteinase inhibitor II, and pathogenesis-related protein P4 in the tomato, *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology*, 1999, 54: 97-114 (doi: 10.1006/pmpp.1998.0192).
100. Farmer E.E., Ryan C.A. Interplant communication: airborne methyl jasmonate induces synthesis of proteinase inhibitors in plant leaves. *Proceedings of the National Academy of Sciences*, 1990, 87(19): 7713-7716 (doi: 10.1073/pnas.87.19.7713).
101. Chen H., Wilkerson C.G., Kuchar J.A., Phinney B.S., Howe G.A. Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. *Proceedings of the National Academy of Sciences*, 2005, 102(52): 19237-19242 (doi: 10.1073/pnas.0509026102).
102. Goodspeed D., Chehab E.W., Min-Venditti A., Braam J., Covington M.F. Arabidopsis synchronizes jasmonate-mediated defense with insect circadian behavior. *Proceedings of the National Academy of Sciences*, 2012, 109(12): 4674-4677 (doi: 10.1073/pnas.1116368109).
103. Okada K., Abe H., Arimura G. Jasmonates induce both defense responses and communication in monocotyledonous and dicotyledonous plants. *Plant Cell Physiology*, 2015, 56(1): 16-27 (doi: 10.1093/pcp/pcu158).

104. Meng Z.J., Yan S.C., Liu D,. Yang C.P. Effects of exogenous jasmonates on free amino acid contents in needles of *Larix olgensis* seedlings. *African Journal of Agricultural Research*, 2012, 7(19): 2995-3006 (doi: 10.5897/AJAR11.2385).
105. Uddin M.R., Thwe A.A., Kim Y.B., Park W.T., Chae S.C., Park S.U. Effects of jasmonates on sorgoleone accumulation and expression of genes for sorgoleone biosynthesis in sorghum roots. *Journal of Chemical Ecology*, 2013, 39(6): 712-722 (doi: 10.1007/s10886-013-0299-7).
106. Wasternack C., Hause B. Jasmonates and octadecanoids: signals in plant stress responses and development. *Progress in Nucleic Acid Research and Molecular Biology*, 2002, 72: 165-221 (doi: 10.1016/S0079-6603(02)72070-9).
107. Howe G.A., Jander G. Plant immunity to insect herbivores. *Annual Review of Plant Biology*, 2008, 59: 41-66 (doi: 10.1146/annurev.arplant.59.032607.092825).
108. Paré P.W., Tumlinson J.H. Plant volatiles as a defense against insect herbivores. *Plant Physiology*, 1999, 121(2): 325-332 (doi: 10.1104/pp.121.2.325).
109. De Moraes C.M., Lewis W.J., Paré P.W., Alborn H.T., Tumlinson J.H. Herbivore-infested plants selectively attract parasitoids. *Letters to Nature*, 1998, 393: 570-573 (doi: 10.1038/31219).
110. Savchenko T., Pearse I.S., Ignatia L., Karban R., Dehesh K. Insect herbivores selectively suppress the HPL branch of the oxylipin pathway in host plants. *The Plant Journal*, 2013, 73(4): 653-662 (doi: 10.1111/tpj.12064).
111. Engelberth J., Alborn H.T., Schmelz E.A., Tumlinson J.H. Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences*, 2004, 101(6): 1781-1785 (doi: 10.1073/pnas.0308037100).
112. Farag M.A., Fokar M., Abd H., Zhang H., Allen R.D., Paré P.W. (Z)-3-Hexenol induces defense genes and downstream metabolites in maize. *Planta*, 2005, 220(6): 900-909 (doi: 10.1007/s00425-004-1404-5).
113. Savchenko T.V., Zastrizhnaya O.M., Klimov V.V. *Biokhimiya*, 2014, 79(4): 458-475 (doi: 10.1134/S0006297914040051) (in Russ.).
114. Pandita D. Chapter 5 - Jasmonates: key players in plant stress tolerance. In: *Emerging plant growth regulators in agriculture*. T. Aftab, M. Naeem (eds.). Academic Press, 2022: 165-192 (doi: 10.1016/B978-0-323-91005-7.00020-5).
115. Savchenko T., Rolletschek H., Heinzel N., Tikhonov K., Dehesh K. Waterlogging tolerance rendered by oxylipin-mediated metabolic reprogramming in *Arabidopsis*. *Journal of Experimental Botany*, 2019, 70(10): 2919-2932 (doi: 10.1093/jxb/erz110).
116. Kazan K. Diverse roles of jasmonates and ethylene in abiotic stress tolerance. *Trends in Plant Science*, 2015, 20(4): 219-229 (doi: 10.1016/j.tplants.2015.02.001).
117. Kim H., Seomun S., Yoon Y., Jang G. Jasmonic acid in plant abiotic stress tolerance and interaction with abscisic acid. *Agronomy*, 2021, 11(9): 1886 (doi: 10.3390/agronomy11091886).
118. Siddiqi K.S., Husen A. Plant response to jasmonates: current developments and their role in changing environment. *Bulletin of the National Research Centre*, 2019, 43(1): 153 (doi: 10.1186/s42269-019-0195-6).
119. Hu Y., Jiang L., Wang F., Yu D. Jasmonate regulates the INDUCER OF CBF EXPRESSION-C-REPEAT BINDING FACTOR/DRE BINDING FACTOR1 cascade and freezing tolerance in *Arabidopsis*. *Plant Cell*, 2013, 25(8): 2907-2924 (doi: 10.1105/tpc.113.112631).
120. Zhu T., Herrfurth C., Xin M., Savchenko T., Feussner I., Goossens A., De Smet I. Warm temperature triggers JOX and ST2A-mediated jasmonate catabolism to promote plant growth. *Nature Communications*, 2021, 12(1): 4804 (doi: 10.1038/s41467-021-24883-2).
121. Riemann M., Dhakarey R., Hazman M., Miro B., Kohli A., Nick P. Exploring jasmonates in the hormonal network of drought and salinity responses. *Frontiers in Plant Science*, 2015, 6: 1077 (doi: 10.3389/fpls.2015.01077).
122. Wang X., Li Q., Xie J., Huang M., Cai J., Zhou Q., Dai T., Jiang D. Abscisic acid and jasmonic acid are involved in drought priming-induced tolerance to drought in wheat. *The Crop Journal*, 2021, 9(1): 120-132 (doi: 10.1016/j.cj.2020.06.002).
123. Savchenko T., Kolla V.A., Wang C.Q., Nasafi Z., Hicks D.R., Phadungchob B., Chehab W.E., Brandizzi F., Froehlich J., Dehesh K. Functional convergence of oxylipin and abscisic acid pathways controls stomatal closure in response to drought. *Plant Physiology*, 2014, 164(3): 1151-1560 (doi: 10.1104/pp.113.234310).
124. Savchenko T., Dehesh K. Drought stress modulates oxylipin signature by eliciting 12-OPDA as a potent regulator of stomatal aperture. *Plant Signaling & Behavior*, 2014, 9(4): e28304 (doi: 10.4161/psb.28304).
125. Delgado C., Mora-Poblete F., Ahmar S., Chen J.-T., Figueroa C.R. Jasmonates and plant salt stress: molecular players, physiological effects, and improving tolerance by using genome-associated tools. *International Journal of Molecular Sciences*, 2021, 22(6): 3082 (doi: 10.3390/ijms22063082).
126. Kang D.-J., Seo Y.-J., Lee J.-D., Ishii R., Kim K.U., Shin D.H., Park S., Jang S.W., Lee I.-J. Jasmonic acid differentially affects growth, ion uptake and abscisic acid concentration in salt-tolerant and salt-sensitive rice cultivars. *Journal of Agronomy and Crop Science*, 2005, 191(4): 273-282 (doi: 10.1111/j.1439-037X.2005.00153.x).

127. Yu H., Wang Y., Xing J., Zhang Y., Duan L., Zhang M., Li Z. Coronatine modulated the generation of reactive oxygen species for regulating the water loss rate in the detaching maize seedlings. *Agriculture*, 2021, 11(7): 685 (doi: 10.3390/agriculture11070685).
128. Ho T.-T., Murthy H.N., Park S.-Y. Methyl jasmonate induced oxidative stress and accumulation of secondary metabolites in plant cell and organ cultures. *International Journal of Molecular Sciences*, 2020, 21(3): 716 (doi: 10.3390/ijms21030716).
129. Kolupaev Yu.E., Yastreb T.O. *Prikladnaya biokhimiya i mikrobiologiya*, 2021, 57(1): 3-23 (doi: 10.31857/S0555109921010281) (in Russ.).
130. Chen Q., Sun J.Q., Zhai Q.Z., Zhou W.K., Qi L.L., Xu L., Wang B., Chen R., Jiang H., Qi J., Li X., Palme K., Li C. The basic helix-loop-helix transcription factor MYC2 directly represses PLETHORA expression during jasmonate-mediated modulation of the root stem cell niche in *Arabidopsis*. *Plant Cell*, 2011, 23(9): 3335-3352 (doi: 10.1105/tpc.111.089870).
131. Goetz S., Hellwege A., Stenzel I., Kutter C., Hauptmann V., Forner S., Mccaig B., Hause G., Miersch O., Wasternack C., Hause B. Role of cis-12-oxo-phytodienoic acid in tomato embryo development. *Plant Physiology*, 2012, 158(4): 1715-1727 (doi: 10.1104/pp.111.192658).
132. Pigolev A., Miroshnichenko D., Dolgov S., Savchenko T. Regulation of sixth seminal root formation by jasmonate in *Triticum aestivum* L. *Plants*, 2021, 10(2): 219 (doi: 10.3390/plants10020219).
133. Hause B., Stenzel I., Miersch O., Maucher H., Kramell R., Ziegler J., Wasternack C. Tissue-specific oxylipin signature of tomato flowers: allene oxide cyclase is highly expressed in distinct flower organs and vascular bundles. *The Plant Journal*, 2000, 24(1): 113-126 (doi: 10.1046/j.1365-313x.2000.00861.x).
134. Feys B., Benedetti C.E., Penfold C.N., Turner J.G. Arabidopsis mutants selected for resistance to the phytotoxin coronatine are male sterile, insensitive to methyl jasmonate, and resistant to a bacterial pathogen. *Plant Cell*, 1994, 6(5): 751-759 (doi: 10.1105/tpc.6.5.751).
135. Krajncic B., Kristl J., Janzekovic I. Possible role of jasmonic acid in the regulation of floral induction, evocation and floral differentiation in *Lemna minor* L. *Plant Physiology and Biochemistry*, 2006, 44(11-12): 752-758 (doi: 10.1016/j.plaphy.2006.10.029).
136. Creelman R.A., Mullet J.E. Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Biology*, 1997, 48: 355-381 (doi: 10.1146/annurev.arplant.48.1.355).
137. Xu Q., Truong T.T., Barrero J.M., Jacobsen J.V., Hocart C.H., Gubler F. A role for jasmonates in the release of dormancy by cold stratification in wheat. *Journal of Experimental Botany*, 2016, 67(11): 3497-3508 (doi: 10.1093/jxb/erw172).
138. Ravnikar M., Rode J., Gogala N., Benedicic D. Regulation of organogenesis with jasmonic acid. *Acta Horticulturae*, 1990, 280: 169-172 (doi: 10.17660/ActaHortic.1990.280.29).
139. Maciejewska B., Kopcewicz J. Inhibitory effect of methyl jasmonate on flowering and elongation growth in *Pharbitis nil*. *Journal of Plant Growth Regulation*, 2002, 21(3): 216-223 (doi: 10.1007/s003440010061).
140. Lalotra S., Hemantaranjan A., Yashu B.R., Srivastava R., Kumar S. Jasmonates: an emerging approach in biotic and abiotic stress tolerance. In: *Plant science — structure, anatomy and physiology in plants cultured in vivo and in vitro*. A. Gonzalez, M. Rodriguez, N.G. Sağlam (eds.). London, IntechOpen, 2020 (doi: 10.5772/intechopen.84608).
141. Kondo S., Roles of jasmonates in fruit ripening and environmental stress. *Acta Horticulturae*, 2010, 884: 711-716 (doi: 10.17660/ActaHortic.2010.884.96).
142. Chiu Y.-C., Matak K., Ku K.-M. Methyl jasmonate treated broccoli: Impact on the production of glucosinolates and consumer preferences. *Food Chemistry*, 2019, 299: 125099 (doi: 10.1016/j.foodchem.2019.125099).
143. Koda Y. Possible involvement of jasmonates in various morphogenic events. *Physiologia Plantarum*, 1997, 100(3): 639-646 (doi: 10.1111/j.1399-3054.1997.tb03070.x).
144. Koda Y., Kikuta Y. Effects of jasmonates on in vitro tuberization in several potato cultivars that differ greatly in maturity. *Plant Production Science*, 2001, 4(1): 66-70 (doi: 10.1626/pps.4.66).
145. Kim S.K., Kim J.T., Jang S.W., Lee S.C., Lee B.H., Lee I.J. Exogenous effect of gibberellins and jasmonate on tuber enlargement of *Dioscorea opposita*. *Agronomy Research*, 2005, 3: 39-44.
146. Debeljak N., Regvar M., Dixon K.W., Sivasithamparam K. Induction of tuberisation in vitro with jasmonic acid and sucrose in an Australian terrestrial orchid, *Pterostylis sanguinea*. *Plant Growth Regulation*, 2004, 36: 253-260 (doi: 10.1023/A:1016570319387).
147. Lulai E.C., Orr P.H., Glynn M.T. *Natural suppression of sprouting in stored potatoes using jasmonates. A01N37/42. North Dakota State University (USA). № US5436226A. Appl. 01.11.93. Publ. 25.07.95.*
148. Savchenko T.V., Rolletschek H., Dehesh K. Jasmonates-mediated rewiring of central metabolism regulates adaptive responses. *Plant and Cell Physiology*, 2019, 60(12): 2613-2620 (doi: 10.1093/pcp/pcz181).
149. Boughton A.J., Hoover K., Felton G.W. Impact of chemical elicitor applications on greenhouse tomato plants and population growth of the green peach aphid, *Myzus persicae*. *Entomologia Experimentalis et Applicata*, 2006, 120(3): 175-188 (doi: 10.1111/j.1570-7458.2006.00443.x).
150. Asghari M. Impact of jasmonates on safety, productivity and physiology of food crops. *Trends in Food Science & Technology*, 2019, 91: 169-183 (doi: 10.1016/j.tifs.2019.07.005).
151. Tassoni A., Fornalи S., Franceschetti M., Musiani F., Michael A.J., Perry B., Bagni M.

Jasmonates and Na-orthovanadate promote resveratrol production in *Vitis vinifera* cv. Barbera cell cultures. *New Phytologist*, 2005, 166(3): 895-905 (doi: 10.1111/j.1469-8137.2005.01383.x).
152. Nimitkeatkai H., Shishido M., Okawa K., Ohara H., Ban Y., Kita M., Moriguchi T., Ikeura H., Hayata Y., Kondo S. Effect of jasmonates on ethylene biosynthesis and aroma volatile emission in japanese apricot infected by a pathogen (*Colletotrichum gloeosporioides*). *Journal of Agricultural and Food Chemistry*, 2011, 59(12): 6423-6429 (doi: 10.1021/jf2010996).
153. Ayala-Zavala J.F., Wang S.Y., Wang C.Y., González-Aguilar G.A. Methyl jasmonate in conjunction with ethanol treatment increases antioxidant capacity, volatile compounds and postharvest life of strawberry fruit. *European Food Research and Technology*, 2005, 221: 731-738 (doi: 10.1007/s00217-005-0069-z).
154. Wang C.Y. Maintaining postharvest quality of raspberries with natural volatile compounds. *International Journal of Food Science & Technology*, 2003, 38(8): 869-785 (doi: 10.1046/j.0950-5423.2003.00758.x).
155. Jin P., Zheng Y.H., Cheng C., Gao H.-Y., Chen W.X., Chen H.J. Effect of methyl jasmonate treatment on fruit decay and quality in peaches during storage at ambient temperature. *Acta Horticulturae*, 2006, 712: 711-716 (doi: 10.17660/ActaHortic.2006.712.90).
156. Meir S., Droby S., Kochanek B., Salim S., Philosoph-Hadas S. Use of methyl jasmonate for suppression of botrytis rot in various cultivars of cut rose flowers. *Acta Horticulturae*, 2005, 669: 91-98 (doi: 10.17660/ActaHortic.2005.669.10).
157. Valent BioSciences. Available: https://www.valentbiosciences.com. Accessed: 01.06.2022.
158. IUPAC. *Prohydrojasmon*. Available: http://sitem.herts.ac.uk/aeru/iupac/Reports/2945.htm. Accessed: 01.06.2022.

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 23-42
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 631.4:631.617:631.672:631.81

doi: 10.15389/agrobiology.2023.1.23eng
doi: 10.15389/agrobiology.2023.1.23rus

POLYMER HYDROGELS IN AGRICULTURE
(review)

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The authors declare no conflict of interests
Acknowledgements:
The work was supported financially by the Government of the Perm Territory within the framework of scientific project No. S-26/507.
Final revision received July 27, 2022
Accepted August 26, 2022

Abstract

Polymer hydrogels (PHGs) are formed by swelling three-dimensionally cross-linked hydrophilic polymers and are usually characterized by high water-holding capacity (K. Rop et al., 2019; N. Singh et al., 2021; A. Sikder et al., 2021). Moisture capacity and a prolonged release of fertilizers, pesticides and bio-preparations make them promising for use in agriculture (P. Rychter et al., 2016; A. Sikder et al., 2021). PHGs reduces the need for frequent irrigation, increases seed germination, plant growth, seedling survival, enhances root growth, prevents soil erosion, pesticide and fertilizer overdose (N. Singh et al., 2021). According to their origin, PHGs are divided into synthetic and natural ones: synthetic hydrogels, mainly polymers and copolymers of acrylamide and acrylic acid, have a high water-holding capacity and strength, however, they are weakly degraded in soils (A.V. Smagin et al., 2014; B. Wilske et al., 2014). It is known that microorganisms are able to use PHGs based on acrylic polymers as a source of nitrogen and/or carbon for growth (H. Matsuoka et al., 2002; M. Bao et al., 2010; F. Yu et al., 2015) due to the presence of amidase activity (F. Yu et al., 2015; A. Nyyssцlд et al., 2019), ensuring gradual decomposition of PHGs in the soil. Natural hydrogels, among which cellulose-based PHGs predominate, have less strength, but are biodegradable and are environmentally friendly (R. Kundu et al., 2022). In addition to cellulose, collagen (Z.-Y. Hu et al., 2021), alginates (B. Tomadoni et al., 2020), chitosan (A. Zinchenko et al., 2022), and other polysaccharides are used as water-retaining strongly swelling agents of natural origin. Hydrogels are promising as carriers for the prolonged release of fertilizers, mainly urea (P. Rychter et al., 2016; W. Tanan et al., 2021), pesticides (C. Xu et al., 2021; C. Bai et al., 2015; F.E. Baloch et al., 2021; D. Zheng et al., 2022), for the introduction of microbial preparations into the soil, including phosphate-mobilizing and nitrogen-fixing bacteria (C.S. Wu, 2008; A.V. Kovrizhnikov et al., 2021). For a more active introduction of PHGs into practice, it is necessary to reduce their cost, mainly by the creation of composite materials based on agricultural and biotechnology industries waste. It is necessary to combine the positive qualities of synthetic and natural PHGs, synthesizing semi-synthetic hydrogels that are biodegradable and do not pollute the environment, have optimal mechanical strength and water-absorbing capacity. As water-retaining and anti-erosion agents, hydrogels based on polymers and copolymers of acrylamide and acrylic acid are more promising (I.G. Panova et al., 2021; N.B. Sadovnikova et al., 2014; A.V. Smagin et al., 2014), and natural and semi-synthetic PHGs are more promising as carriers of fertilizers and pesticides (P. Jungsinyatam et al., 2022; A. Di Martino et al., 2021). This review summarizes current information on the use of PHGs of various compositions in agriculture, provides data on the positive effect of PHGs on soil water balance, productivity, growth, survival of various crops, seed germination and commercial quality of root crops, as well as the prospects for the PHGs development.

Keywords: polymer hydrogels, water-retaining capacity, biological preparations, fertilizers, pesticides

Hydrophilic polymers with multiple bonds between macromolecules can

absorb water volume exceeding their own dry mass 1000-fold or more. The resulting polymer hydrogels (PHGs) retain their swollen state even under pressure. The ability to absorb and retain water is due to the presence of hydrophilic groups −OH, −CONH−, $-CONH_2$, $-SO_3H$, $-NH_2$, −COOH, −OH in the polymer molecule. Despite their high moisture holding capacity, PHGs do not dissolve due to their three-dimensionally cross-linked structure [1, 2].

The purpose of this review is to summarize and analyze data regarding the use of hydrophilic polymers in agriculture, to show their advantages and problems when introduced into practice, and to provide examples of application.

In agriculture, hydrogels are used for several purposes. Firstly, in crop production, their superabsorbent qualities are realized, mainly moisture capacity, the ability to retain and gradually release water; secondly, hydrogels can be carriers for minerals, growth stimulants, and pesticides; thirdly, biological products are introduced into the soil with hydrogels.

PHGs are soil conditioners that improve its physical and chemical properties, and therefore fertility. By serving as a water reservoir near the plant root zone, hydrogels reduce soil osmotic pressure and improve water supply, with 95% of the water absorbed by the hydrogel remaining available to the plant [3]. Upon PHG incorporation of into the soil, ventilation and root development, the viability of plants in general, the rate of seed germination, productivity increase, and, as a result, the cost of crop production decreases [2, 4]. PHGs are used as water-retaining agents in agriculture in arid regions, as well as in urban landscape and home gardens, and are used to improve the survival rate of seedlings [5]. PHGs prevent crusting and soil erosion on irrigated lands [6, 7]. Hydrogels are promising in soil-free cultivation technologies, when growing plants hydroponically on vermiculite, perlite, sand and other substrates [8]. In addition, hydrogels serve as a material for encapsulating mineral fertilizers [9, 10], promote their gradual release into soils and provide a prolonged effect [11-13].

Figure 1 shows various applications of PHGs in agriculture.

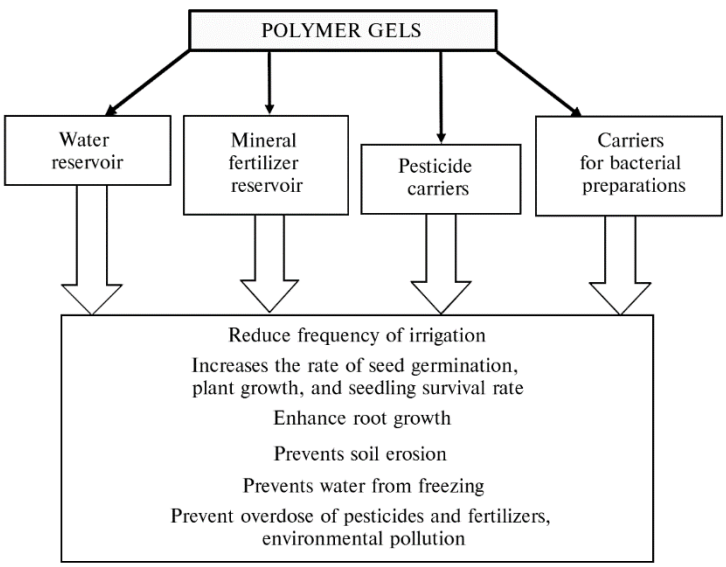

Fig. 1. Application of polymer hydrogels in agriculture.

Classification of hydrogels. Methods of obtaining. Physicochemical characteristics. According to their origin, PHGs are synthetic and natural. Synthetic PHGs are characterized by significant water absorption capacity, long shelf life and fairly high mechanical strength. In turn, natural

materials are safe for the environment, have better biodegradability, but at the same time they have less mechanical strength and moisture retention [1, 14, 15].

Synthetic polyacrylamide and polyacrylate hydrogels. Among the numerous soil conditioners used in practice to optimize the physical state of soils, hydrophilic highly swelling polymer hydrogels based on polyacrylamide and polyacrylates are of particular interest. Such hydrogels have a very high degree of swelling in water (up to 1000 g H_2O/g dry polymer) and are effectively used to regulate the water-holding capacity of light-textured soils [16].

To obtain synthetic hydrogels, acrylamide and acrylic acid are mainly used. Chemical cross-linking of polymers is accomplished through various methods, such as free radical polymerization, condensation polymerization, ultraviolet irradiation, and small-molecule cross-linking [17].

Acrylamide-based hydrogels are the most commonly used. They are capable of significant changes in volume in response to physical and chemical influences. Polyacrylamide hydrogels are traditionally produced by free radical polymerization, the initiation process of which uses a combination of ammonium persulfate and tetramethylethylenediamine. Free radicals, which are formed from the initiator, break double bonds in monomers. In addition, these radicals act on the double bond of the crosslinking agent N,N′-methylene-bis-acrylamide, resulting in the formation of covalent bonds between it and the monomers. The hydrolytic stability of the gel (the ability to resist chemical degradation in the presence of water) can be further improved by the introduction of acrylamides with groups such as alkyl and hydroxyalkyl [17].

Acrylic acid contains a vinyl radical connected to a carboxyl group, which ensures a reaction with electrophilic agents and free radicals. Polyacrylates are synthesized by free radical polymerization from acrylic acid monomers or by their combination with other monomers. Polymerization of acrylic acid can also occur in an acidic environment using sulfuric acid and chlorosulfonic acid. In addition, polymerization is possible in the presence of alkalis, iron salts, high temperature, light, and peroxide compounds [17].

Polyacrylamide derivatives are divided into cationic, anionic, nonionic and polyampholytes. An additional stabilizing effect can be achieved by binding anionic and cationic polymers. Such an interpolyelectrolyte complex is stabilized by the electrostatic interaction of cationic and anionic units and contains hydrophobic and hydrophilic segments. The former are represented by blocks with mutually neutralized charges of both polymers, the latter by free fragments of polymer chains. Modification of an anionic hydrogel with a linear cationic polymer increases the strength of polymer-soil formations, only slightly reducing the ability of the hydrogel to swell and retain moisture. Micro-sized hydrogels of this type seem to be the most promising for suppressing erosion processes in the soil and creating favorable conditions for plant development, especially in areas with insufficient moisture [7].

When synthetic polyacrylate and polyacrylamide hydrogels were added in doses of 0.2-0.3%, an increase in the water-holding capacity of soils of different genesis, composition and dispersion and a heavier granulometric composition were revealed, and the greatest effect was achieved in non-saline sandy substrates. In this case, water retention increased 3-5 times, reaching the level in natural sandy loams and loams [18].

Degradation of PHGs in soils is one of the main factors limiting its effectiveness. Biodegradation depends on climatic conditions and soil type. Thus, in humid climatic conditions, the PHG destruction proceeds more slowly than in arid irrigated soils, and 10-13% of the applied amount is lost during the growing season. The greatest stability of PHG is observed at 20 °C, while the period of

95% decomposition of the hydrogel introduced at a concentration of 0.05-0.2% is 2-15 years, respectively, while at 37 °C it is 1.7-7.5 years [19].

Hydrogels based on biopolymers. Superabsorbent hydrogels can be synthesized based on polysaccharides - cellulose, starch, guar gum, cyclodextrin, xanthan [15, 20, 21], alginate, chitosan, κ-carrageenan [22-24]. Biodegradability, biocompatibility, non-toxicity and insolubility in most solvents, as well as the ability to be obtained from natural and renewable raw materials, make cellulose an attractive source for hydrogel production. Cellulose hydrogels are used in agriculture due to their superabsorbent properties and environmental friendliness. They are usually synthesized by free radical polymerization using cellulose derivatives such as carboxymethylcellulose (CMC) and its sodium salt [25]. Cellulose-based PHGs can be synthesized from lignocellulosic biomass, rice straw, pulp and paper industry waste, and agricultural waste [25-27]. In the structure of plant cellulose, elementary fibrils are distinguished, organized into larger microfibrils. Plant cellulose is a crystallizable polymer with crystalline and amorphous parts [28]. In addition, PHG can be obtained from bacterial cellulose, in which case the material has a number of advantages over cellulose hydrogels of plant origin due to its crystalline nanofibrillar structure, which increases its strength. Cellulose of this type is produced extracellularly by representatives of the genus *Gluconacetobacter* and some other bacteria, and is characterized by high purity, since it does not contain hemicellulose and lignin, tensile strength, and greater water-holding capacity. On average, the length of a nanofiber is 100-1000 nm, its diameter is 20-100 nm, and its crystallinity is 50-60% [29].

In addition to the function of swelling and water retention, cellulose-based PHGs can perform the function of slow release of some nitrogen fertilizers. Thus, an anionic hydrogel was synthesized from cellulose nanofibers oxidized with 2,2,6,6-tetramethyl-1-piperidinyloxy radical in an aqueous solution of NaOH/urea and epichlorohydrin as a cross-linker. This hydrogel had a microporous structure and high hydrophilicity, excellent water absorption properties with controlled release of urea, and supported the process of seed germination and plant growth [30].

A hydrogel fertilizer based on skin waste has been proposed for the controlled release of mineral matter. The addition of skin waste hydrolyzate promoted the formation of a porous structure without any subsequent treatment or the use of a special porogen. This hydrogel had a high swelling rate and was biodegradable by microorganisms in the soil, which led to the gradual release of fertilizer when growing plants. The collagen-based hydrogel fertilizer demonstrated high water absorption capacity reaching 2208 g/g, as well as controlled release of nitrogen and potassium for more than 40 days [31].

Sodium alginate is a biopolymer of natural origin, a linear polysaccharide consisting of (1-4)-linked residues β-D-mannuronate and α-L-guluronate with two main functional groups (—COOH and —OH), which is hydrophilic, biocompatible and biodegradable. Calcium cations bind guluronic acid residues through carboxyl groups, which leads to gelation. $CaCl_2$ concentration and cross-linking time were the most important variables affecting the swelling properties of alginate-based hydrogels. The degree of swelling of the alginate hydrogel prepared from a 2-3% alginate solution cross-linked with 0.2% $CaCl_2$ for 10 minutes was 55 g of distilled water per 1 g of dry hydrogel in 24 hours. The experiment demonstrated the ability of this PHG to control soil moisture and support lettuce growth under drought conditions [32].

To prevent wind and water erosion of soil, a method was developed based on in situ gelation of a polyion complex formed from chitosan and CMC. This gel was prepared by sequentially adding chitosan powder and D-(+)-glucono- -

lactone to a 1% CMC solution with vigorous stirring. Hydrolysis of the acidifying agent, glucono-δ-lactone, reduces the pH of the dispersion from neutral to slightly acidic, initiating the gradual dissolution of chitosan caused by the protonation of its amino groups. Gradually dissolving cationic chitosan electrostatically interacts with negatively charged CMC and forms a CMC-chitosan polymer network. Soil particles formed a composite with polymer films and microfibers through electrostatic interactions. The mechanical properties of such soils depended on the structure and stability of the polyionic network of carboxymethylcellulose and chitosan and were controlled by the degree of polymerization of macromolecules. The hydrogel-like polymer network has been shown to be equally suitable for mechanical amendment of soil containing large amounts of water and dry soil material [33].

Hydrogels of mixed nature (a semi-synthetic PHG). When using synthetic materials as carriers for fertilizers, two main disadvantages can be noted: firstly, non-renewable resources are used to obtain them, and secondly, the remains of the covering shells are not biodegradable and can be potentially harmful to the soil environment. In this regard, there is a need for inexpensive biodegradable materials based on renewable resources that provide controlled release of mineral fertilizers [34, 35]. Macromolecules of natural origin are copolymerized with chemical compounds to improve the technical characteristics of polymer networks and are used for moisture retention and prolonged release of nutritional compounds and plant protection products [36-39]. New commercially available biodegradable copolymers of cassava starch, acrylic acid, natural rubber and vinyl alcohol (VA) were developed by varying the ratio of starch to acrylic acid, but with a fixed ratio of rubber to VA. Properties of this material include good water retention, high swelling ability, acceptable thermal stability, and satisfactory biodegradability [40]. As the rubber to VA ratio increases, the tensile strength, water absorption, and biodegradability of hydrogels tend to decrease, but advantages such as higher thermal stability and denser structure emerge. These hydrogels have been used to slowly release urea [41].

Superabsorbent composites based on sugarcane bagasse (bagasse), polyacrylamide and attapulgite have been developed for controlled release of urea. The composites were prepared by graft copolymerization of acrylamide on bagasse in the presence of attapulgite using N,N′-methylene-bis-acrylamide as a cross-linker and potassium persulfate as a polymerization initiator [42].

For planting guava under drought conditions, PHGs based on a natural product, guar gum, covalently cross-linked with various acrylate monomers (acrylamide, acrylic acid and N-isopropylacrylamide) was used [43], and for planting sugar cane, PHG based on guar gum with polyethylene glycol [44]. The hydrogel composite with urea and biochar obtained by slow pyrolysis of switchgrass had a high water absorption capacity and ensured the gradual release of nitrogen. To prepare PHG, urea, cellulose, acrylamide, potassium persulfate, N,N′-methylene-bis-acrylamide, biochar (2.5-7.5%) were mixed, purged with nitrogen gas and irradiated in a microwave oven at a power of 495-825 W. There was no obvious cross-linking between the biochar and other materials in the composite, and the urea-bound biochar was gradually released during the swelling of the composite. The water absorption capacity increased with increasing biochar dosage. When applied to soil, such composites can release nutrients along with some of the biochar while increasing soil moisture [45]. A hydrogel based on cellulose extracted from sugarcane pulp with sulfuric acid and sodium hydroxide, modified with polyvinyl alcohol and the cross-linking agent glutaraldehyde, quickly swelled (compared to regenerated cellulose without additives), had a structure resistant to mechanical destruction, and the ability to biodegrade. The swelling rate was 135% [46].

PHGs synthesized from cellulose, acrylamide and acrylic acid provided controlled release of urea and calcium superphosphate. These fertilizer granules were sprayed with a solution of epoxy resin in acetone to improve the adhesion of the hydrogel, after which the hydrogel was sprayed to obtain a two-layer coating. The combined PHG had a high swelling ratio (88.8%) and not only released nutrients but also increased water availability to plants [47].

Copolymerization of starch with acrylamide or acrylic acid provides production of superabsorbents with high water absorption capacity. Graft copolymerization is a widely used chemical method for preparing starch-based hydrogels. Starch molecules and monomers of acrylamide or acrylic acid are covalently grafted under the action of an initiator (potassium persulfate) and a cross-linking agent N,N′-methylene-bis-acrylamide.

Starch-grafted acrylamide hydrogel swelled up to 905% at pH 11 [48]. Superabsorbents based on modified carboxymethylated corn starch and partially neutralized acrylic acid were obtained by radical copolymerization of its monomers and modified starch in a parts ratio of 3.75:1. With the highest degree of carboxymethylation of starch and a decrease in the concentration of the crosslinking agent N,N′-methylene-bis- acrylamide from 0.0480 to 0.0047 mol% water absorption increased from 232 to 1203 g/g, that is, the amount of absorbed water increased with decreasing amount of cross-linking agent [49].

Problems of biodestruction of PHG. Polymer hydrogels, including those based on polymers and copolymers of polyacrylamide and polyacrylic acid, when added to soils, should perform the function of retaining moisture and gradually supplying plants with water, but at the same time undergo gradual decomposition in the soil without polluting the environment. When using gels based on polymers and copolymers of acrylamide and acrylic acid, this is especially true because the monomers acrylamide and acrylic acid are highly toxic to living organisms [50-52]. The question arises whether such gels will undergo decomposition with the release of monomers and whether their degradation is exclusively a physicochemical process or is supplemented by biodegradation with the participation of microorganisms.

In the biodegradation of polymers, the following stages can be distinguished: fragmentation of polymers under the influence of various physical, chemical and biological factors; the formation of oligomers, dimers and monomers under the influence of microbial enzymes; penetration of small molecules into the cytoplasm of microbial cells; bioassimilation, i.e., integration of molecules into microbial metabolism to obtain energy and synthesize new biomass macromolecules; mineralization, i.e., the release of simple and complex metabolites into the environment, the release of water and simple compounds of carbon and nitrogen [53].

To date, microbial degradation of polyacrylamide and polyacrylate gels remains poorly studied. It is known that the structural stability of superabsorbent polymers increases with increasing density of three-dimensional cross-links [17]. It was reported that polyacrylate PHGs are practically not biodegraded and the polyacrylate backbone decomposes in loamy soils at a rate of no more than 0.12-0.24% in 6 months [54]. However, there is an opposing view that these synthetic hydrogels undergo physicochemical degradation, after which short stretches of the polymer are destroyed by soil microbiota [19, 55]. The limited information on bacterial strains that degrade polyacrylamide confirms the possibility of microorganisms using this polymer as a source of carbon and nitrogen and its complete utilization [56-58] or carbon [56, 59-61] for growth. Obviously, the possibility of utilizing polyacrylamide as a source of energy and/or nitrogen nutrition is associated with the presence of amidase activity [61-63]. It has been shown that among

eukaryotic organisms, only the white rot pathogen *Phanerochaete chrysosporium* can use polyacrylamide and polyacrylate as a source of carbon and nitrogen. Apparently, these polymers are destroyed by enzymes that oxidize lignin (in particular, peroxidases), and subsequently the formation of soluble polymer degradation products, the hydroxylated derivatives of carboxylic acids and amino derivatives occurs with their further use as a substrate and mineralization by prokaryotes and plants. Toxic acrylic derivatives are not formed in this case [63, 64].

Hydrogels based on biopolymers are decomposed by enzymes of soil microorganisms. Starch is mainly broken down by glycoside hydrolases, a large group of enzymes that catalyze the hydrolysis of glycosidic bonds. The α-amylase performs the primary cleavage of long starch polymers to form shorter fragments, which are then hydrolyzed by β-amylase, glucoamylase and α-glucosidase. Starch hydrolyzing enzymes are present in a variety of soils and are produced by both bacteria and fungi. In addition to glycoside hydrolases, polysaccharide monooxygenases are capable of oxidative degradation of starch [65]. Cellulase enzymes, which catalyze the cleavage of β-glycosidic bonds, are responsible for the degradation of extracellular cellulose. There are endoglucanases, which hydrolyze the internal bonds of cellulose, and cellobiohydrolases, which attack only the terminal groups. These enzymes are produced by cellulolytic microorganisms, which are dominated by fungi and eubacteria [66].

Chitosan and alginate are also subject to biodegradation. Depolymerization of chitosan is carried out by hydrolases — highly specific chitosanases and nonspecific enzymes such as lipases and cellulases. Microbial chitosanases are extracellular inducible enzymes secreted into the environment by both bacteria and fungi [67]. Alginate lyases are also widespread in nature: they are synthesized in seaweeds, invertebrates, bacteria, and fungi. Endoalginate lyases are divided into polyguluronate lyases, polymannuronate lyases, and bifunctional alginate lyases, which are specific for 1→4-glycosidic bonds between both mannuronic and guluronic acid residues. Exoalginate lyases (oligouronide lyases) break down alginic acid oligosaccharides to monosaccharides [68].

Thus, in contrast to synthetic PHGs, the biodegradation of hydrogels of natural origin is not questioned. The biodegradation of hydrogels of mixed nature depends on the ratio of natural and synthetic components, and the first stage of this process is the decomposition of such a polymer into shorter units. Moreover, both the ability to swell and retain water, and the rate of biodegradation depend on the type of cross-linking agent. For example, in the work of P. Jungsinyatam et al. [69] it was shown that glutaraldehyde, but not ethylene glycol dimethacrylate or N,N′-methylenebisacrylamide, can be considered the most preferred cross-linking agent for the synthesis PHG from cassava starch, acrylic acid, natural rubber and vinyl alcohol, which provides the best biodegradability of this PHG.

Hydrogels with biologically active fillers. The problem of low efficiency of fertilizer use can be solved by creating a new technology for their controlled release, which will increase the efficiency of consumption of nitrogen, phosphorus, potassium and other elements by plants and reduce environmental pollution. In this case, fertilizers are encapsulated in shells made of polymeric materials and are released during their gradual destruction (Fig. 2).

There are two methods for loading hydrogels with nutrients: during synthesis of the cross-linked copolymer (in situ loading) and after the hydrogel is prepared (ex situ loading) [31]. Ammonium nitrate, ammonium phosphate, and potassium chloride included in the hydrogel matrix are available to plants for a much longer time than when these minerals are directly added to soils [70]. Urea is a commonly used nitrogen fertilizer, but has rather low efficiency due to its high rate of decomposition and volatilization. One solution to this problem may be the

gradual release of urea into the soil from hydrogels, in which case two goals achieved are water retention and supply of nitrogen fertilizer to crops.

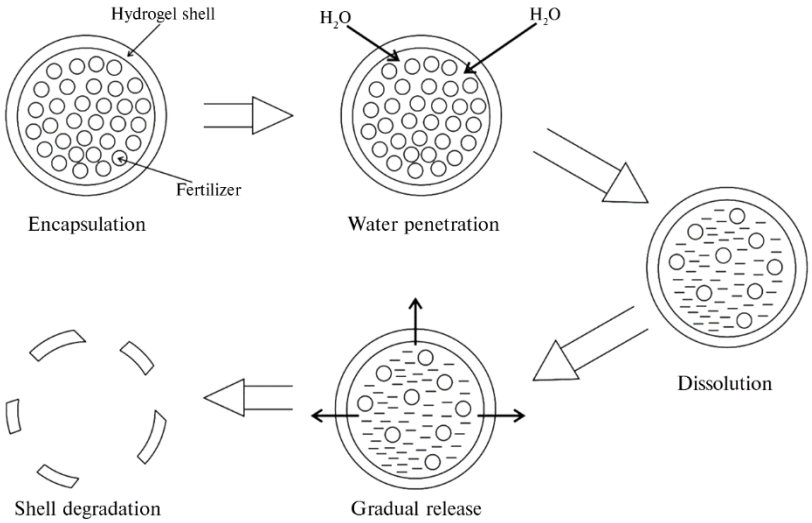

Fig. 2. The mechanism of release of compounds encapsulated in a polymer hydrogel.

Fertilizers that combine water holding capacity, sustained release, and safety for rhizosphere bacteria have been developed based on polyacrylonitrile and acrylic acid hydrogels. Urea granules were coated with oleic acid, soybean oil, and linseed oil, and the outer shell was acrylonitrile, polymerized alone or in combination with acrylic acid [71].

New composite hydrogels were synthesized from sodium alginate, cellulose nanofibers and polyvinyl alcohol with the addition of fertilizer containing nitrogen, phosphorus and potassium. Such hydrogels had good swelling ability in water and slow release of minerals [72]. Soil conditioners based on whey alginic acid hydrogel have been developed for moisture retention and sustained release of urea [73].

Nanoparticles made from biopolymers can be used for the controlled release of mineral fertilizers and pesticides. Nanosized carriers can be used to deliver pesticides with a controlled and gradual release profile, thereby achieving the goals of so-called precision agriculture, which aims to deliver a specific substance directly to the plant without causing water and soil pollution [74]. A naturally occurring polymer, chitosan can be used in agriculture as a matrix for encapsulating plant nutrients. Thus, it was demonstrated that wheat growth was accelerated with an increase in yield and a reduction in the life cycle from 170 to 130 days when using chitosan nanoparticles with nitrogen, phosphorus and potassium, which were applied to the surface of the leaves and penetrated into the stomata when absorbing gas. In this case, direct interaction of these nanoparticles with soil was excluded [75].

By controlling the release of pesticides from the hydrogel matrix, the efficiency of their use can be increased [76]. Composite hydrogels based on alginate and acrylamide have been developed for controlled release of glyphosate [77], acrylic nanocomposite hydrogels of starch with montmorillonite and chlorpyrifos [78], and hydrogels consisting of calcium alginate, attapulgite, polydopamine with pH-sensitive controlled release of this insecticide [79]. Starch-based PHG was synthesized for the controlled release of the fungicide carbendazim [80], as well as CMC polymers containing the insecticide thiamethoxam cross-linked with boric acid [81] and hydrogel composites obtained by cross-linking CMC with citric acid in the presence of bentonite [82].

In the process of obtaining polyacrylamide and polyacrylate hydrogels, various fillers can be used. Thus, the biotechnological synthesis of acrylamide from acrylonitrile is carried out with a biocatalyst based on nitrile hydrolyzing bacteria [83], and the waste of this process is the spent sludge of the biocatalyst. When using biocatalyst sludge as a filler, several goals can be achieved, including reducing the cost of the water-retaining preparation, recycling biotechnological production waste, and introducing an additional organic food source for soil bacteria [16, 84].

Hydrogels as carriers of bacterial preparations (biofertilizers). The use of biofertilizers is emerging as an alternative practice that promotes sustainable agriculture and restoration of degraded soils. Biofertilizers based on living microorganisms are applied to seeds or soil for subsequent colonization of the rhizosphere. To immobilize bacteria that stimulate plant growth, inert carriers are required that will protect microorganisms from adverse environmental influences, ensure their gradual release and the possibility of long-term storage of such a preparation without loss of viability of immobilized microorganisms.

The most common way to isolate microorganisms from the environment is their encapsulation. Microorganisms are coated with a semi-permeable polymer through which they are released under certain conditions. An example is the development of a biodegradable and biocompatible hydrogel with ionic cross-links based on a mixture of chitosan and starch for the immobilization of bacteria which attach to the outer surface of the granules and the walls of the channels of this polycomplex and form agglomerates and multilayer biofilms. The hydrogel was prepared as follows: the tripolyphosphate anion interacted with the protonated amino groups of chitosan, which was then mixed with starch, pregelatinized by heating [85).

Calcium alginate has been proposed as a matrix for encapsulating azospirillum. Such immobilized bacterial preparations retained proliferative function and metabolic activity [86]. A composite of polybutylene succinate grafted with acrylic acid and starch was proposed, containing phosphate and an encapsulated bacterial fertilizer based on the phosphate-mobilizing bacterium *Bacillus* sp. PG01. Starch introduced into the matrix improved its biodegradability. The release of bacteria into the soil occurred during the destruction of the composite, so the rate of this process and the amount of released bacterial fertilizer can be adjusted by changing the ratio of starch and polybutylene succinate in the hydrogel composition [87].

Soil microbiota significantly affects plant growth, their productivity and resistance to stress, so a natural question arises about the impact of GHGs of various origins on environment-forming soil microorganisms. Synthetic polyacrylate hydrogels have been shown to have no negative effects on microbiota [84]. The addition of rice straw-based hydrogels to the soil improved the microbial composition of the soil, with an increase in the proportion of *Azotobacter* spp., as well as phosphate-mobilizing bacteria, fungi, and actinomycetes [88], but the acrylic-based polymer did not affect the content of actinomycetes and fungi in the soil [89]. It has also been shown that the addition of alginate-based PHG has a positive effect on soil microbiota: the addition of this polymer in an amount of 5% led to an increase in the abundance of fungi, bacteria and actinomycetes, respectively, by 28, 30 and 38% [32].

The influence of hydrogels on the growth of agricultural crops and ornamental plants. The table shows the effect of some hydrogels used as water-retaining agents on plant growth, crop survival and productivity.

Effect of polymer hydrogels (PHGs) on plant growth and development

Hydrogel	Plant	Observed effect	References
(RITEK-ENPC LLC, Russia), copolymer of acrylamide and potassium acrylate Aquasorb (SNF s.a.s., France)	Spring wheat (Triticum aestivum L.), Esther variety	A significant increase in grain yield up to 35.70 c/ha when using the gel together with the application of nitrogen fertilizer	[90]
PHG granulated (composition and manufacturer not specified)	Winter wheat (*Triticum aestivum* L.), variety Donskoy Surprise	When PHG was applied at a dose of 80 kg/ha, the yield was 3.047-3.222 t/ha (an increase of 0.210-0.280 t/ha relative to the control), the wet gluten content increased by 0.8-1.0%	[91]
Polyacrylamide Praestol 650 (WaterChem LLC, Russia)	Winter wheat (*Triticum aestivum* L.), variety Bezenchukskaya 308; spring barley (*Hordeum vulgare* L.), variety Nutans 553; peas (*Pisum* L.), variety Flagman 12	Increase in yield for winter wheat is 0.24-0.72 t/ha, spring barley 0.14-0.41 t/ha, peas 0.02-0.07 t/ha. In combination with manure and mineral fertilizers, the increase in yield for wheat is 1.30-1.56 t/ha, barley 1.20-1.29 t/ha, peas 0.19-0.23 t/ha	[92]
Ritin-10 (RITEK-ENPC LLC, Russia)	Winter wheat (*Triticum aestivum* L.), variety Bagheera	Increase in crop productivity with the combined use of hydrogel (200-300 kg/ha) and fertilizers by 8.1-17.4 c/ha (31.9-68.6%); increase in raw gluten content by 0.2-3.6%	[93]
PHG (composition and manufacturer not specified)	Winter wheat (*Triticum aestivum* L.), variety Bagheera	In the 4th year after the use of PHG (400 kg/ha), the yield increased by 6.1-6.5 c/ha	[94]
PHG (composition and manufacturer not specified)	Winter wheat (*Triticum aestivum* L.), variety Bagheera	In the 3rd year after applying PHG (100-400 kg/ha), the yield increased by 4.0-24.7 c/ha (8.8-44.0%)	[95]
Pusa Hydrogel (India) based on cellulose and anionic polyacrylate	Wheat (*Triticum aestivum* L.),	Increased growth rate	[114]
PHG based on acrylamide and acrylates, 0.1-0.3% (synthesized in the laboratory by polymerization of N,N-methyl-bis-acrylamide and a mixture of Na and K salts of acrylic acid)	Common barley (*Hordeum vulgare* L.)	Increasing the rate of seed germination	[96]
Lignin hydrogel (synthesized in the laboratory from alkali lignin and polyethylene glycol diglycidyl ether)	Corn (*Zea mays* L.)	Increased plant height, biomass and phosphorus content using hydrogel under drought conditions	[97]
Hydrogel ша carbamate starch phosphate with urea (synthesized in the laboratory)	Corn (*Zea mays* L.)	Increase in the number of leaves, average leaf length and stem width of seedlings	[98]
Cross linked potassium polyacrylate hydrogel (manufacturer not specified)	Corn (*Zea mays* L.)	Increase in plant height by 26.3% on loamy soil with the application of 0.5% PHG, more than 2 times on sandy soil with the application of 1% PHG	[99]
Acrylamide polymer water-absorbing AK-639 series (Akripol LLC, Russia	Carrot (*Daucus carota* L.)	Increased field germination by 33%, plant density by 24%, yield by 32%, and marketable root crop yield by 12%	[100]
Ritin-10 (RITEK-ENPC LLC, Russia)	Carrot (*Daucus carota* L.), variety Samson (Nantskaya type)	When seeds are inlaid with gel, the increase in yield relative to the control is 93.7 c/ha	[101]
Water-absorbing acrylamide polymer series AK-639, grade B-415K (Akripol LLC, Russia)	Carrot (*Daucus carota* L.), beet (*Beta vulgaris* L.)	Increase in field germination of carrots by more than 3 times, yield - up to 90.5 t/ha, marketability of root crops - up to 84.3% (control - 57.2 t/ha and 77.1%, respectively). Beet yield - 55.4 t/ha with marketability of root crops 87.5% (control - 35.6 t/ha and 75.0%)	[102]
PHG (composition and manufacturer not specified)	Potato (*Solanum tuberosum* L.), varieties Zhukovskii rannii, Udacha abd Nevskii	Increase in the yield of the Zhukovskii rannii variety to 31.0-33.0 t/ha (by 3.6-11.2% of the control), of the Udacha variety to 33.4-36.9 t/ha (by 13.2-25.2%), varieties Nevskii up to 28.5-31.6 t/ha (by 9.9-22.0%)	[103]
Ritin-10 (RITEK-ENPC LLC, Russia)	White cabbage (*Brassica oleracea* L.), variety Kuizor	When treating the roots with gel, the increase in yield is 20.2 c/ha vs. control, when adding the gel to the soil is 6.0 c/ha	[101]
Cellulose-based PHG (synthesized in the laboratory)	Cucumber (*Cucumis sativus* L.)	Increase in plant growth rate and height, leaf fresh weight and area	[104]

Continued Table

PHG based on chiosan with copper nanoparticles (synthesized in pilot plant of the Research Center for Applied Chemistry, Mexico)	Tomato (*Lycopersicon esculentum* L.)	Increased yield and improved taste	[105]
Polyurethane hydrogel (synthesized in the laboratory)	Tomato (*Lycopersicon esculentum* L.)	Increase in plant height by the 90th day of the growing season in relation to the control (240 cm): 300 cm for 1-2% PHG, 505 cm for PHG with fertilizer	[106]
Polyacrylamide gel Acrylex P-150 (JSC VECTON, Russia)	Radish (*Raphanus sativus* var. radicula Pers.)	Seed germination under irrigation (light chestnut soil) and the use of PHG is 82.5% (control 45%), an increase in the content of organic carbon in rain-fed aquaculture, there is no deformation of the root crop in the PHG variant with irrigation. Germination of seeds with PHG on the solonchak is 90% (control 5%), on the solonetz 50% (control 0)	[107, 108]
PHG based on serum, cellulose derivatives and polylactid (synthesized in the laboratory)	Radish (*Raphanus sativus* L.), common beans (*Phaseolus vulgaris* L.)	Increase in growth rate and survival rate by 20% when applying 1-2% PHG under drought conditions compared to control	[109]
PHG based cellulose (synthesized in the laboratory)	Radish (*Raphanus sativus* L.)	Increasing the rate of seed germination	[104]
Stockosorb® («Evonik Industries AG», Germay)	Chickpeas (*Cicer arietinum* L.)	No effect on yield found	[116]
PHG (composition and manufacturer not specified)	Chickpeas (*Cicer arietinum* L.)	Increasing the yield of peas up to 1555 kg/ha (control 1306 kg/ha), straw up to 2053 kg/ha (control 1593 kg/ha) when applying 10 kg/ha PHG	[117]
Agarose hydrogel with selenium (synthesized in the laboratory)	Mung beans (*Vigna radiata* L.)	Obtaining plants enriched with selenium	[113]
Pusa hydrogel (India)	Senna alexandria (*Cassia angustifolia* Vahl.)	Increased yield of leaves and pods (2324.7 and 675.7 kg/ha, respectively) with a hydrogel dose of 3 kg/ha compared to the control (1611.6 and 433.5 kg/ha)	[115]
Alginate (synthesized in the laboratory)	Lettuce (*Lactuca sativa* L.)	Increased biomass	[32]
Ritin-10 (RITEK-ENPC LLC, Russia)	Oilseed radish (*Raphanus sativus* L. var. Oleifera Metzg.), variety Tambovchanka	Yield increase by 15-27% with 200 and 300 kg/ha hydrogel	[110]
Ritin-10 (RITEK-ENPC LLC, Russia) and V415-K (Akripol LLC, Russia)	Perennial herbs	Increase in hay yield with Ritin-10 (200 kg/ha) by 1.2 t/ha, with B-415K (300 kg/ha) by 2.9 t/ha	[111]
Water-absorbing polymer PR3005 (SNF Holding Company, France)	Rosella (*Hibiscus sabdariffa* L.)	Increase in chlorophyll content index, leaf area, number of calyxes under drought conditions	[118]
Acrylic acid polymer Aquasin (Tampomechanika-Moscow LLC, Russia)	Pilea Cadieux (*Pilea cadierei*), striped tradescantia (*Tradescantia zebrina*)	When 5 g/kg PHG was added, the height of the Cadieu pilea increased more than 2 times vs. control, 4 g/kg PHG led to an increase in the number of shoots and leaves of tradescantia vs. control	[112]

Grain crops tested were spring [90] and winter wheat [91-95], common barley [96], corn [97-99]; vegetable crops were carrots [100-102], potatoes [103], white cabbage [101], cucumber [104], tomato [105, 106], radish [107, 108], radish [104, 109]. Among the PHGs, synthetic polymers based on acrylamide and acrylic acid [90, 92, 93, 96, 99-102, 107, 108, 110-112], polymers based on natural raw materials, the cellulose [104], chitosan [105], agarose [113], alginate [32], lignin [97], and semi-synthetic hydrogels [114] were tested. The vast majority of experiments have shown the positive effect of hydrogels on crop productivity [90-95, 100-103, 110, 111, 115], seed germination and plant growth [96, 97, 99, 102, 104, 106-108, 114], especially in drought conditions. It was shown that when there was a lack of moisture, PHG provided a significant increase in yield compared to the control (up to 70%).

So, the main property of polymer hydrogels is their ability to hold an

amount of water many times greater than their mass, which allows them to gradually release moisture to the plant. In addition, hydrogels are used as a depot of mineral fertilizers and pesticides (herbicides, insecticides, fungicides) with the possibility of their prolonged release. The use of GHGs in agricultural practice, despite their positive impact on the chemical and physical properties of the soil, the environment, soil water balance and productivity, is insufficient. This is mainly due to the cost of PHGs, which can be reduced by various additives, e.g., waste (for example, organic mass of spent biocatalyst from biotechnological production), added to the composition of hydrogels. Both synthetic and natural PHGs have their drawbacks: synthetic hydrogels decompose slowly due to their low biodegradability, while natural PHGs characterized by a high rate of biodegradation, do not have sufficient mechanical strength and moisture capacity. In this case, the solution to the problem may be the creation of GHGs of a mixed nature, containing synthetic and natural components. Hydrogels based on polymers and copolymers of acrylamide and acrylic acid are more promising as moisture-retaining and anti-erosion agents, and natural and semi-synthetic PHGs are more promising as carriers of fertilizers and pesticides. A fairly promising direction can be considered the introduction of bacterial preparations immobilized in hydrogel matrices into soils for the subsequent reproduction of bacteria in the soil, however, this is not sufficiently covered in the scientific literature and is still poorly implemented in practice.

REFERENCES

1. Rop K., Mbui D., Njomo N., Karuku G.N., Michira I., Ajayi R.F. Biodegradable water hyacinth cellulose-graft-poly(ammonium acrylate-co-acrylic acid) polymer hydrogel for potential agricultural application. *Heliyon*, 2019, 5(3): e01416 (doi: 10.1016/j.heliyon.2019.e01416).
2. Singh N., Agarwal S., Jain A., Khan S. 3-Dimensional cross linked hydrophilic polymeric network "hydrogels": an agriculture boom. *Agricultural Water Management*, 2021, 253: 106939 (doi: 10.1016/j.agwat.2021.106939).
3. Al-Jabari M., Abu Ghyadah R., Alokely R. Recovery of hydrogel from baby diaper wastes and its application for enhancing soil irrigation management. *Journal of Environmental Management*, 2019, 239: 255-261 (doi: 10.1016/j.jenvman.2019.03.087).
4. Sikder A., Pearce A.K., Parkinson S.J., Napier R., O'Reilly R.K. Recent trends in advanced polymer materials in agriculture related applications. *ACS Applied Polymer Materials*, 2021, 3(3): 1203-1217 (doi: 10.1021/acsapm.0c00982).
5. Nascimento C.D.V., Simmons R.W., de Andrade Feitosa J.P., dos Santos Dias C.T., Cristina M., Costa G. Potential of superabsorbent hydrogels to improve agriculture under abiotic stresses. *Journal of Arid Environments*, 2021, 189: 104496 (doi: 10.1016/j.jaridenv.2021.104496).
6. Green V.S., Stott D.E. Polyacrylamide: a review of the use, effectiveness, and cost of a soil erosion control amendment. In: *Sustaining the global farm*. D.E. Stott, R.H. Mohtar, G.C. Steinhardt (eds.). USA, West Lafayette, 2001.
7. Panova I.G., Ilyasov L.O., Khaidapova D.D., Bashina A.S., Smagin A.V., Ogawa K., Adachi Y., Yaroslavov A.A. Soil conditioners based on anionic polymer and anionic micro-sized hydrogel: a comparative study. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2021, 610: 125635 (doi: 10.1016/j.colsurfa.2020.125635).
8. Cao L., Li N. Activated-carbon-filled agarose hydrogel as a natural medium for seed germination and seedling growth. *International Journal of Biological Macromolecules*, 2021, 177: 383-391 (doi: 10.1016/j.ijbiomac.2021.02.097).
9. Rychter P., Kot M., Bajer K., Rogacz D., Šišková A., Kapuśniak J. Utilization of starch films plasticized with urea as fertilizer for improvement of plant growth. *Carbohydrate Polymers*, 2016, 137: 127-138 (doi: 10.1016/j.carbpol.2015.10.051).
10. Kiran, Tiwari R., Krishnamoorthi S., Kumar K. Synthesis of cross-linker devoid novel hydrogels: Swelling behaviour and controlled urea release studies. *Journal of Environmental Chemical Engineering*, 2019, 7(4): 103162 (doi: 10.1016/j.jece.2019.103162).
11. Guo Y., Guo R., Shi X., Lian S., Zhou Q., Chen Y., Liu W., Li W. Synthesis of cellulose-based superabsorbent hydrogel with high salt tolerance for soil conditioning. *International Journal of Biological Macromolecules*, 2022, 209(Part A): 1169-1178 (doi: 10.1016/j.ijbiomac.2022.04.039).
12. Lin X., Guo L., Shaghaleh H., Hamoud Y.A., Xu X., Liu H. A TEMPO-oxidized cellulose

nanofibers/MOFs hydrogel with temperature and pH responsiveness for fertilizers slow-release. *International Journal of Biological Macromolecules*, 2021, 191: 483-491 (doi: 10.1016/j.ijbiomac.2021.09.075).
13. Wang Y., Shaghaleh H., Hamoud Y.A., Zhang S., Li P., Xu X., Liu H. Synthesis of a pH-responsive nano-cellulose/sodium alginate/MOFs hydrogel and its application in the regulation of water and N-fertilizer. *International Journal of Biological Macromolecules*, 2021, 187: 262-271 (doi: 10.1016/j.ijbiomac.2021.07.154).
14. Zhang Z., Wang X., Liu T., Liu L., Yu C., Tian Y., Zhang X., Shen J. Al^{3+} coordinated chitosan hydrogel with ultrahigh water absorbency and environmental response. *Materials & Design*, 2022, 214: 110390 (doi: 10.1016/j.matdes.2022.110390).
15. Qureshi M.A., Nishat N., Jadoun S., Ansari M.Z. Polysaccharide based superabsorbent hydrogels and their methods of synthesis: a review. *Carbohydrate Polymer Technologies and Applications*, 2020, 1: 100014 (doi: 10.1016/j.carpta.2020.100014).
16. Sadovnikova N.B., Smagin A.V., Sidorova M.A. *Pochvovedenie*, 2014, 4: 455-465 (doi: 10.7868/S0032180X14040066) (in Russ.).
17. Sennakesavan G., Mostakhdemin M., Dkhar L.K., Seyfoddin A., Fatihhi S.J. Acrylic acid/acrylamide based hydrogels and its properties — a review. *Polymer Degradation and Stability*, 2020, 180: 109308 (doi: 10.1016/j.polymdegradstab.2020.109308).
18. Smagin A.V., Sadovnikova N.B., Nikolaeva E.I. *Pochvovedenie*, 2014, 2: 192-202 (doi: 10.7868/S0032180X14020117) (in Russ.).
19. Smagin A.V., Sadovnikova N.B., Smagina M.V. *Pochvovedenie*, 2014, 6: 716-723 (doi: 10.7868/S0032180X14060100) (in Russ.).
20. Bauli C.R., Lima G.F., de Souza A.G., Ferreira R.R., Rosa D.S. Eco-friendly carboxymethyl cellulose hydrogels filled with nanocellulose or nanoclays for agriculture applications as soil conditioning and nutrient carrier and their impact on cucumber growing. *Colloids and Surfaces A: Physicochemical and Engineering Aspects*, 2021, 623: 126771 (doi: 10.1016/j.colsurfa.2021.126771).
21. Rather R.A., Bhat M.A., Shalla A.H. An insight into synthetic and physiological aspects of superabsorbent hydrogels based on carbohydrate type polymers for various applications: a review. *Carbohydrate Polymer Technologies and Applications*, 2022, 3: 100202 (doi: 10.1016/j.carpta.2022.100202).
22. Wang Y., Liu M., Ni B., Xie L. K-carrageenan-sodium alginate beads and superabsorbent coated nitrogen fertilizer with slow-release, water-retention, and anticompaction properties. *Industrial & Engineering Chemistry Research*, 2012, 51(3): 1413-1422 (doi: 10.1021/ie2020526).
23. Arafa E.G., Sabaa M.W., Mohamed R.R., Elzanaty A.M., Abdel-Gawad O.F. Preparation of biodegradable sodium alginate/carboxymethylchitosan hydrogels for the slow-release of urea fertilizer and their antimicrobial activity. *Reactive and Functional Polymers*, 2022, 174: 105243 (doi: 10.1016/j.reactfunctpolym.2022.105243).
24. Arafa E.G., Sabaa M.W., Mohamed R.R., Kamel E.M., Elzanaty A.M., Mahmoud A.M., Abdel-Gawad O.F. Eco-friendly and biodegradable sodium alginate/quaternized chitosan hydrogel for controlled release of urea and its antimicrobial activity. *Carbohydrate Polymers*, 2022, 291: 119555 (doi: 10.1016/j.carbpol.2022.119555).
25. Kundu R., Mahada P., Chhirang B., Das B. Cellulose hydrogels: green and sustainable soft biomaterials. *Current Research in Green and Sustainable Chemistry*, 2022, 5: 100252 (doi: 10.1016/j.crgsc.2021.100252).
26. Meng Y., Liu X., Li C., Liu H., Cheng Y., Lu J., Zhang K., Wang H. Super-swelling lignin-based biopolymer hydrogels for soil water retention from paper industry waste. *International Journal of Biological Macromolecules*, 2019, 135: 815-820 (doi: 10.1016/j.ijbiomac.2019.05.195).
27. Li S., Chen G. Agricultural waste-derived superabsorbent hydrogels: Preparation, performance, and socioeconomic impacts. *Journal of Cleaner Production*, 2020, 251: 119669 (doi: 10.1016/j.jclepro.2019.119669).
28. Voskoboynikov I.V., Konstantinova S.A., Korotkov A.N., Mikhaylov A.I., Nikol'skiy S.N. *Lesnoy vestnik*, 2010, 6: 151-153 (in Russ.).
29. Ventura-Cruz S., Tecante A. Nanocellulose and microcrystalline cellulose from agricultural waste: review on isolation and application as reinforcement in polymeric matrices. *Food Hydrocolloids,* 2021, 118: 106771 (doi: 10.1016/j.foodhyd.2021.106771).
30. Zhang H., Yang M., Luan Q., Tang H., Huang F., Xiang X., Yang C., Bao Y. Cellulose anionic hydrogels based on cellulose nanofibers as natural stimulants for seed germination and seedling growth. *Journal of Agricultural and Food Chemistry,* 2017, 65(19): 3785-3791 (doi: 10.1021/acs.jafc.6b05815).
31. Hu Z.-Y., Chen G., Yi S.-H., Wang Y., Liu Q., Wang R. Multifunctional porous hydrogel with nutrient controlled-release and excellent biodegradation. *Journal of Environmental Chemical Engineering*, 2021, 9(5): 106146 (doi: 10.1016/j.jece.2021.106146).
32. Tomadoni B., Salcedo M.F., Mansilla A.Y., Casalongué C.A., Alvarez V.A. Macroporous alginate-based hydrogels to control soil substrate moisture: effect on lettuce plants under drought stress. *European Polymer Journal*, 2020, 137: 109953 (doi: 10.1016/j.eurpolymj.2020.109953).
33. Zinchenko A., Sakai T., Morikawa K., Nakano M. Efficient stabilization of soil, sand, and clay

by a polymer network of biomass-derived chitosan and carboxymethyl cellulose. *Journal of Environmental Chemical Engineering*, 2022, 10(1): 107084 (doi: 10.1016/j.jece.2021.107084).
34. Zhang S., Yang Y., Gao B., Wan Y., Li Y.C., Zhao C. Bio-based Interpenetrating network polymer composites from locust sawdust as coating material for environmentally friendly controlled-release urea fertilizers. *Journal of Agricultural and Food Chemistry*, 2016, 64(28): 5692-5700 (doi: 10.1021/acs.jafc.6b01688).
35. Chiaregato C.G., França D., Messa L.L., dos Santos Pereira T., Faez R. A review of advances over 20 years on polysaccharide-based polymers applied as enhanced efficiency fertilizers. *Carbohydrate Polymers*, 2022, 279: 119014 (doi: 10.1016/j.carbpol.2021.119014).
36. Raafat A.I., Eid M., El-Arnaouty M.B. Radiation synthesis of superabsorbent CMC based hydrogels for agriculture applications. *Nuclear Instruments and Methods in Physics Research Section B: Beam Interactions with Materials and Atoms*, 2012, 283: 71-76 (doi: 10.1016/j.nimb.2012.04.011).
37. Guo L., Wang Y., Wang M., Shaghaleh H., Hamoud Y.A., Xu X., Liu H. Synthesis of bio-based MIL-100(Fe)@CNF-SA composite hydrogel and its application in slow-release N-fertilizer. *Journal of Cleaner Production*, 2021, 324: 129274 (doi: 10.1016/j.jclepro.2021.129274).
38. Chaudhuri S.D., Dey A., Upganlawar S., Chakrabarty D. Influence of clay concentration on the absorption and rheological attributes of modified cellulose /acrylic acid based hydrogel and the application of such hydrogel. *Materials Chemistry and Physics*, 2022, 282: 125942 (doi: 10.1016/j.matchemphys.2022.125942).
39. Zhang Y., Tian X., Zhang Q., Xie H., Wang B., Feng Y. Hydrochar-embedded carboxymethyl cellulose-g-poly(acrylic acid) hydrogel as stable soil water retention and nutrient release agent for plant growth. *Journal of Bioresources and Bioproducts*, 2022, 7(2): 116-127 (doi: 10.1016/j.jobab.2022.03.003).
40. Tanan W., Panichpakdee J., Saengsuwan S. Novel biodegradable hydrogel based on natural polymers: Synthesis, characterization, swelling/reswelling and biodegradability. *European Polymer Journal*, 2019, 112: 678-687 (doi: 10.1016/j.eurpolymj.2018.10.033).
41. Tanan W., Panichpakdee J., Suwanakood P., Saengsuwan S. Biodegradable hydrogels of cassava starch-g-polyacrylic acid/natural rubber/polyvinyl alcohol as environmentally friendly and highly efficient coating material for slow-release urea fertilizers. *Journal of Industrial and Engineering Chemistry*, 2021, 101: 237-252 (doi: 10.1016/j.jiec.2021.06.008).
42. Kenawy E.-R., Seggiani M., Cinelli P., Elnaby H.M.H., Azaam M.M. Swelling capacity of sugarcane bagasse-g-poly(acrylamide)/attapulgite superabsorbent composites and their application as slow release fertilizer. *European Polymer Journal*, 2020, 133: 109769 (doi: 10.1016/j.eurpolymj.2020.109769).
43. Abdel-Raouf M.E., El-Saeed S.M., Zaki E.G., Al-Sabagh A.M. Green chemistry approach for preparation of hydrogels for agriculture applications through modification of natural polymers and investigating their swelling properties. *Egyptian Journal of Petroleum*, 2018, 27(4): 1345-1355 (doi: 10.1016/j.ejpe.2018.09.002).
44. Songara J.C., Patel J.N. Synthesis of guar gum-based hydrogel for sugarcane field solid conditioning. *Journal of the Indian Chemical Society*, 2021, 98(11): 10022 (doi: 10.1016/j.jics.2021.100220).
45. Wu Y., Brickler C., Li S., Chen G. Synthesis of microwave-mediated biochar-hydrogel composites for enhanced water absorbency and nitrogen release. *Polymer Testing*, 2021, 93: 106996 (doi: 10.1016/j.polymertesting.2020.106996).
46. Ban M.T., Mahadin N., Karim K.J.A. Synthesis of hydrogel from sugarcane bagasse extracted cellulose for swelling properties study. *Materials Today: Proceedings*, 2022, 50: 2567-2575 (doi: 10.1016/j.matpr.2021.08.342).
47. Rizwan M., Gilani S.R., Durrani A.I., Naseem S. Kinetic model studies of controlled nutrient release and swelling behavior of combo hydrogel using *Acer platanoides* cellulose. *Journal of the Taiwan Institute of Chemical Engineers*, 2022, 131: 104137 (doi: 10.1016/j.jtice.2021.11.004).
48. Cui C., Jia Y., Sun Q., Yu M., Ji N., Dai L., Wang Y., Qin Y., Xiong L., Sun Q. Recent advances in the preparation, characterization, and food application of starch-based hydrogels. *Carbohydrate Polymers*, 2022, 291: 119624 (doi: 10.1016/j.carbpol.2022.119624).
49. Kowalski G., Ptaszek P., Kuterasiński Ł. Swelling of hydrogels based on carboxymethylated starch and poly(acrylic acid): nonlinear rheological. *Approach Polymers*, 2020, 12: 2564 (doi: 10.3390/polym12112564).
50. Kusnin N., Syed M.A., Ahmad S.A. Toxicity, pollution and biodegradation of acrylamide — a mini review. *Journal of Biochemistry, Microbiology and Biotechnology*, 2015, 3(2): 6-12.
51. Tepe Y., Çebi A. Acrylamide in environmental water: a review on sources, exposure, and public health risks. *Expo Health*, 2019, 11: 3-12 (doi: 10.1007/s12403-017-0261-y).
52. Khan H., Iram, Gul K., Ara B., Khan A., Ali N., Ali N., Bilal M. Adsorptive removal of acrylic acid from the aqueous environment using raw and chemically modified alumina: Batch adsorption, kinetic, equilibrium and thermodynamic studies. *Journal of Environmental Chemical Engineering*, 2020, 8(4): 103927 (doi: 10.1016/j.jece.2020.103927).
53. Chen J., Wua J., Raffa P., Picchioni F., Koning C.E. Superabsorbent Polymers: From long-established, microplastics generating systems, to sustainable, biodegradable and future proof alternatives. *Progress in Polymer Science*, 2022, 125: 101475 (doi: 10.1016/j.progpolymsci.2021.101475).
54. Wilske B., Bai M., Lindenstruth B., Bach M., Rezaie Z., Frede H.-G., Breuer L. Biodegradability

of a polyacrylate superabsorbent in agricultural soil. *Environmental Science and Pollution Research*, 2014, 21: 9453-9460 (doi: 10.1007/s11356-013-2103-1).
55. Smagin A.V. *Teoriya i praktika konstruirovaniya pochv* [Theorrical and practicfal soil engineering]. Moscow, 2012 (in Russ.).
56. Matsuoka H., Ishimura F., Takeda T., Hikuma M. Isolation of polyacrylamide-degrading microorganisms from soil. *Biotechnology and Bioprocess Engineering*, 2002, 7: 327-330 (doi: 10.1007/BF02932844).
57. Bao M., Chen Q., Li Y., Jiang G. Biodegradation of partially hydrolyzed polyacrylamide by bacteria isolated from production water after polymer flooding in an oil field. *Journal of Hazardous Materials*, 2010, 184(1-3): 105-110 (doi: 10.1016/j.jhazmat.2010.08.011).
58. Maksimova Yu.G., Gorshkova A.A., Demakov V.A. *Vestnik Permskogo universiteta. Seriya biologiya*, 2017, 2: 200-204 (in Russ.).
59. Wen Q., Chen Z., Zhao Y., Zhang H., Feng Y. Biodegradation of polyacrylamide by bacteria isolated from activated sludge and oil-contaminated soil. *Journal of Hazardous Materials*, 2010, 175(1-3): 955-959 (doi: 10.1016/j.jhazmat.2009.10.102).
60. Sipulinov R.B., Karagaycheva Yu.V., Kozulina T.N., Rogacheva S.M., Otradnova M.I. *Uchenye zapiski Tavricheskogo natsional'nogo universiteta im. V. I. Vernadskogo*, 2014, 27(66)(2): 150-156 (in Russ.).
61. Yu F., Fu R., Xie Y., Chen W. Isolation and characterization of polyacrylamide-degrading bacteria from dewatered sludge. *International Journal of Environmental Research and Public Health*, 2015, 12(4): 4214-4230 (doi: 10.3390/ijerph120404214).
62. Kay-Shoemake J.L., Watwood M.E., Sojka R.E., Lentz R.D. Soil amidase activity in polyacrylamide-treated soils and potential activity toward common amide-containing pesticides. *Biology and Fertility of Soils*, 2000, 31: 183-186 (doi: 10.1007/s003740050643).
63. Nyyssölä A., Ahlgren J. Microbial degradation of polyacrylamide and the deamination product polyacrylate. *International Biodeterioration & Biodegradation*, 2019, 139: 24-33 (doi: 10.1016/j.ibiod.2019.02.005).
64. Xiong B., Loss R.D., Shields D., Pawlik T., Hochreiter R., Zydney A.L., Kumar M. Polyacrylamide degradation and its implications in environmental systems. *npj Clean Water*, 2018, 1: 17 (doi: 10.1038/s41545-018-0016-8).
65. Polman E.M.N., Gruter G.-J.M., Parsons J.R., Tietema A. Comparison of the aerobic biodegradation of biopolymers and the corresponding bioplastics: a review. *Science of the Total Environment*, 2021, 753: 141953 (doi: 10.1016/j.scitotenv.2020.141953).
66. Pérez J., Muñoz-Dorado J., de la Rubia T., Martínez J. Biodegradation and biological treatments of cellulose, hemicellulose and lignin: an overview. *International Microbiology*, 2002, 5(2): 53-63 (doi: 10.1007/s10123-002-0062-3).
67. Aktuganov G.E., Melent'ev A.I., Varlamov V.P. *Prikladnaya biokhimiya i mikrobiologiya*, 2019, 55(4): 315-337 (doi: 10.1134/S0555109919040020) (in Russ.).
68. Belik A.A., Sil'chenko A.S., Kusaykin M.I., Zvyagintseva T.N., Ermakova S.P. *Bioorganicheskaya khimiya*, 2018, 44(4): 382-393 (doi: 10.1134/S0132342318040048) (in Russ.).
69. Jungsinyatam P., Suwanakood P., Saengsuwan S. Multicomponent biodegradable hydrogels based on natural biopolymers as environmentally coating membrane for slow-release fertilizers: Effect of crosslinker type. *Science of the Total Environment*, 2022, 843: 157050 (doi: 10.1016/j.scitotenv.2022.157050).
70. Ni B., Liu M., Lü S. Multifunctional slow-release urea fertilizer from ethylcellulose and superabsorbent coated formulations. *Chemical Engineering Journal,* 2009, 155(3): 892-898 (doi: 10.1016/j.cej.2009.08.025).
71. Ghobashy M.M., Mousaa I.M., El-Sayyad G.S. Radiation synthesis of urea/hydrogel core shells coated with three different natural oils via a layer-by-layer approach: an investigation of their slow release and effects on plant growth-promoting rhizobacteria. *Progress in Organic Coatings*, 2021, 151: 106022 (doi: 10.1016/j.porgcoat.2020.106022).
72. Liu S., Wu Q., Sun X., Yue Y., Tubana B., Yang R., Cheng H.N. Novel alginate-cellulose nanofiber-poly(vinyl alcohol) hydrogels for carrying and delivering nitrogen, phosphorus and potassium chemicals. *International Journal of Biological Macromolecules*, 2021, 172: 330-340 (doi: 10.1016/j.ijbiomac.2021.01.063).
73. Di Martino A., Khan Y.A., Durpekova S., Sedlarik V., Elich O., Cechmankova J. Ecofriendly renewable hydrogels based on whey protein and for slow release of fertilizers and soil conditioning. *Journal of Cleaner Production*, 2021, 285: 124848 (doi: 10.1016/j.jclepro.2020.124848).
74. Sampathkumar K., Tan K.X., Loo S.C.J. Developing nano-delivery systems for agriculture and food applications with nature-derived polymers. *iScience*, 2020, 23(5): 101055 (doi: 10.1016/j.isci.2020.101055).
75. Abdel-Aziz H.M.M., Hasaneen M.N.A., Omer A.M. Nano chitosan-NPK fertilizer enhances the growth and productivity of wheat plants grown in sandy soil. *Spanish Journal of Agricultural Research*, 2016, 14(1): e0902 (doi: 10.5424/sjar/2016141-8205).
76. Xu C., Cao L., Bilal M., Cao C., Zhao P., Zhang H., Huang Q. Multifunctional manganese-based carboxymethyl chitosan hydrogels for pH-triggered pesticide release and enhanced fungicidal

activity. *Carbohydrate Polymers*, 2021, 262: 117933 (doi: 10.1016/j.carbpol.2021.117933).
77. Zheng D., Wang K., Bai B., Hu N., Wang H. Swelling and glyphosate-controlled release behavior of multi-responsive alginate-g-P(NIPAm-co-NDEAm)-based hydrogel. *Carbohydrate Polymers*, 2022, 282: 119113 (doi: 10.1016/j.carbpol.2022.119113).
78. Baloch F.E., Afzali D., Fathirad F. Design of acrylic acid/nanoclay grafted polysaccharide hydrogels as superabsorbent for controlled release of chlorpyrifos. *Applied Clay Science*, 2021, 211: 106194 (doi: 10.1016/j.clay.2021.106194).
79. Xiang Y., Zhang G., Chen C., Liu B., Cai D., Wu Z. Fabrication a pH-responsively controlled-release pesticide using an attapulgite-based hydrogel ACS. *Sustainable Chemistry & Engineering*, 2018, 6(1): 1192-1201 (doi: 10.1021/acssuschemeng.7b03469).
80. Bai C., Zhang S., Huang L., Wang H., Wang W., Ye Q. Starch-based hydrogel loading with carbendazim for controlled-release and water absorption. *Carbohydrate Polymers*, 2015, 125: 376-383 (doi: 10.1016/j.carbpol.2015.03.004).
81. Sarkar D.J., Singh A. pH-triggered Release of boron and thiamethoxam from boric acid cross-linked carboxymethyl cellulose hydrogel based formulations. *Polymer-Plastics Technology and Engineering*, 2019, 58(1): 83-96 (doi: 10.1080/03602559.2018.1466165).
82. Sarkar D.J., Singh A. Base triggered release of insecticide from bentonite reinforced citric acid crosslinked carboxymethyl cellulose hydrogel composites. *Carbohydrate Polymers*, 2017, 156: 303-311 (doi: 10.1016/j.carbpol.2016.09.045).
83. Debabov V.G., Yanenko A.S. Biocatalytic hydrolysis of nitriles. *Review Journal of Chemistry*, 2011, 1(4): 385-402 (doi: 10.1134/S2079978011030010).
84. Maksimova Yu.G., Maksimov A.Yu., Demakov V.A., Budnikov V.I. *Vestnik Permskogo universiteta. Seriya Biologiya*, 2010, 1(1): 45-49.
85. Perez J.J., Francois N.J., Maroniche G.A., Borrajo M.P., Pereyra M.A., Creus C.M. A novel, green, low-cost chitosan-starch hydrogel as potential delivery system for plant growth-promoting bacteria. *Carbohydrate Polymers*, 2018, 202: 409-417 (doi: 10.1016/j.carbpol.2018.07.084).
86. Kovrizhnikov A.V., Pylaev T.E., Zakharevich A.M., Konnova S.A., Kupryashina M.A. *Seriya: Khimiya. Biologiya. Ekologiya*, 2021, 21(3): 298-303 (dol: 10.18500/1816-9775-2021-21-3-298-303) (in Russ.).
87. Wu C.-S. Controlled release evaluation of bacterial fertilizer using polymer composites as matrix. *Journal of Controlled Release*, 2008, 132(1): 42-48 (doi: 10.1016/j.jconrel.2008.08.015).
88. El-Saied H., El-Hady O.A., Basta A.H., El-Dewiny C.Y., Abo-Sedera S.A., Bio-chemical properties of sandy calcareous soil treated with rice straw-based hydrogels. *Journal of the Saudi Society of Agricultural Sciences*, 2016, 15(2): 188-194 (doi: 10.1016/j.jssas.2014.11.004).
89. Mikiciuk G., Mikiciuk M., Hawrot-Paw M. Influence of superabsorbent polymers on the chemical composition of strawberry (*Fragaria* × *ananassa* Duch.) and biological activity in the soil. *Folia Horticulturae*, 2015, 27: 63-69 (doi: 10.1515/fhort-2015-0015).
90. Danilova T.N., Tabynbaeva L.K. Polymer gels to manage water availability for wheat (*Triticum aestivum* L.) under various environment conditions. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2019, 54(1): 76-83 (doi: 10.15389/agrobiology.2019.1.76eng).
91. Tibir'kov A.P., Filin V.I. *Izvestiya Nizhnevolzhskogo agrouniversitetskogo kompleksa*, 2012, 3(27): 1-5 (in Russ.).
92. Kuzin E.N., Aref'ev A.N. *Zemledelie*, 2013, 2: 12-14 (in Russ.).
93. Godunova E.I., Gundyrin V.N., Shkabarda S.N. *Dostizheniya nauki i tekhniki APK*, 2014, 1: 24-27 (in Russ.).
94. Godunova E.I., Gundyrin V.N., Shkabarda S.N. *Dostizheniya nauki i tekhniki APK*, 2017, 31(5): 16-19 (in Russ.).
95. Gundyrin V.N., Godunova E.I., Shkabarda S.N. *Dostizheniya nauki i tekhniki APK*, 2016, 30(8): 37-39 (in Russ.).
96. Akhter J.A, Akhter J., Mahmood K.A, Malik K.A., Mardan A., Ahmad M., Iqbal M.M. Effects of hydrogel amendment on water storage of sandy loam and loam soils and seedling growth of barley, wheat and chickpea. *Plant, Soil and Environment*, 2004, 50(10): 463-469 (doi: 10.17221/4059-pse).
97. Mazloom N., Khorassani R., Zohury G.H., Emami H., Whalen J. Lignin-based hydrogel alleviates drought stress in maize. *Environmental and Experimental Botany*, 2020, 175: 104055 (doi: 10.1016/j.envexpbot.2020.104055).
98. Dong G., Mu Z., Liu D., Shang L., Zhang W., Gao Y., Zhao M., Zhang X., Chen S., Wei M. Starch phosphate carbamate hydrogel based slow-release urea formulation with good water retentivity. *International Journal of Biological Macromolecules*, 2021, 190: 189-197 (doi: 10.1016/j.ijbiomac.2021.08.234).
99. Albalasmeh A.A., Mohawesh O., Gharaibeh M.A., Alghamdi A.G., Alajlouni M.A., Alqudah A.M. Effect of hydrogel on corn growth, water use efficiency, and soil properties in a semi-arid region. *Journal of the Saudi Society of Agricultural Sciences*, 2022, 21(8): 518-524 (doi: 10.1016/j.jssas.2022.03.001).
100. Azopkov M.I. *Kartofel' i ovoshchi*, 2012, 7: 21 (in Russ.).
101. Danilova T.N. *Izvestiya Sankt-Peterburgskogo gosudarstvennogo agrarnogo universiteta*, 2018, 52:

47-53 (in Russ.).
102. Bykovskiy Yu.A., Azopkov M.I., Fefelova S.V., Akimov D.S., Bagrov R.A. *Kartofel' i ovoshchi*, 2018, 1: 18-21 (in Russ.).
103. Starovoytov V.I., Starovoytova O.A., Manokhina A.A. *Vestnik Federal'nogo gosudarstvennogo obrazovatel'nogo uchrezhdeniya vysshego professional'nogo obrazovaniya «Moskovskiy gosudarstvennyy agroinzhenernyy universitet imeni V.P. Goryachkina»*, 2015, 1(65): 15-19 (in Russ.).
104. Montesano F.F., Parente A., Santamaria P., Sannino A., Serio F. Biodegradable superabsorbent hydrogel increases water retention properties of growing media and plant growth. *Agriculture and Agricultural Science Procedia*, 2015, 4: 451-458 (doi: 10.1016/j.aaspro.2015.03.052).
105. Hernández H.H., Benavides-Mendoza A., Ortega-Ortiz H., Hernández-Fuentes A.D., Juárez-Maldonado A.D. Cu nanoparticles in chitosan-PVA hydrogels as promoters of growth, productivity and fruit quality in tomato. *Emirates Journal of Food and Agriculture*, 2017, 29(8): 573-580.
106. Tanasić J., Erceg T., Tanasić L., Baloš S., Klisurić O., Ristić I. The influence of reaction conditions on structural properties and swelling kinetics of polyurethane hydrogels intended for agricultural purposes. *Reactive and Functional Polymers*, 2021, 169: 105085 (doi: 10.1016/j.reactfunctpolym.2021.105085).
107. Okolelova A.A., Rachimova N.A., Egorova G.S., Kasterina N.G., Zaikina V.N. Influence of hydrogels on productivity of light-brown soils. *International Journal of Environmental Problems*, 2015, 2(2): 117-135 (doi: 10.13187/ijep.2015.2.117).
108. Voskoboynikova T.G., Okolelova A.A., Manov R.O. *Nauchnye vedomosti. Seriya Estestvennye nauki*, 2015, 9(31): 37-42 (in Russ.).
109. Durpekova S., Bergerova E.D., Hanusova D., Dusankova M., Sedlarik V. Ecofriendly whey/polysaccharide-based hydrogel with poly(lactic acid) for improvement of agricultural soil quality and plant growth. *International Journal of Biological Macromolecules*, 2022, 212: 85-96 (doi: 10.1016/j.ijbiomac.2022.05.053).
110. Gundyrin V.N., Godunova E.I., Shkabarda S.N. *Zemledelie*, 2014, 6: 37-38 (in Russ.).
111. Danilova T.N., Olenchenko E.A. *Melioratsiya i vodnoe khozyaystvo*, 2016, 2: 22-25 (in Russ.).
112. Voropaeva E.V., El'shaeva I.V. *Izvestiya Sankt-Peterburgskogo gosudarstvennogo agrarnogo universiteta*, 2021, 2(63): 84-91 (doi: 10.24412/2078-1318-2021-2-84-91) (in Russ.).
113. Cao L., Zhu J., Li N. Selenium-agarose hybrid hydrogel as a recyclable natural substrate for selenium-enriched cultivation of mung bean sprouts. *International Journal of Biological Macromolecules*, 2022, 194: 17-23 (doi: 10.1016/j.ijbiomac.2021.11.091).
114. Suman A., Verma P., Yadav A.N., Srinivasamurthy, Singh A., Prasanna R. Development of hydrogel based bio-inoculant formulations and their impact on plant biometric parameters of wheat (*Triticum aestivum* L.). *International Journal of Current Microbiology and Applied Sciences*, 2016, 5(3): 890-901 (doi: 10.20546/ijcmas.2016.503.103).
115. Jnanesha A.C., Kumar A., Lal R.K. Hydrogel application improved growth and yield in Senna (*Cassia angustifolia* Vahl.). *Industrial Crops & Products*, 2021, 174: 114175 (doi: 10.1016/j.indcrop.2021.114175).
116. Farjam S., Kenarsari M.J., Rokhzadi A., Yousefi B. Effects of inter-row spacing and superabsorbent polymer application on yield and productivity of rainfed chickpea. *Journal of Biodiversity and Environmental Sciences*, 2014, 5(3): 316-320.
117. Kumar M.S., Kumar G.V.S., Mrudula K.A., Kumar G.V. Effect of hydrophilic polymer and farmyard manure on yield attributes and yields of rainfed chickpea. *International Journal of Current Microbiology and Applied Sciences*, 2020, 9(8): 4003-4007 (doi: 10.20546/ijcmas.2020.908.461).
118. Besharati J., Shirmardi M., Meftahizadeh H., Ardakani M.D., Ghorbanpour M. Changes in growth and quality performance of Roselle (*Hibiscus sabdariffa* L.) in response to soil amendments with hydrogel and compost under drought stress. *South African Journal of Botany*, 2022, 145: 334-347 (doi: 10.1016/j.sajb.2021.03.018).

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 43-59
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.37:631.52:631.524.6:631.559

doi: 10.15389/agrobiology.2023.1.43eng
doi: 10.15389/agrobiology.2023.1.43rus

OBJECTIVES OF GUAR BREEDING IN THE RUSSIAN FEDERATION IN CONNECTION WITH THE PROSPECTS OF DOMESTIC GUAR GUM PRODUCTION

(review)

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The authors declare no conflict of interests
Acknowledgements:
Supported financially by Russian Science Foundation, project No. 21-16-00084
Final revision received December 23, 2022
Accepted January 21, 2023

Abstract

Guar (*Cyamopsis tetragonoloba* (L.) Taub is a source of guar gum extracted from the endosperm of this annual legume plant (D. Mugdil et al., 2014; R. Pathak et al., 2015). Guar gum has a wide range of applications as gel-forming agent in gas/oil industry and as emulsifier and thickener of substances in food, cosmetic, textile and paper industries (R.J. Chudzikowski et al., 1971; N.Thombare et al., 2016; A.M.A. Hasan et al., 2018). Guar has moderate drought-resistance, it is tolerant to salinized soils and has low demands to soil fertility (D.J. Undersander et al., 1991; R.K. Bhatt et al., 2017). Domestication of guar took place in India and Pakistan where the plant was used as a forage crop (N. Thombare et al., 2016). These countries are the main manufacturers and exporters of Guar gum in the world market today. Guar was introduced into Russian Federation in terms of import submission. Production batches of conditioned seeds were obtained in some regions of South Federal Part and Lower Volga adjacent area last years. Experimental batches of gum extracted from native seeds have demonstrated that guar gum fits the quality standards (I.V. Kruchina-Bogdanov et al., 2019) and in the nearest future guar gum would be produced in Russia. There is a high demand in industrialized-type guar cultivars, well adopted to diverse local conditions in the Russian Federation. Guar was introduced into culture in the Russian Federation, 4 cultivars among 10 registered in the State Register of Breeding achievements were originated by VIR (Vavilov Institute of Plant Genetic Resources). Gum production depends upon guar yield productivity so the problem of high yield is urgent (A.K. Jukanti et al., 2019). However, in the Russian Federation, the most limiting factor for guar production is high temperature demand of the crop (D.V. Lebed et al., 2017), so precocity becomes a priority feature, which, in turn, is associated with the sensitivity of the plant to the photoperiod. Therefore, it is necessary to search in the gene pool for forms with reduced sensitivity to photoperiod, capable for forming full-fledged seeds in a relatively short summer (S.B. Teplyakova et al., 2019). Being introduced to northern altitudes, guar plants may form blackened seeds in pods in conditions of prolonged vegetative period combined with low night temperatures and extra moisture (T. Hymovitz et al., 1963; D.V. Lebed et al., 2018). Due to this fact it is important to use most early guar varieties in Russia. The question arises about the optimal plant architectonics for the conditions of the Russian Federation, contributing to the formation of high productive agrocenosis (M.I. Voloshin et al., 2019). One-stem and few-branched guar plants are recommended as most early and better adopted to mechanized harvesting, such type of plants fit the model of industrial variety (F. Gresta F. et al., 2018; C.M.G. Reis et al., 2021). Plants with determinated type of growth are also early maturing (E.A. Dzyubenko et al., 2017). Guar, like most annual crops, is self-pollinated (R.E. Stafford et al., 1975), its hybridization is a complicated procedure, and the rate of successful crosses is very low (R.E. Stafford et al., 1980). Main breeding method in guar is selection of outstanding genotypes (A.K. Jukanti et al., 2019). The most effective selection index for breeding for high yield in guar is the number of pods per plant (F. Gresta et al., 2013). The diversity of plant genetic resources and the evolving genomic resources allow the use of traditional, biotechnological and molecular approaches in guar breeding (S. Kumar et al., 2017). Some types of molecular markers are identified but not too

much compared to other legume cultures (W. Ravelombola et al., 2021). It is also necessary to be ready to resist to the effects of biotic stressors — diseases and pests, it is urgent to create resistant varieties with a broad genetic basis (E.E. Radchenko et al., 2018). The whole range of these issues is outlined in this review as the objectives that domestic guar breeders face when creating gum-forming varieties. Prospects of marker-assisted selection and genomic breeding are under discussion (S. Kumar et al., 2020; E. Gigoreva et al., 2021; S. Pareek et al., 2022). The created domestic varieties of guar were obtained by traditional breeding, but the active development of genomic, metabolomic and transcriptomic resources of the species allow us to hope for a quick practical application of breakthrough methods of crop breeding that will increase crop yield and adaptation in the conditions of the Russian Federation.

Keywords: guar, guar gum, introduction, breeding, seed yield, early maturation

Guar *Cyamopsis tetragonoloba* (L.) Taub is an annual leguminous crop that in recent decades has become a world's most sought after sources of galactomannan, a seed polysaccharide that, after extraction and processing, is used as guar gum (guaran). In terms of the scale of application in various industries, plant galactomannans and their derivatives come after cellulose and starch. These polysaccharides, due to the unique properties of their aqueous solutions and lack of toxicity, are used as food additives, stabilizers, flocculants, thickeners and gelling agents in binary mixtures [1].

Guar gum is the most sought after galactomannan in the world due to its safety and non-toxicity, the extraction from renewable natural resources, and is easily available in large quantities. It is widely used as a natural thickener, stabilizer and sealant in the paper, textile, pharmaceutical, food and cosmetics industries, but is especially in demand in the oil and gas industry [2-6]. Domestication of guar occurred in South Asia, and for a long time the main producers of the crop, primarily as a forage plant, were India and Pakistan. These countries remain the leaders in guar production today, having the status of leading exporters of seeds and the gum obtained from them. To date, the production range of guar has expanded, but is limited mainly to the tropical regions of the USA, Australia, Brazil and some African countries. The annual production of guar seeds worldwide is approximately 3.4 million tons [7]. The Russian Federation, along with Germany, the Netherlands, Italy, France, Spain and the UK, is the largest importer of guar gum in Europe [8]. Dependence on imported guarana prompted Russian scientists and farmers to take active steps to introduce the crop into Russia. In the Krasnodar Territory, Rostov Provunce and Crimea, private farms and breeders have appeared, trying to implement guar production and selection. This served as an impetus for entrying new accessions into the VIR collection (Vavilov All-Russian Institute of Plant Genetic) and initiating crop study by Russian scientists. There are reports on various aspects of the guar biology in different locations [9-11], on the traits intraspecific variability when growing guar in the Russian Federation [12], on guar pathogens and the susceptibility of the guar gene pool to these pathogens [13, 14].

For any introduced species, when cultivated in atypical conditions, problems arise in selecting agrotechnology, combating diseases and pests, and improving existing varieties. High seed productivity and quality are the key issues in gum production. Varieties introduced into the Russian Federation from tropical and subtropical regions, even high-productive, may not correspond to local environmental and climatic conditions, the length of the photoperiod. In addition, they may be susceptible to local biotic and abiotic stressors. It is necessary to create domestic varieties of guar, adapted to the specific conditions of the regions.

This article presents an analysis of the results of guar cultivation in the Russian Federation, considers the limiting factors for crop production and outlines the range of tasks facing guar breeders when creating domestic varieties that produce guar gum.

Guar gum: chemical composition and properties. The main component of guar gum is the polysaccharide galactomannan, consisting of D-mannose (Man) and D-galactose (Gal). The chemistry of galactomannan involves the presence of multiple hydroxyl groups, which allow it to combine with other polymers to create new chemically modified compounds with desirable properties that are less expensive, biodegradable, and environmentally friendly [15].

Highly purified guar gum is used in the food industry, while lower quality guarana has many other uses, including as a drilling fluid additive. When used during oil drilling, guar gum prevents water loss from viscous drilling fluid and suspends bentonite clay. Guarana is less expensive than most other drilling fluid thickeners. Of particular importance is that the rheological properties, solution viscosity, and emulsification tendency of natural and chemically modified galactomannans can be altered through interaction with other monomers or polymers of the carbohydrate base [16].

Galactomannans are localized in the cell membranes of endosperm tissue and serve as an energy reserve and regulator of the water balance of the seed during germination. In a number of leguminous plants (e.g., peas, beans), the endosperm is absent, in others it constitutes a small percentage of the seed mass, 14% in fenugreek (*Trigonella foenum-graecum* L.), no more than 6% in alfalfa (*Medicago sativa* L.) [17]. In the guar seed, the endosperm is large, spherical and accounts for 38-45% of its mass [18]. According to various sources, the percentage of structural components of the seed and the biochemical composition of the guar endosperm may vary somewhat (Table 1).

1. Quantitative ratio of structural components of guar *Cyamopsis tetragonoloba* (L.) Taub seed and the endosperm biochemical composition

Component	Percentage
Structural components of a seed [16, 19, 20]	
Testa	14-16
Germ	45
Endosperm	38-45
Biochemical composition of endosperm [18]	
Galactomannan	75-85
Protein	5-6
Ash	0.5-1
Cellulose	2-3
Fat	0.5-0.9
Moisture	8-14

The Man/Gal ratio affects the viscosity of guar gum. This value is species-specific and determines various industrial use of seed galactomannans. Like all biochemical indicators, the galactomannan content (percentage of the dry weight of the seed) depends on the growing conditions and, according to different reports, varies significantly, e.g., within 15.9-31.8% [21], 21.8-34.4% [22], 28.5-32.9% [23], 35.0% [5], 16.8-36.7% [24].

The guar is on average 67-73% mannose and 27-33% galactose, respectively, that is, 2:1 [18]. In other legumes which serve as the main sources of gum the ratio of these components was ~ 1.1:1 in *Trigonella foenum-graecum* L. [25], ~ 3:1 in *Caesalpinia spinosa* (Molina) Kuntze [26], and ~ 4:1 in *Ceratonia siliqua* L. [27]. These differences determine the use of the gum as various additives in the food industry and gelling additives in cosmetics. Galactomannans with Man/Gal proportions of 1.15-2.30 have been identified in the seeds of many wild legume species of the domestic flora [28].

The gum is obtained mainly from crushed endosperm after removing the seed coat [19]. During processing, guar grains are split into two halves of galactomannan-containing endosperm (the so-called split) using special mechanisms, and the embryo and seed coat, which contain protein, are then used in animal

feeding [29].

Experience of introduction and production of guar in the Russian Federation. Guar has moderate drought resistance, is tolerant of soil salinity and is undemanding to soil fertility. This plant is 57-110 cm high, blooming mainly with white-pink flowers collected in erect racemes (Fig. 1, a). Mature beans 6-8 cm long contain from 5 to 12 seeds, varying in size between genotypes, the weight of one seed is from 11 to 51 mg [30]. The beans are collected in dense clusters (see Fig. 1, b), due to which the English name of the plant sounds like cluster bean. Guar seeds contain 27-37% protein, concentrated mainly in the germ and seed coat [31]. The color of the seeds varies from dull white to pink, light gray or black, but is predominantly beige (see Fig. 1, c). It should be noted that guar is an excellent soil-improving crop and fits well into crop rotation with wheat, cotton, grain sorghum, and vegetables [32, 33].

Fig. 1. Inflorescences (a)**, beans** (b) **and seeds** (c) **of the guar** ***Cyamopsis tetragonoloba*** **(L.) Taub from the VIR collection** (FRC All-Russian Institute of Plant Genetic Resources named after N.I. Vavilov, St. Petersburg) (photo by E.A. Dzyubenko).

In the Russian Federation, the main limiting factor for guar is heat supply. The sum of effective air temperatures above 10 °C during the growing season should be at least 3400-3500 °C. In terms of summer air temperatures and other climatic indicators, the agricultural regions of the North Caucasus and Crimea are inferior to India, but are close to the United States, where guar is successfully grown in the southern states. The optimal time for sowing guar is when the temperature of the topsoil passes through 20 °C. In this case, 350-500 mm of precipitation during the growing season is sufficient. Consequently, the flat part of the Stavropol and Krasnodar Territories is sufficiently provided with natural moisture to grow guar; in Crimea and the Rostov region it is advisable to carry out additional irrigation [34, 35]. It should be noted that in India, where guar for grain is cultivated mainly on non-irrigated lands in the north-west of the country, in the Thar Desert, varieties intended for food consumption as a vegetable crop are grown in the southern states only under irrigation [36].

The first guar crops in the Krasnodar Territory and Rostov Region appeared in 2011-2014. The maximum seed yield reached 24 c/ha. Physiological maturity of seeds occurred 100-130 days after germination [9, 34]. The dates of timely sowing and harvesting turned out to be critical for the successfu guar l cultivation.

To search for samples adapted to the conditions of the Russian Federation at the Vavilov Research Institute of Plant Genetic Resources for a number of years carried out ecological and geographical testing of guar samples in four geographical points where experimental stations are located: Krasnodar Territory (Krymsk and the village of Gulkevichi) and the Lower Volga region (Volgograd and Astrakhan) [11]. Gum yield and viscosity of samples grown in these regions were assessed. It has been proven that the yield of gum from guar seeds is determined not only by

genetic factors, but is also influenced by external conditions.The quality of gum produced from domestic seeds meets the necessary requirements [11, 29].

As the genotypes most adapted to the conditions of the Russian Federation were identified, the breeding domestic guar varieties first started by M.I. Voloshin and Z.S. Vinogradov in 2011-2014, then revived. VIR developed a methodology for assessing guar varieties for distinctiveness, uniformity and stability for the State Commission of the Russian Federation for Variety Testing [37]. In 2018-2019, the State Register of Breeding Achievements was replenished with varieties of domestic selection, including those created by VIR employees, e.g., Vavilovsky 130, VIR 1, Kaspiets, Nakhodka [38]. This is a significant contribution to the country's crop production, considering that there are only 10 zoned varieties in the State Register of Breeding Achievements of the Russian Federation (Table 2).

2. Characteristics of guar *Cyamopsis tetragonoloba* (L.) Taub varieties from the State Register of Breeding Achievements approved for use

Variety	1	2	3	4	5	6	7
Judd	12.5	–	120	–	Her	2014	West Texas Group
Vavilovsky 130	28.5	32.0	120	Branched	Yes	2018	Vavilov VIR
VIR 1	29.0	41.0	109	Single-stem	Yes	2019	Vavilov VIR
Nakhodka	27.0	–	115	Few-branched	Yes	2019	Vavilov VIR
Kaspiec	27.0	37.1	110	Branched	No	2019	Vavilov VIR
Kubansky	26.0	32.0	120	Branched	Yes	2018	LLC Agroalliance, LLC Nika-Petrotek
Kuban yubileinyi	25.5	45.0	120	Branched	Yes	2018	LLC Agroalliance, LLC Nika-Petrotek
Sinus	28.5	33.8	115	Branched	Yes	2018	Private person
Vector	23.5	32.7	120	Single-stem	Yes	2018	Private person
Pobeda	25.5	40.4	102	Single-stem	No	2020	Private person

N o t e. 1 — declared seed yield, c/ha; 2 — declared gum content, %; 3 — growing season, days; 4 — type of branching; 5 — pubescence; 6 — year of registration; 7 — originator. Dashes mean no data.

Breeding improvement of a crop should be facilitated by significant polymorphism of traits characteristic of the species. Intervarietal differences in the duration of the growing season, plant height, their pubescence, number and shape of leaves, branches, beans in a cluster, brushes and beans on a plant, dry biomass and seed weight, shape and size of seeds, root length, bean size have been repeatedly noted [12 , 22, 33, 39, 41].

It should be noted that the increased demand for guar in recent years has led to its introduction into a number of countries, e.g., in Italy, Spain, Portugal, China, where intensive study of the culture began.

Guar breeding methodoloy. Like many annual legumes, guar is a self-pollinator; the percentage of natural cross-pollination is insignificant, from 0.3 to 4.4%. Under uncontrolled conditions, the maximum cross-pollination reached 9%, which can be neglected when maintaining the genetic integrity of breeding lines and varieties [42].

Similar to some legumes (soybeans, beans, peanuts, etc.), guar is characterized by bud autogamy, i.e., self-pollination in a closed flower. The guar flower is delicate, with a small stigma, and a tight stamen-pistillate column [43], so the guar hybridization technique is very complex; the proportion of successful crosses with castration is about 4% [44]. An original method of in vivo hybridization of guar was developed - using manipulations with the pistil of a flower, when pollen grains were introduced directly into the tissue of the lower part of the style or ovary through microholes made. The method was developed to produce an interspecific hybrid of *C. tetragonoloba* × *C. serrata* in an attempt to transfer early maturation genes from a wild relative to a cultivated one. One interspecific hybrid plant was obtained. The hybridization method also turned out to be effective for intraspecific hybridization of guar plants, but apparently is not widely practiced [45].

Chemical and physical mutagenesis of guar was used to expand the genetic basis of the crop but no new economically useful traits were obtained [46].

Due to difficulties with hybridization, the main method of guar breeding at present is individual selection of valuable genotypes [43].

Guar genomics and transcriptomics research began only in the 21st century. The size of the guar genome has so far been determined by the amount of DNA, and information is contradictory. Several types of molecular markers have been identified in guar. These are RAPD (random amplified polymorphic DNA), AFLP (amplified fragment length polymorphism), SSR (simple sequence repeats), SNP (single-nucleotide polymorphism) [7], and SCAR (sequence characterized amplified region) [47] markers. Using AFLP markers, a number of agronomically valuable traits were studied, namely, the number of seeds per bean and beans per plant, growth pattern [48]. SSR was used to study genetic diversity [49]. SNPs were identified that can be effective in relation to the number of ripened beans per plant as a trait that determines high guar seed productivity [50]. However, the the number of guar genome markers obtained is still much less than those for other legumes with sequenced genomes, and is not yet sufficient for next-generation breeding programs.

Recent transcriptome sequencing has identified genes involved in biological processes, molecular functions, galactomannan biosynthesis, cellular functions, and stress tolerance pathways [51]. The development of molecular breeding methods, understanding of metabolic pathways, and further marker-mediated selection of guar now rely on next-generation sequencing platforms [47, 52].

The process of galactomannan synthesis in developing guar seeds was also studied using RNA sequencing. To identify genes involved in synthesis, RNA was extracted separately from the embryo and endosperm of the seed 30 and 40 days after flowering. As a result of transcriptomic sequencing of guar endosperm RNA on days 30 and 40, 2535 and 2724 genes with specific expression in the endosperm were identified. Of these, one mannan synthetase gene (Unigene5327), three galactosyltransferase genes (Unigene7196, Unigene23466, Unigene8081) (UniGene database, NCBI), as well as four genes that may be involved in the synthesis of guar galactomannan, were identified based on the degree of expression. According to the authors, this information will be useful for genome editing of crops [53].

Priority traits for improving guar culture in the Russian Federation. In the guar gene pool, like many leguminous crops, there is differentiation into fodder, grain and vegetable varieties. The source of guar gum is grain varieties. It can be noted that in India, the largest exporter of guar gum to the world market, until recently universal grain-feed varieties were cultivated for grain, but in recent decades, targeted selection has been carried out for seed productivity and gum yield [12].

It has been shown that, regardless of the amount of galactomannan in seeds of different genotypes, seed yield is much more influences the yield from the sown area: with an increase in seed yield, the yield of protein and galactomannan per hectare also increases, while an increase in only the content of galactomannan in the seed does not lead to a general increase in the yield of galactomannan [54]. The key property that determines the quantitative yield of galactomannan from a plant is the number of beans per plant [54, 55]. Therefore, the search in the gene pool for genotypes with the maximum yield of gum from the seed is an optional stage in the selection of source material for breeding. Meanwhile, more and more researchers are coming to recognize the number of beans per plant as the most effective breeding criterion by which selection should be made [54-57]. For the Russian Federation, this criterion should be adjusted and take into account the number of ripened beans on the plant, since unripe green

beans do not contribute to the harvest.

With the introduction of guar into countries of temperate latitudes, selection for early ripening becomes relevant, since the growing season of the crop in tropical and subtropical conditions is too extended. A comparative study of the duration of the growing season in 68 guar genotypes of different geographical origins in the conditions of Southern Italy showed that this trait varied from 155-163 days in the earliest ripening varieties, to 175-184 days in the latest ripening ones. Under these conditions, guar plants ended their growing season from mid-October to early November, exposed to autumn rains, which negatively affected the quality of the crop [56]. Guar has an indeterminate growth pattern, and the plants continue to flower and set pods until a critical drop in temperature or senescence [48]. Flowers and green beans formed on second-order shoots of branched guar plants significantly complicate the harvesting of ripened beans.

All Russian varieties require a growing season of more than 100 days to ripen seeds. Therefore, early ripening becomes a key trait for relatively northern regions, more important than the selection of highly productive varieties, since physiologically ripe seeds are used to extract gum.

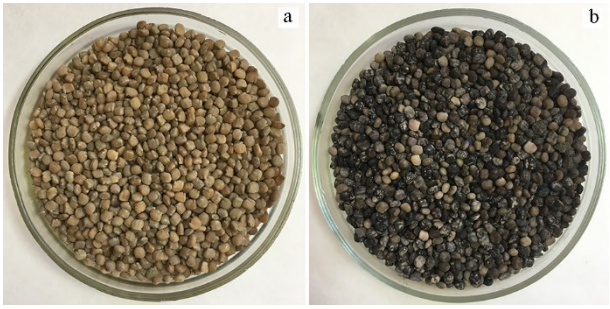

Fig. 2. Guar *Cyamopsis tetragonoloba* (L.) Taub variety Kuban seeds depending on sowing dates: a — normal seed color at optimal sowing time and timely harvesting, b — immature seeds with blackened seed coat as a result of late sowing (Kuban experimental station VIR, 2018; photo by E.A. Dzyubenko).

A serious problem when growing guar for grain under unfavorable conditions for the crop is incomplete seed ripening, which manifests itself, in particular, in the unformed seed coat. During the period of autumn temperature decrease and high humidity, the seeds in the beans turn black (Fig. 2). In this case, one can observe different ratios of ripened, with a normally formed seed coat and with its color characteristic of the genotype, and dark-colored immature seeds in the harvest. This is the first time this problem has been encountered in the United States. Black seed coat has been shown to be caused by external factors [58]. It was later established that seeds with blackened seed coats have a mature endosperm with the usual content of galactomannan; laboratory germination of blackened and light-colored seeds turned out to be close [59]. However, since the seed coat of blackened seeds is not properly formed, they absorb moisture more quickly and cannot be stored for a long time.

In Russia, the phenomenon of blackening of seeds was observed when growing guar in the Krasnodar Territory in a number of areas, including at the Kuban experimental station of the VIR during late harvesting (see Fig. 2). If there are black seeds in industrial crops, it is recommended to sort the seed material using optical separators [60]. To avoid this problem, the earliest ripening varieties should be used in production crops.

Early maturity is largely determined by photoperiod sensitivity. Guar is a plant with a short photoperiod. The critical duration of the photoperiod in different guar varieties varies from 12-13 to 13-15 hours; with longer daylight hours, plants begin flowering with a strong delay, although there are also genotypes that are weakly sensitive to photoperiod [61]. In the Krasnodar Territory and Rostov Region, where the main guar crops in the Russian Federation are located, the duration of daylight hours in May-June varies between 14.3-15.6 hours.

In the VIR guar collection, polymorphism of the species in response to photoperiod was revealed and genotypes contrasting for this trait were found. They studied metabolomic and transcriptomic profiles, which made it possible to determine the sequences of new genes and allelic variants of genes responsible for the photoperiodic response, the timing of the onset of flowering, and the duration of the growing season [62). Key metabolites associated with the onset of flowering in guar have been identified [63]. A transcriptomic-metabolomic analysis of early flowering loci was performed [64], early and late flowering genotypes were determined, and a model of gene regulation of flowering was proposed [62]. The main result of this research for breeding is the identification of material in the gur gene pool for the creation of early ripening varieties.

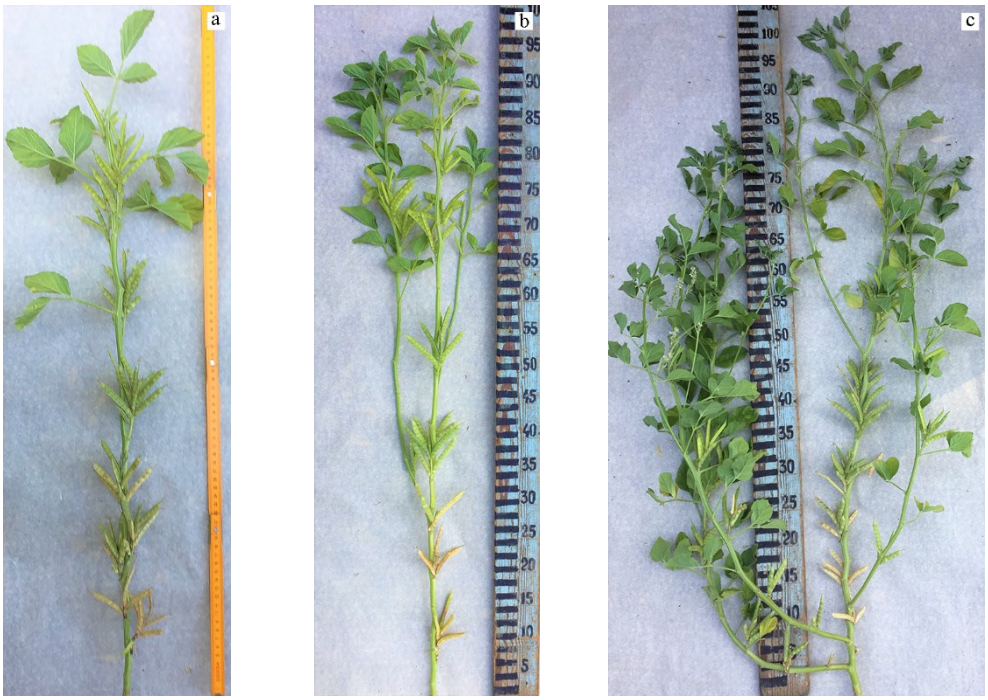

Fig. 3. Samples of guar *Cyamopsis tetragonoloba* (L.) Taub plants from the VIR collection (Vavilov FRC All-Russian Institute of Plant Genetic Resources, St. Petersburg): a — single-stemmed, b — small-stemmed, c — branched (Kuban experimental station VIR, 2018; photo by E.A. Dzyubenko).

The precocity of guar is also associated with plant architecture. The guar gene pool is differentiated into two morphotypes: branching and single-stem (Fig. 3). Branched forms have different types of branching (see Fig. 3, b, c), which were described in detail in the study of samples from the American collection [65]. For varieties of each morphotype, approximate ranges of plant density have been established in order to obtain maximum seed yield. In the conditions of the Krasnodar Territory, this is 200-250 thousand branching and 250-300 thousand single-stem plants per hectare. The yield of branched forms is ultimately slightly higher than that of single-stem forms. However, single-stem guar has a competitive advantage in weed control when planted in dense rows [66]. In addition, beans on single-stemmed plants ripen more evenly, plants can be harvested earlier, which, in conditions of a relatively short summer, makes single-stemmed and small-stemmed forms of guar preferable. Breeders in Italy, India and Portugal have recently paid increasing attention to early maturing single-stem lines [41, 67, 68]). Of the Russian varieties, two varieties bred by VIR, the VIR 1 and Nakhodka belong to the single-stem and small-stem type (see Fig. 3, a, b; Fig. 4, a, b); the

varieties Vector and Pobeda are also characterized by one stem (see. Table 2).

At VIR, when creating the Kaspiets variety, forms with a determinant growth type were isolated from varieties of American selection, which favors precocity [69] (see Fig. 4, c). Under US conditions, determinate varieties ripen in 60-90 days, indeterminate varieties in 120-150 days [70].

A number of productive branched varieties of guar have a low location of the first cluster, from 1 to 4 cm from the ground surface, which entails losses during mechanized harvesting. In this regard, the task is to develop varieties with a higher location of the lower fruit node [48, 68]. In thickened crops of single-stem varieties, for example the Vector variety, under conditions of plant competition, the first fruiting node is placed higher [66]. When creating domestic varieties, in particular VIR 1 and Nakhodka, the selection of source material from the VIR collection was carried out, among other things, based on the highest possible location of the first fruit node.

Fig. 4. Single-stem morphotype of the guar Cyamopsis tetragonoloba (L.) Taub in the field (samples from the VIR collection, FRC Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg): a — k-52779 from India (Kuban experimental station VIR), b — k-52589, variety Kubansky 1B (Astrakhan experimental station VIR), c — k-54214, variety Kaspiets, branched morphotype with a determinant type of stem growth (2018, photo by E.A. Dzyubenko).

Introduced plants inevitably encounter new diseases and pests. Phytosanitary monitoring of ecological and geographical crops of the VIR guar collection in four geographical locations revealed the main range of guar pests in the Russian Federation. Among the insects, representatives of the family *Aphididae* (true aphids) dominated: the alfalfa aphid *Aphis craccivora* (Koch) and the bean aphid *A. fabae* Scopoli [13]. Aphids are the most harmful because they carry viral infections. After the mass reproduction of aphids on environmental crops, severe focal viral damage to plants, yellowing and marbling of leaves were observed. After insecticidal treatments in August, only single colonies of aphids were detected on the crops [13, 14].

Among fungal diseases, the overwhelming majority of cases were Alternaria blight, caused predominantly by the species *Alternaria tenuissima* (Nees & T. Nees: Fr.) Wiltshire [14]. Analysis of rhizosphere pathogenic mycoflora showed

the dominance of fungi from the genera *Verticillium* Nees and Fusarium Link. The most harmful disease of guar, including in the Russian Federation, is bacteriosis (pathogen *Xanthomonas cyamopsidis*). Massive drying out and death of plants was noted in a number of samples. The disease, transmitted through seeds, can lead to the death of plants at all stages of growth and development. Symptoms include large, angular, necrotic lesions on leaf tips that cause defoliation and black streaking on stems. This potentially poses the greatest threat to the guar. Resistant plants have been identified, suggesting the possibility of selecting for individual resistant genotypes [71].

The differential interaction between parasite and host is shown. Therefore, to prevent epiphytoties, varieties protected by non-identical resistance genes should be grown and the maximum possible number of genetically heterogeneous accessions should be involved in breeding.

Heritability and associations of guar productivity traits. The heritability of most productivity traits has been studied, which has made it possible to predict the success of selection for them. Various methods of data analysis were used, including analysis of path coefficients, ANOVA, etc. The additive effect of genes for the following traits was established: the number of branches per plant on the 90th day after sowing, the number of brushes per plant, pod length, bean weight, bean weight on the plant, seed productivity of the plant, gum content in the endosperm of the seed. It is concluded that genetic improvement and selection for these traits will be effective. Low genetic additive variation was observed for traits such as protein content and number of seeds per bean, meaning selection for these traits may be ineffective [72-74]. Low heritability and high environmental influence on the trait number of seeds in a pod, as well as plant height on day 90, were also shown in other studies [41, 75, 76]. The traits number of seeds per bean, number of beans per plant, number of beans per cluster, number of clusters per plant, number of days to 50% flowering, number of days to ripening have significant positive correlations with seed productivity [68].

In experiments on 43 guar samples conducted in the Indian state of Karnataka, the additive genetic variation (GAM) and heritability of traits that make up guar yield were determined. The coefficient of variation indicates how much variation is present in the germplasm for various traits, but heritability coefficients are useful in predicting the success of selection. In the experiment, high (more than > 80%) heritability was noted for the traits number of branches on the plant, type of growth, number of days before flowering, number of clusters on the plant, number of beans on the plant, weight of 100 seeds, gum content in the endosperm (65). The high heritability of the traits the number of days before beans ripen and the number of beans per plant was also established in other works [41, 75, 76].

When studying the mechanism of inheritance of guar gum content, it was found that the manifestation of the trait is under the control of additive, dominant and epistatic effects of genes and is modified by external factors [77].

In none of the guar cultivation areas in the Russian Federation were nitrogen-fixing nodules found on its roots. This is explained by the absence of bacteria in the soil that can enter into symbiosis with guar. Meanwhile, inoculation of legume seeds with biological preparations of root nodule bacteria provides intensive biological nitrogen fixation, which enhances photosynthesis, increases yield, protein content in seeds and green mass, etc. [78]. Based on this, another significant trait for legumes was introduced into the list of selectively significant traits—efficiency of interaction with beneficial soil bacteria (ESBM) [79, 80]. This is especially important when cultivating legumes in new territories, the soils of which do not contain the necessary microsymbionts. For the successful introduction

of guar to Russia, along with the above-described measures to improve the culture, selection of effective microsymbionts is required for the purpose of creating biological products.

Scientists from the All-Russian Research Institute for Agricultural Microbiology (ARRIAM, St. Petersburg) was able to identify in the rhizosphere of guar grown in a vegetation experiment with the addition of soil from India, nitrogen-fixing bacteria of two species, the *Bradyrhizobium retamae* and *Ensifer aridi*, which were characterized, patented by the authors and deposited in the Departmental collection beneficial microorganisms for agricultural purposes (ARRIAM) [81, 82]. Both strains showed the ability to form an effective symbiosis with guar and the promise of their further testing in field experiments with the aim of creating biological products to improve the nitrogen nutrition of plants.

Thus, when breeding domestic varieties of guar, a new introduced species on the territory of the Russian Federation, the set of key traits that require improvement is dictated by the main limiting factors for the cultivation of this tropical crop in new soil and climatic conditions. These include: lack of heat and, in some places, moisture supply, long photoperiod, the presence of pathogens, and the absence of nitrogen-fixing microsymbionts in the soil. This determines the search in the gene pool as the starting material for the selection of guar forms with reduced photosensitivity, a short growing season, resistant to certain and/or complex pathogens, and also requires the creation and use of complementary rhizobial preparations. This approach will contribute to the production of productive genotypes that have time to form full-fledged seeds under relatively short summer conditions. The main sign of guar seed productivity is the number of beans on the plant, which determines the yield and yield of gum both from the plant and from industrial crops. Single-stem and low-branched forms are recognized as the best morphotype for Russian conditions, which ensure uniform distribution of beans, suitability for mechanized harvesting and early ripening. The combination of the listed characteristics in one genotype corresponds to the model of an industrial guar variety for the conditions of our country. The created domestic varieties of guar were obtained using traditional breeding methods, but the active development of genomic, metabolomic and transcriptomic resources of the species allows us to hope that in the foreseeable future varieties of this valuable crop for the Russian Federation will be created using marker-mediated and genomic selection.

REFERENCES

1. Shcherbukhin V.D., Anulov O.V. *Prikladnaya biokhimiya i mikrobiologiya*, 1999, 35(3): 257-274 (in Russ.).
2. Chudzikowski R.J. Guar gum and its applications. *Journal of the Society of Cosmetic Chemists* 22, 1971: 43-60.
3. Mudgil D., Barak, S., Khatkar B.S. Guar gum: processing, properties and food applications — a review. *Journal of Food Science and Technology*, 2014, 51: 409-418 (doi: 10.1007/s13197-011-0522-x).
4. Hasan A.M.A., Abdel-Raouf M.E. Applications of guar gum and its derivatives in petroleum industry: a review. *Egyptian Journal of Petrolium*, 2018, 27(4): 1043-1050 (doi: 10.1016/j.ejpe.2018.03.005).
5. Pathak R. Clusterbean gum and by-product. In: *Clusterbean: physiology, genetics and cultivation*. Springer, Singapore, 2015: 33-60 (doi: 10.1007/978-981-287-907-3_3).
6. Thombare N., Jha U., Mishra S., Siddiqui M.Z. Guar gum as a promising starting material for diverse applications. *International Journal of Biological Macromolecules*, 2016, 88: 361-372 (doi: 10.1016/j.ijbiomac.2016.04.001).
7. Ravelombola W., Manley A., Adams C., Trostle C., Ale S., Shi A., Cason J. Genetic and genomic resources in guar: a review. *Euphytica*, 2021, 217(11): 119 (doi: 10.1007/s10681-021-02929-2).
8. Europe guar market — analysis, trends, and forecasts (2023-2028). Available: https://www.mordorintelligence.com/industry-reports/europe-guar-market. No date.
9. Bulyntsev S.V., Val'yanikova T.I., Silaeva O.I., Kopot' E.I., Pimonov K.I. *Materialy Vserossiiskoy konferentsii «Innovatsii v tekhnologiyakh vozdelyvaniya sel'skokhozyaystvennykh kul'tur»* [Proc. of

Russian Conf. "Innovative technologies for crop cultivation"]. Persianovka, 2017: 167-172 (in Russ.).
10. Kantemirova E.N., Shaukharov R.A. *Sbornik tezisov Mezhdunarodnoy konferentsii «125 let prikladnoy botaniki v Rossii»* [Abstracts of the International Conference "125 Years of Applied Botany in Russia"]. St. Petersburg, 2019: 139 (doi: 10.30901/978-5-907145-39-9) (in Russ.).
11. Kruchina-Bogdanov I.V., Miroshnichenko E.V., Shaukharov R.A., Kantemirova E.N., Golovina M.A., Abdullaev K.M., Balashov A.V., Rusinova E.V., Rusinov P.G., Potokina E.K. Impact of growing conditions on the gum properties of different genotypes of guar (*Cyamopsis tetragonoloba* (L.) Taub.). *Vavilov Journal of Genetics and Breeding*, 2019, 23(7): 941-948 (doi: 10.18699/VJ19.570).
12. Dzyubenko E.A. *Vavilovia*, 2021, 4(3): 16-39 (doi: 10.30901/2658-3860-2021-3-16-39) (in Russ.).
13. Radchenko E.E., Sokolova D.V. Resistance of guar *Cyamopsis tetragonolob*a (L.) Taub. to harmful organisms (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2018, 53(5): 897-906 (doi: 10.15389/agrobiology.2018.5.897eng).
14. Radchenko E.E., Abdulaev R.A., Alpat'eva N.V., Putina O.V., Gasich E.L. *Sbornik tezisov Mezhdunarodnoy konferentsii «125 let prikladnoy botaniki v Rossii»* [Abstracts of the International Conference "125 Years of Applied Botany in Russia"]. St. Petersburg, 2019: 179 (doi: 10.30901/978-5-907145-39-9) (in Russ.).
15. Tripathy S., Das M.S. Guar gum: present status and applications. *Journal of Pharmaceutical and Scientific Innovations* 2013, 2(4): 24-28 (doi: 10.7897/2277-4572.02447).
16. Srivastava M., Kapoor V.P. Seed galactomannans: an overview. *Chemistry & Biodiversity*, 2005, 2(3): 295-317 (doi: 10.1002/cbdv.200590013).
17. Hegnauer R. Uber das Endosperm der Leguminosen. *Pharmazeutische Zentralhalle für Deutschland*, 1957, 9(4): 141-143 (in German).
18. Murwan K.S., Abdalla A.H., Nouri S.H. Quality assessment of guar gum (endosperm) of guar (*Cyamopsis tetragonoloba*). *ISCA Journal of Biological Science*, 2012, 1(1): 67-70.
19. Maier H., Anderson M., Karl C., Magnuson K., Whistler R.L. Chapter 8 — Guar, locust bean, tara, and fenugreek gums. In: *Industrial gums (Third edition): Polysaccharides and their derivatives*. Cambridge, Academic Press, 1993: 181-226 (doi: 10.1016/B978-0-08-092654-4.50012-7).
20. Mirhosseini H., Amid B.T. A review study on chemical composition and molecular structure of newly plant gum exudates and seed gums. *Food Research International*, 2012, 46(1): 387-398 (doi: 10.1016/j.foodres.2011.11.017).
21. Dabas B.S., Mital S.P., Arunachalam V. An evaluation of germplasm accessions in Guar (*Cyamopsis tetragonoloba* L.). *Indian Journal of Genetics and Plant Breeding*, 1982, 42: 56-59.
22. Dwivedi N., Bhandari D.C., Bhatnagar N., Dabas B.S. Characterizing cluster bean [*Cyamopsis tetragonaloba* (L.) Taub] germplasm for yield components and quality traits. *Annals of Arid Zone*, 1999, 38(2): 151-156.
23. Naik C.S.R., Ankaiah R., Sudhakar P., Reddy D.T., Murthy V.R., Spandana V., Jatothu J. Variation in the protein and galactomannan content in guar seeds of different genotypes. *Plant Archives*, 2013, 13(1): 247-252.
24. Wadhwa N., Joshi U.N. Galactomannan content and key enzymes of its metabolism in seeds of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.]. *Legume Research*, 2017, 40: 462-469 (doi: 10.18805/lr.v0iOF.10758).
25. Zandi P., Basu S., Cetzal-Ix. W., Kordrostami M., Chalaras S., Khatibai L. Fenugreek (*Trigonella foenum-graecum* L.): an important medicinal and aromatic crop. In: *Active ingredients from aromatic and medicinal plants*. H.A. El-Shemy (ed.). In Tech, 2017: 217-223 (doi: 10.5772/66506).
26. Rigano L., Deola M., Zaccarioto F., Colleoni T., Lionetti N. A new gelling agent and rheology modifier in cosmetics: *Caesalpinia spinosa* Gum. *Cosmetics*, 2019, 6(2): 34 (doi: 10.3390/cosmetics6020034).
27. Batal H.E., Hasib A., Ouatmane A., Boulli A., Dehbi F., Jaouad A. Yield and composition of carob bean gum produced from different Moroccan populations of carob (*Ceratonia silique* L.). *Journal of Materials and Environmental Science*, 2013, 4: 309-314.
28. Anulov O.V., Ponomarenko S.F., Egorov A.V., Shcherbukhin V.D. *Rastitel'nye resursy*, 2003, 39(1): 80-82 (in Russ.).
29. Dzyubenko N.I., Dzyubenko E.A., Potokina E.A., Bulyntsev S.V. Clusterbeans *Cyamopsis tetragonoloba* (L.) Taub. — properties, use, plant genetic resources and expected introduction in Russia (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2017, 52(6): 1116-1128 (doi: 10.15389/agrobiology.2017.6.1116rus).
30. Khan S.D., Jahan I., Akhtar L.H., Minhas R. Seed mass variation in seed lots of fifteen gerplasms of guar [*Cyamopsis tetragonoloba* (L.) Taub.]. *International Journal of Biology and Biotechnology*, 2018, 15(4): 711-720.
31. Whistler R.L., Hymowitz T. *Guar: agronomy, production, industrial use, and nutrition*. Purdue University Press, 1979.
32. Bhatt R.K., Jukanti A., Roy M.M. Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.], an important industrial arid legume: a review. *Legume Research*, 2017, (40): 207-214 (doi:

10.18805/lr.v0iOF.11188).
33. Sortino O., Gresta F. Growth and yield performances of five guar cultivars in a Mediterranean environment. *Italian Journal of Agronomy*, 2007, 4: 359-364 (doi: 10.4081/ija.2007.359).
34. Lebed' D.V., Kostenkova E.V., Voloshin M.I. *Tavricheskiy vestnik agrarnoy nauki*, 2017, 1(9): 53-64 (in Russ.).
35. Startsev V.I., Livanskaya G.A., Kulikov M.A. *Nauchnyy zhurnal VRGAZU*, 2017, 24(29): 11-15 (in Russ.).
36. Kumar V., Ram R.B., Yadav R.K. Genetic diversity in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] for morphological characters. *Indian Journal of Science and Technology*, 2014, 7(8): 1144-1148 (doi: 10.17485/ijst/2014/v7i8.17).
37. *Ofitsial'nyy byulleten'*. FGBU «Gosudarstvennaya komissii Rossiyskoy Federatsii po ispytaniyu i okhrane selektsionnykh dostizheniy» [Official bulletin. FSBI State Commission of the Russian Federation for Testing and Protection of Breeding Achievements]. Moscow, 2017, 10(230): 686-694 (in Russ.).
38. *Gosudarstvennyy reestr selektsionnykh dostizheniy, dopushchennykh k ispol'zovaniyu. Tom 1. «Sorta rasteniy» (ofitsial'noe izdanie)* [State register of selection achievements approved for use. V. 1. "Plant Varieties" (official publication]. Moscow, 2022 (in Russ.).
39. Stafford R.E., Barker G.L. Heritability and interrelationships of pod length and seed weight in guar. *Plant Breeding*, 2006, 103(1): 47-53 (doi: 10.1111/j.1439-0523.1989.tb00350.x).
40. Singh R., Singh P., Jaiswal A., Kumar Y. Heritability and genetic advance for yield and related traits in [*Cyamopsis tetragonoloba* (L.) Taub]. *JNKVV Research Journal*, 2010, 44(2): 127-130.
41. Gresta F., Avola G., Cannavò S., Santonoceto C. Morphological, biological, productive and qualitative characterization of 68 guar (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes. *Industrial Crops and Products*, 2018, 114: 98-107 (doi: 10.1016/j.indcrop.2018.01.070).
42. Stafford R.E., Lewis C.R. Natural crossing in guar *Cyamopsis tetragonoloba* (L.) Taub. *Crop Science*, 1975, 15(6): 876-877 (doi: 10.2135/cropsci1975.0011183X001500060042x).
43. Jukanti A.K., Pathak R., Mushyam C. Cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] breeding. In: *Advances in plant breeding strategies: legumes*. J.M. Al-Khayri, S.M. Jain, D.V. Johnson (eds.). Springer, Cham, 2019: 113-149 (doi: 10.1007/978-3-030-23400-3_4).
44. Stafford R.E., Hymowitz T. Guar. In: *Hybridization of crop plants*. American Society of Agronomy-Crop Science Society of America, Madison, 1980: 381-392.
45. Ahlawat A., Pahuja S.K., Dhingra H.R. Overcoming interspecific hybridization barriers in *Cyamopsis* species. *International Journal of Biotechnology and Bioengineering Research*, 2013, 4(3): 181-190.
46. Arora R.N., Pahuja S.K. Mutagenesis in guar [*Cyamopsis tetragonoloba* (L.) Taub.]. *Plant Mutation Reports*, 2008, 2(1): 7-9.
47. Kumar S., Modi A.R., Parekh M.J., Mahla H.R., Sharma R., Fougata R.S., Yadav D., Yadav N.R., Patil G.B. Role of conventional and biotechnological approaches for genetic improvement of cluster bean. *Industrial Crops and Products*, 2017, 97: 639-648 (doi 10.1016/j.indcrop.2017.01.008).
48. Gresta F., Mercati F., Santonoceto C., Abenavoli M.R., Ceravolo G., Araniti F., Anastasi U., Sunseri F. Morphoagronomic and AFLP characterization to explore guar (*Cyamopsis tetragonoloba* L.) genotypes for the Mediterranean environment. *Industrial Crops Products*, 2016, 86: 23-30.
49. Pareek S., Jaiswal P., Shrivastava D. Guar genes to genome and meta-analysis of SSR markers in sequencing studies. *Genetic Resources Crop Evolution*, 2022, 69: 2279-2290 (doi: 10.1007/s10722-022-01420-8).
50. Grigoreva E., Barbitoff Y., Changalidi. A., Karzhaev D., Volkov V., Shadrina V., Safronycheva E., Ben C., Gentzbittel L., Potokina E.K. Development of SNP set for the marker-assisted selection of guar (*Cyamopsis tetragonoloba* (L.) Taub.) based on a custom reference genome assembly. *Plants*, 2021, 10(10): 2063 (doi: 10.3390/plants10102063).
51. Chaudhury A., Kaila T., Gaikwad K. Elucidation of galactomannan biosynthesis pathway genes through transcriptome sequencing of seeds collected at different developmental stages of commercially important indian varieties of cluster bean (*Cyamopsis tetragonoloba* L.). *Sci. Rep.*, 2019, 9: 11539 (doi: 10.1038/s41598-019-48072-w).
52. Kumar S., Palve A.S., Patel S.K., Selvanayagam S., Sharma R., Rathore A. Development of genomic microsatellite markers in cluster bean using next generation DNA sequencing and their utility in diversity analysis. *Current Plant Biology*, 2020, 21: 100134 (doi: 10.1016/j.cpb.2019.100134).
53. Hu H., Wang H., Zhang Y., Kan B., Ding Y., Huang J. Characterization of genes in guar gum biosynthesis based on quantitative RNA-sequencing in guar bean (*Cyamopsis tetragonoloba*). *Scientific Reports*, 2019, 9: 10991 (doi: 10.1038/s41598-019-47518-5).
54. Gresta F., Sortino O., Santonoceto C., Issi L., Formantici C., Galante Y.M. Effects of sowing times on seed yield, protein and galactomannans content of four varieties of guar (*Cyamopsis tetragonoloba* L.) in a Mediterranean environment. *Industrial Crops and Products*, 2013, 41(1): 46-52 (doi: 10.1016/j.indcrop.2012.04.007).
55. Manivannan A., Anandakumar C.R., Ushakumari R., Dahiya G.S. Genetic diversity of guar genotypes (*Cyamopsis tetragonoloba* (L.) Taub.) based on agro-morphological traits. *Bangladesh Journal of Botany*, 2015, 44(1): 59-65 (doi: 10.3329/bjb.v44i1.22724).

56. Avola G., Riggi E., Trostle C., Sortino O., Gresta F. Deficit irrigation on guar genotypes (*Cyamopsis tetragonoloba* (L.) Taub.): effects on seed yield and water use efficiency. *Agronomy*, 2020, 10(6): 789 (doi: 10.3390/agronomy10060789).
57. Meftahizade H., Ghorbanpour M., Asarehc M.H. Changes in phenological attributes, yield and phytochemical compositions of guar (*Cyamopsis tetragonoloba* L.) landaraces under various irrigation regimes and planting dates. *Scientia Horticulturae*, 2019, 256: 108577 (doi: 10.1016/j.scienta.2019.108577).
58. Hymovitz T., Matlock R.S. Guar: seed, plant and population studies. *Technical Bulletin (Oklahoma Agricultural Experiment Station)*, 1964, V108.
59. Liu W., Peffley E.B., Powell R.J., Auld D.L, Hou A. Association of seedcoat color with seed water uptake, germination, and seed components in guar (*Cyamopsis tetragonoloba* (L.) Taub). *Journal of Arid Environments*, 2007, 70(1): 9-38 (doi: 10.1016/j.jaridenv.2006.12.011).
60. Lebed' D.V., Voloshin M.I., Bespalov E.A., Kostenkova E.V. *Tavricheskiy vestnik agrarnoy nauki*, 2018, 2(14): 54-63 (doi: 10.25637/TVAN.2018.02.05) (in Russ.).
61. Lubbers E.L. Characterization and inheritance of photoperiodism in guar, *Cyamopsis tetragonoloba* (L.) Taub. Univ. of Arizona, 1987.
62. Teplyakova S.B., Volkov V.A., Dzyubenko E.A., Potokina E.K. Variability of photoperiod response in guar (*Cyamopsis tetragonoloba* (L.) Taub.) genotypes of different geographic origin. *Vavilov Journal of Genetics and Breeding*, 2019, 23(6): 730-737 (doi: 10.18699/VJ19.547).
63. Arkhimandritova S., Shavarda A., Potokina E. Key metabolites associated with the onset of flowering of guar genotypes (*Cyamopsis tetragonoloba* (L.) Taub). *BMC Plant Biology*, 2020, 20(Suppl 1): 291 (doi: 10.1186/s12870-020-02498-x).
64. Grigoreva E., Tkachenko A., Arkhimandritova S., Beatovic A., Ulianich P., Volkov V., Karzhaev D., Ben C., Gentzbittel L., Potokina E.K. Identification of key metabolic pathways and biomarkers underlying flowering time of guar (*Cyamopsis tertragonoloba* (L.) Taub.) via integrated transcriptome-metabolome analysis. *Genes*, 2021, 12(7): 952 (doi: 10.3390/genes12070952).
65. Morris J.B. Morphological and reproductive characterization of guar (*Syamopsis tetragonoloba*) genetic resources regenerated in Georgia, USA. *Genetic Resources Crop Evolution*, 2010, 57: 985-993 (doi: 10.1007/s10722-010-9538-8).
66. Voloshin M.I., Bespalov E.A. *Tavricheskiy vestnik agrarnoy nauki*, 2019, 2(18): 15-22 (in Russ.).
67. Vir O., Singh A.K. Variability and correlation analysis in the germplasm of cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] in hyper hot arid climate of Western India. *Legume Research*, 2015, 38(1): 37-42 (doi: 10.5958/0976-0571.2015.00006.5).
68. Reis C.M.G., Almeida C.M., Peças L.F.V., Almeida J.P.F. Yield evaluation of guar genotypes (*Cyamopsis tetragonoloba* L. Taub.) selected for high-density planting and mechanical harvesting. *Bulgarian Journal of Agriculture Science*, 2021, 27(5): 926-932.
69. Dzyubenko E.A., Dzyubenko N.I. *Tezisy dokladov IV Vavilovskoy mezhdunarodnoy nauchnoy konferentsii «Idei N.I. Vavilova v sovremennom mire»* [Abstracts of the IV Vavilov Innternational Conference "Ideas of N.I. Vavilov in the modern world']. St. Petersburg, 2017: 118 (in Russ.).
70. Undersander D.J., Putnam D.H., Kaminski A.R., Kelling K.A., Doll J.D., Oplinger E.S., Gunsolus J.L. Guar. *Alternative field crops manual*. University of Wisconsin, 1991. Available: https://hort.purdue.edu/newcrop/afcm/guar.html. No date.
71. Radchenko E.E. Incidence of insect pests and diseases of guar in Krasnodar area of Russia. In: *International congress on oil and protein crops*. 20-24 mai 2018, Chişinău. Chişinău, 2018: 158.
72. Pathak R., Singh M., Henry A. Genetic diversity and interrelationship among clusterbean [*Cyamopsis tetragonoloba* (L.) Taub.] genotype for qualitative traits. *Indian Journal of Agricultural Science*, 2011, 81(5): 402-406.
73. Santhosha G.R., Evoor Shashikanth E., Gasti V.D., Prabhuling G., Rathod V.D, Mulge R. Genetic variability studies in cluster bean [*Cyamopsis tetragonaloba* (L.) Taub.] for growth, yield and quality parameters. *Legume Research*, 2017, 40(2): 232-236 (doi: 10.18805/lr.v0i0.7296).
74. Deepashree G., Gasti D., Raut N., Chittapur R., Reddi S.G., Kustagi G. Genetic variability studies in cluster bean [*Cyamopsis tetragonoloba* (L.) Taub.] for growth, yield and quality parameters. *The Pharma Innovation Journal*, 2021, 10(4): 666-670 (doi: 10.22271/tpi.2021.v10.i4j.6033).
75. Prakash R.K.R., Prasanthi L., Sekhar M.R. Genetic variability studies for seed yield, physiological and quality attributes in guar. *The Asian and Australasian Journal of Plant Science and Biotechnology*, 2008, 2: 36-38.
76. Manivannan A., Anandakumar C.R. Genetic variability, character association and path analysis in cluster bean. *Journal of Food Legumes*, 2013, 26(3-4): 34-37.
77. Liu W-kh., Hou A.-f., Peffley E.B., Auld D.L. The inheritance and variation of gum content in guar [*Cyamopsis tetragonoloba* (L.) Taub.]. *Agricultural Sciences in China*, 2009, 8(12): 1517-1522 (doi: 10.1016/S1671-2927(08)60367-3).
78. Zhukov V.A., Akhtemova G.A., Zhernakov A.I., Sulima A.S., Shtark O.Yu., Tikhonovich I.A. Evaluation of the symbiotic effectiveness of pea (*Pisum sativum* L.) genotypes in pot experiment. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2017, 52(3): 607-614 (doi: 10.15389/agrobiology.2017.3.607eng).

79. Shtark O.Y, Borisov A.Y, Zhukov V.A, Tikhonovich IA. Mutually beneficial legume symbioses with soil microbes and their potential for plant production. *Symbiosis*, 2012, 58: 51-62 (doi: 10.1007/s13199-013-0226-2).
80. Shtark O.Yu., Zhukov V.A., Sulima A.S., Singkh R., Naumkina T.S., Akhtemova G.A., Borisov A.Yu. Prospects for the use of multi-component symbiotic systems of the Legumes. *Ekologicheskaya genetika*, 2015, 13(1): 33-46 (doi: 10.17816/ecogen13133-46).
81. Kuznetsova I.G., Sazanova A.L., Safronova V.I., Popova Zh.P., Sokolova D.V., Tikhomirova N.Yu., Osledkin Yu.S., Karlov D.S., Belimov A.A. Isolation and identification of root nodule bacteria from guar *Cyamopsis tetragonoloba* (L.) Taub. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2018, 53(6): 1285-1293 (doi: 10.15389/agrobiology.2018.6.1285eng).
82. Ul'yanich P.S., Belimov A.A., Kuznetsova I.G., Sazanova A.L., Yuzikhin O.S., Laktionov Yu.V., Karlov D.S., Vishnyakova M.A., Safronova V.I. Effectiveness of nitrogen-fixing symbiosis of guar (*Cyamopsis tetragonoloba*) with strains *Bradyrhizobium retamae* RCAM05275 and *Ensifer aridi* RCAM05276 in pot experiment. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2022, 57(3): 555-565 (doi: 10.15389/agrobiology.2022.3.555eng).

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 60-74
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Toward sustainable farming

UDC 631.62:58.05:551.58.056:631.421

doi: 10.15389/agrobiology.2023.1.60eng
doi: 10.15389/agrobiology.2023.1.60rus

THE IMPACT OF CLIMATE CHANGE ON CROP FARMING IN THE DRAINED LANDS OF THE EUROPEAN NON-CHERNOZEM REGION OF RUSSIA: VULNERABILITY AND ADAPTATION ASSESSMENT

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Final revision received November 29, 2022
Accepted January 13, 2023

Abstract

The impact of climate change on agricultural sustainability has become a particularly acute and global problem. The human activity increasingly significant contributes to such changes. Therefore, investigations have been intensified worldwide to assess the regional agro-climatic consequences of global climate change in order to find ways to adapt to them. This work aimed to assess the vulnerability and adaptation of field crops to a changing weather in the zone of drained lands of the European Non-Chernozem Region, which is characterized by a humid climate and limited thermal resources. Although the thermal conditions for growing crops here are becoming more favorable due to the increase in heat supply, more frequent incessant and heavy rains lead to a sharp overwetting of crops, causing significant crop shortages and quality losses. The novelty of our study lies in the evaluation of a shift in the boundaries of vulnerable territories. The conclusions we came to resulted from the subdivision of the zone into subzones by natural environment attributes, given changes in existing agrolandscapes and farming systems in latitudinal zonality, including thermally deficient areas. In the European Non-Chernozem Region of Russia, with a warming climate, the altitudes of drained lands were shown to affect the magnitude and frequency of heavy precipitation and should be taken into account when regionalizing adaptation measures and strategies, which is done for the this zone for the first time. The paper submits the analysis of thermal changes and atmospheric moisture changes during grain formation in winter cereals and fodder cereals and during intensive accumulation of biomass of silage and hay crops for two periods that differ in the degree of anthropogenic influence on the climate, 1945-1980 and 1981-2017. Using a set of analytical methods (selection of agro-climatic indicators, frequency analysis) and mathematical methods (trend analysis and smoothing of time series, functional analysis, etc.), we revealed that the coverage of areas subject to an increasing risk of overwetting of crops is expanding to the north. An increase in high temperatures leads to an intensification of evaporation and, as a result, to an increased convection. Based on simulation modeling of agro-climatic conditions until 2030, it is also shown that in the future, the northern and marshy areas are the most vulnerable to sudden overwetting. That is, along with the atmospheric circulation (i.e., a higher cyclonic activity), the thermal factor combined with the moisture content of the underlying surface in the soil contribute more and more significantly to the aggravation of overwetting. Soil texture also plays an important role in the manifestation of the effects of atmospheric overwetting on crops. Adaptation of field crop growing to climate change includes a set of measures aimed at effective management of interrelated changes in the thermal regime in the active layer of the atmosphere and the water balance components of the underlying surface and upper layers of the soil. The adaptation measures should regard the agro-climatic, soil and landscape features of the natural and agricultural subzones of the European Non-Chernozem Region drained lands and consists both in optimizing reclamation techniques and in improving crop cultivation technologies and land use planning. It is also extremely important that elevated concentrations of technogenic pollutants in the atmosphere have a negative impact on the quantity and quality of precipitation. Therefore, targeted monitoring of compliance with environmental standards is mandatory.

Keywords: European Non-Chernozem Region, climate change, drained lands, vulnerability, adaptation

The problem of sustainable agriculture has acquired a global scale due to increasing climate change and an increase in the frequency of extreme weather events, including those arising from anthropogenic activities [1, 2]. In humid regions, such phenomena include ultra-intense precipitation and heat waves [3-5]. The latter are associated with an increase in temperature to extremely high values. At low latitudes, tropical cyclones of exceptional power arise [6, 7] with the contribution of the anthropogenic factor to their formation [8]. In the middle latitudes of both hemispheres, cyclonic activity intensifies, which also extends to high latitudes [9, 10].

One of these regions in the middle and high latitudes includes the zone of drained lands of the European Non-Black Earth Region. In agricultural terms, this territory differs from other regions of Russia in its moderately warm and humid climate. The factors influencing sustainable farming here, on the one hand, are limited heat resources, and on the other, excess atmospheric moisture. Based on natural landscape characteristics, the region is divided into three agricultural subzones: mid-taiga (60-63°N, 29-51°E), southern taiga (54-60°N, 28-47°E) and coniferous-deciduous (52-54°N, 31-33°E). Landscape features consist of a predominance of lowlands and lowlands and an increasing proportion of wetlands in the direction from the southwestern regions to the northeastern ones [11).

The soils of the region is podzolic with a wide distribution of loamy and heavy loamy soils with difficult permeability of moisture from the surface layers to the underlying ones. In addition, heavily waterlogged peaty soils are often found in all subzones, and in river floodplains the soil cover is represented by alluvial soils [12].

Soil and climatic conditions determine the set of field crops, their placement and directions of crop production. Along with the cultivation of cool-climate food crops, the region has developed grass sowing and the production of forage crops, which serve as fodder for dairy farming. The selection focus in regional field farming is high-yielding and flexible varieties that are quite unpretentious to soil conditions, but at the same time tolerant to the effects of biotic and abiotic stresses. However, when optimizing habitats from the point of view of economically justified placement of crops and varieties, the manifestation of global and regional climate changes should be accounted [13, 14].

This paper analyzes the interrelated changes in thermal conditions and the nature of atmospheric moisture in the zone of drained lands of the European Non-Black Earth Region of Russia under conditions of increasing anthropogenic influence on the climate. Unlike air temperature fluctuations, which are determined to a certain extent, the amount of precipitation is characterized by high stochasticity in spatiotemporal resolution. In a changing climate, there is a tendency to increase the heat supply of crops, but the appearance of extremely heavy precipitation leads to a sharp waterlogging of crops, causing significant crop shortages and loss of quality. The study focused on the changing conditions of July, the period of formation of the final harvest of winter grains and forage cereals, as well as the intensive accumulation of biomass of silage and hay crops, when air temperatures are high and rainfall can be very intense.

The novelty of the presented results lies in the establishment of a shift in the boundaries of such territories by differentiating the zone of drained lands of the European Non-Black Earth Region into subzones based on natural landscape characteristics with a change in the existing agricultural landscapes and farming systems in latitudinal zones, including thermally deficient areas. For the first time, it has been shown that the altitudinal layering of landscapes in the zone of drained lands in a warming climate affects the magnitude and frequency of heavy precipitation and should be taken into account when developing adaptation measures and strategies. Adaptation of regional field farming to climate change was assessed

from the standpoint of effective management of climate-related agricultural risks, effective management of the productivity of agricultural lands and crops, as well as possible control of the quantity and quality of moisture coming from the atmosphere through compliance with environmental standards. Thus, the aspect of increasing anthropogenic impact on the humidity characteristics of the regional climate is added, which was not previously focused on.

The purpose of the work is i) to analyze the recurrence of adverse weather events associated with an excess of atmospheric moisture in combination with a thermal background that becomes more favorable for the cultivation of crops, and ii) to identify agricultural areas in the zone of drained agricultural lands of the European Non-Black Earth Region of Russia that are vulnerable to climate change.

Materials and methods. Data provided by temporary series of average monthly air temperatures and monthly precipitation were obtained from 1945 to 2017 based on observations at agrometeorological stations in the middle taiga subzone (Vyborg, Sortavala, Petrozavodsk, Vytegra, Shenkursk, Kotlas, Syktyvkar), in the southern taiga subzone (Pskov, Velikiye Luki, Tikhvin, Staraya Russa, Smolensk, Bologoe, Kostroma, Vologda, Totma, Nikolsk), and in the subzone of coniferous-deciduous forests (Bryansk). The location of the stations in space quite fully reflected the intrazonal features of the soil cover and landscapes. Data homogeneity in the observation series was achieved through a system for introducing corrections for improving instruments and refining measurement techniques, developed at the All-Russian Research Institute of Hydrometeorological Information — World Data Center (VNIIGMI-WCD, Obninsk, Kaluga Province). However, for the comparability with shorter series of other indicators (atmospheric circulation indices and simulated characteristics of the current climate), the study also used shortened initial data.

We combined analytical (selection of agroclimatic indicators, comparative frequency analysis) and mathematical methods of data analysis and processing. The latter included trend analysis and smoothing of time series, functional analysis (with access to the construction of regression dependencies), a method of bilinear interpolation of model estimates from nodes of regular climate model grids into station coordinates, and the use of a quadratic spline to smooth out isolines when delineating the boundaries of vulnerable areas.

As agroclimatic indicators, we used threshold values of the G.T. Selyaninov's hydrothermal coefficient, a complex indicator of moisture availability which also accounts the influence of thermal conditions [15). The indicator has a general form:

$$\mathrm{HTC}_{i,j} = \frac{\Sigma P_{i,j}}{0.1 \cdot \Sigma t_{i,j}},$$

where $\sum P$ is the sum of precipitation, mm, $\sum t$ is the sum of air temperatures, °C for the growing season (time period) with an average daily air temperature above 10 °C, i is the selected year, j is the duration of the selected growing season (time period) .

Threshold HTC values of 1.8, 2.5 and 3.5 met the criteria for the occurrence of varying degrees of waterlogging of crops [16]. For a comparative analysis of the frequency of occurrence of waterlogging effects, HTC values were selected that were in the ranges between the threshold values: $1.8 \leq \mathrm{HTC} < 2.5$, $2.5 \leq \mathrm{HTC} \leq 3.5$ and $3.5 < \mathrm{HTC} \leq 4.5$ and higher. This differentiates the HTC values, classifying them into the categories of pronounced, sharp and very sharp waterlogging of crops.

Results. Based on the analysis of long-term data, it was established that with the observed climate change, the increase in positive air temperatures was

most pronounced in July compared to other warm months. Although July temperatures varied from year to year, there was a general upward trend from 1950 to 2017, with a maximum in 2010. One of the reasons behind this trend is the more frequent penetration of heat waves and their simultaneous intensification. According to studies conducted in Poland between 1951 and 2015, heat waves have intensified since the mid-1990s, spreading to northeastern Europe, with 49% of heat waves occurring in July during the May-September period [17].

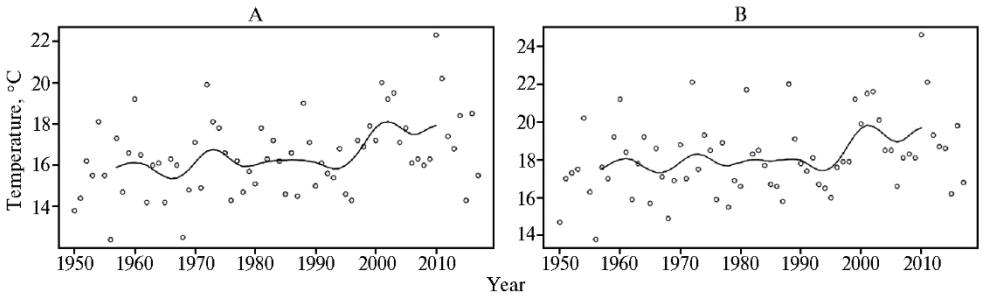

Fig. 1. Time course of average monthly air temperature in July (dots) **and 7-year moving averages** (lines) **in Petrozavodsk** (61.8°N, 34.3°E, altitude 110 m, average taiga subzone) (A) **and Kostroma** (57.7°N; 40.8°E, altitude 125 m above sea level, southern taiga subzone) (B).

A comparison of the course of moving averages (Fig. 1) showed that the average air temperature in July in Petrozavodsk since the late 1990s has been approaching the average air temperature in Kostroma in early decades, reaching the background level of 17.7 °C, which in terms of the amount temperatures ($\sum t_{VII}$) was 549 °C. The increase in heat supply noted at other northern stations also brought heat supply conditions in July closer to those in earlier decades in the territory of more southern stations. Thus, the sum of July temperatures in Vytegra (middle taiga subzone, altitude 55 m above sea level) since the late 1990s has reached the background value $\sum t_{VII}$ = 557 °C. This value was even slightly higher than the background value in Staraya Russa (southern taiga subzone, altitude 24 m above sea level) in early decades $\sum t_{VII}$ = 541 °C. At the eastern mid-taiga stations (Shenkursk and Kotlas), located in depressions of the relief, and in Vologda (southern taiga subzone, altitude 125 m above sea level), the sums of July temperatures since the late 1990s reached background values, $\sum t_{VII}$ = 570 °C, $\sum t_{VII}$ = 564 °C and $\sum t_{VII}$ = 567 °C, respectively. They are comparable to the value of heat supply in early decades in Bryansk (coniferous-broad-leaved subzone, altitude 214 m), for which $\sum t_{VII}$ was about 560 °C.

This spatio-temporal analogy can be considered as a factor contributing to the expansion of plantings of more productive varieties and valuable crops to the north of the traditional areas of their cultivation, given that the total contribution of other warm months to the increase in heat supply is approximately equal to the contribution of July conditions.

The amount of precipitation that falls in July is largely determined by air masses of oceanic origin [18]. Using data from 1950 to 2017 [19], the time course of the July East Atlantic Oscillation (EA) index was plotted and 7-year moving averages were obtained (Fig. 2). The index of this mode of climate variability, like the North Atlantic Oscillation (NAO) index, is calculated from the decomposition of the atmospheric pressure field into orthogonal components [20]. However, unlike the NAO index which characterizes the westerly transport as a whole; the EA index reflects changes in the intensity and number of cyclones [21, 22].

Since the late 1990s, there has been a sharp predominance of positive values of the EA index (Fig. 2), which corresponds to increased cyclonic activity

in a warming climate [21], which is associated with increased surface water temperatures in the North Atlantic. This increase is manifested in the increasing advection of air masses saturated with moisture, which cause heavy rains while simultaneously lowering the air temperature due to cloudy weather.

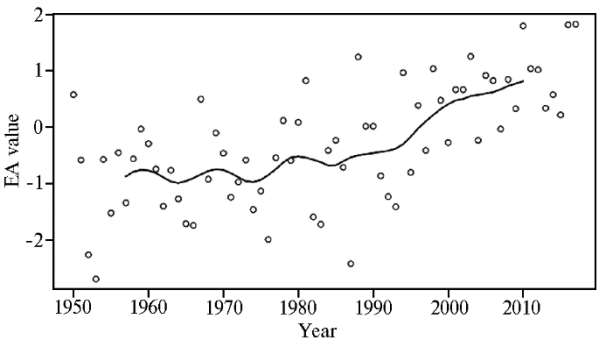

Fig. 2. Time course of July values of the East Atlantic Oscillation (EA) **index and 7-year moving averages** (according to reanalysis data from NCAR — National Center for Atmospheric Research, USA).

The development of convection processes, caused by intense evaporation at rising July temperatures, led to increased thunderstorm activity with heavy rainfall, especially in areas further inland, where daytime maximum July air temperatures were slightly higher.

In a changing climate, record amounts of precipitation were observed both at sums of air temperatures below and above the long-term average (Table 1).

1. Absolute maximum precipitation in July with sums of air temperatures below and above average long-term values, observed in early and late decades in the zone of drained lands of the European Non-Black Earth Region of Russia

Station	Coordinates, height above sea level	$\sum P_{average}$, mm	$\sum t_{average}$, °C	Year	$\sum P_i$, mm	$\sum t_i$, °C	Year	$\sum P_i$, mm	$\sum t_i$, °C
Stations with average long-term annual precipitation of more than 600 mm									
Staraya Russa	58.0°N, 31.2°E, 24 m	80[a]	553	1953	176	546	1990	234[a]	518[a]
Smolensk	54.5°N., 32.9°E, 236 m	92	539	1962	197[a]	515	1998	234[a]	518[a]
Bryansk	53.2°N, 34.2°E, 214 m	90	572	1980	197[a]	477	1999	215	670
Vytegra	61.0°N, 36.4°E, 55 m	80[a]	524	1961	171	521	2017	226	488
Stations with average long-term annual precipitation less than 600 мм									
Vyborg	60.7°N, 28.8°E, 10 m	74	551	1966	150	558	2012	169	564
Kotlas	61.2°N, 46.7°E, 55 m	76	532	1951	187	465	2000	254	623
Kostroma	57.7°N, 40.8°E, 125 m	77	560	1968	184	462	2008	204	567
Shenkursk	62.1°N, 42.9°E, 40 m	70	539	1978	154	459	1998	164	583

Note. $\sum P_{average.}$ and $\sum t_{average.}$ — long-term average sums of precipitation and air temperatures in July for 1945-2017, $\sum Pi$ and $\sum ti$ — sums of precipitation and air temperatures in July in the indicated years; [a] — close values, differing in tenths. Absolute maximums of July precipitation also occurred in Bologoye, Tikhvin, Sortavala, Petrozavodsk, Pskov and Totma.

The absolute maximum precipitation in July in recent decades (1981-2017) exceeded the values of previous decades (1945-1980) (see Table 1). However, in the more moist part of the zone of drained lands, the absolute maximum of July precipitation usually corresponded to a reduced thermal background, while in the less moist part, on the contrary, to an increased thermal background. For stations located in lowlands and lowlands, there was a greater increase in the amount of extreme precipitation compared to stations located at higher elevations.

Based on data obtained at agrometeorological stations in different agricultural subzones, we compared the frequency of years when the amount of precipitation in July exceeded its long-term average amount (taken as 100%) by 1.5 times or more for two periods, 1945-1980 and 1981-2017. These periods were selected based on the characteristics of the anomalies of globally averaged air temperature from 1880 to 2020 [22]. Until the end of the 1970s, the anomalies were negative, with the exception of positive peaks in 1939-1944 (up to +0.2 °C). Since 1981, these peaks have been exceeded and the anomalies have had a sharp increase with a maximum in 2017 of +1.0 °C. This growth was closely linked to the impact of industrialization [23].

For these periods, which differ in the degree of anthropogenic influence

on the climate, the proportion of years with heavy precipitation was calculated using the gradation of their anomalies and differentiation of the zone of drained lands (Table 2).

2. Proportion of years with an anomalous amount of July precipitation during periods differing in the degree of anthropogenic influence on the climate in different agricultural subzones

Natural landscape, agricultural subzone	Anomaly in precipitation from the long-term average value			
	150-200%	201-250%	251-300%	301-350%
	Years 1945-1980			
Middle taiga	39%	17%	3%	
South taiga	53%	25%	3%	6%
Coniferous-broadleaf	22%	3%		
	Years 1981-2017			
Middle taiga	35%	19%	6%	3%
South taiga	54%	22%	11%	3%
Coniferous-broadleaf	14%	8%		

In all natural landscape agricultural subzones, a shift towards an increase in the frequency of years with increasing rainfall was observed. At the same time, for the middle taiga subzone, the appearance of an additional range with the frequency of years with super-heavy precipitation was observed. In general, in the tiered differentiation of landscapes in the zone of drained lands, the largest increase in the percentage of years was noted for stations located in lowlands (height above sea level less than 70 m) and some lowland stations (height above sea level less than 143 m).

The European Environment Agency [24] also records changes in the dynamics of intense precipitation events. It was reported [25] that during the period 1981-2013, compared with the previous period (1951-1980), the number of days with very intense precipitation in Europe increased by 45% due to a sharp increase in the number of such precipitation events in the northern and northeastern parts of Europe. It has also been shown [26, 27] that, under expected climatic conditions, the most pronounced increase in the number of days with very intense precipitation, which is simultaneously contributed by an increasing temperature background, will be characteristic of northeastern Europe in the summer season.

To assess the changing contribution of humidity and thermal factors to the values of the hydrothermal coefficient G.T. Selyaninov, functional analysis was applied. Its essence is that the HTC is presented as a fractional function of two variables, the amount of precipitation and the air temperatures. In a three-dimensional image, this is a surface whose slope is determined by the magnitude of the partial derivatives with respect to the arguments. Horizontal lines are drawn on the surface. Using them, it is possible to count the number of cases where July HTC values fall into the ranges corresponding to the categories of waterlogging. The assessment of the contribution of each of these factors to the value of the HTC, as well as the change in this ratio over periods that differ in the degree of anthropogenic influence on the climate, is carried out by projecting the surface onto the corresponding plane [16].

Using the described method, we identified an increasing time-increasing contribution of the amounts of heavy July precipitation to high values of the HTC in July (HTC_{VII}), which was expressed in a decrease in the residual dispersion, that is, a decrease in the scatter of plotted points that lie closely along a straight line. The changing contribution of sums of air temperatures to high values of the HTC was manifested in a general shift of the cloud of points towards increasing sums of temperatures.

Thus, closer regression connections have been established between increasing amounts of July precipitation with the manifestation of their extremeness and high values of HTC_{VII}. For example, the coefficient of determination

increased from 0.78 to 0.95 for Vytegra, from 0.90 to 0.97 for Smolensk and from 0.89 to 0.92 for Kostroma with a decrease in the standard deviation, respectively, from 0.24 to 0.16, from 0.21 to 0.16 and from 0.28 to 0.15. A similar pattern was revealed for other stations, which increases the reliability of extrapolation of moisture supply conditions.

The effects of waterlogging were also reflected in crop losses. It is convenient to present the latter in the form of relative deviations from the technological trend, described by a parabolic function, based on the characteristics of the dynamics of economic productivity.

Thus, in the excessively wet year of 1998 in the Smolensk region, the loss of winter rye yield relative to the trend level reached 54%. For the Smolensk station (medium loamy gleyed soil), moisture conditions were characterized by the following indicators: total precipitation for the summer months ($\sum P_{VI\text{-}VIII}$) is 459 mm (with $\sum P_{VII}$ = 234 mm), $HTC_{V\text{-}VI}$ = 2.19, HTC_{VII} = 4.64, $HTC_{VII\text{-}VIII}$ = 4.29. The July EA index value was 0.77. In the same year, in the Pskov region, the loss of winter rye yield was 37%. For the Pskov station (sandy loam soil) $\sum P_{VI\text{-}VIII}$ was 457 mm ($\sum P_{VII}$ = 174 mm), $HTC_{V\text{-}VI}$ = 3.32, HTC_{VII} = 3.42, $HTC_{VII\text{-}VIII}$ = 2.79. In the excessively wet 2017 in the Vologda region, the loss of winter rye yield relative to the trend level reached 40%. For the Vytegra station (medium loamy gley soil), the moisture conditions were as follows: $\sum P_{VI\text{-}VIII}$ = 406 mm ($\sum P_{VII}$ = 226 mm); $HTC_{V\text{-}VI}$ = 2.40; HTC_{VII} = 4.61; $HTC_{VII\text{-}VIII}$ = 3.15. The value of the July EA index this year is 1.96.

When comparing indicators throughout the growing season, it was found that excessively wet conditions in July made the most significant contribution to the loss of the final harvest in the zone of drained lands of the European Non-Black Earth Region. The above estimates of crop losses are comparable with estimates obtained earlier at the All-Russian Research Institute of Agricultural Meteorology for the Non-Black Earth Zone as a whole. According to these estimates, prolonged rains and downpours can lead to a reduction in yield vs. its average value for sown grasses by 25-35%, for winter cereals by 40-60%, and for corn hybrids in some years by 80% [28]. It should be noted that on soils with difficult water permeability, super-abundant moisture accumulates in the upper layers, sharply exacerbating root-stem lodging, which simultaneously leads to loss of food quality of the final harvest.

As for the periods without rain, they were short-lived and affected the upper layers of the soil. In the exceptionally warm and dry year of 2010, the loss of the winter rye harvest in the Smolensk and Pskov regions amounted to about 30% of its trend value, which is less than in the excessively wet 1998.

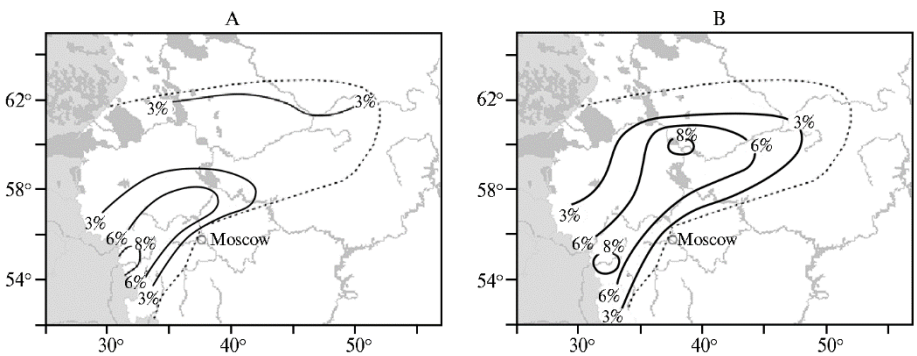

FIg. 3. Frequency of years (%) with very sharp waterlogging of crops in July in the zone of drained lands of the European Non-Black Earth Region of Russia: A — 1945-1980, B — 1981-2017. The dotted line is the border of the zone of drained lands.

The maps (Fig. 3) with isolines display the spatial change in the frequency

of years with very sharp waterlogging of crops in July ($3.5 < HTC_{VII} \leq 4.5$ and above) in the zone of drained lands of the European Non-Black Earth Region. On the maps, this zone (outlined by a dotted line) corresponds to the classification of G.T. Selyaninov zone of excess moisture, for which the HTC values exceed 1.3 [29]. From the north, it is limited by the zone with the occurrence of July frosts, and from the southeast, it is surrounded by territories with conditions of sufficient moisture where the effects of waterlogging were weaker and much less frequent.

Due to local geomorphological features of the surface (undulation of the relief) and the increasing frequency of heavy precipitation, for greater completeness of cartographic estimates, data from additional stations with homogeneous and continuous series of observations from 1981 to 2017 were used: Pushkinskie Gory, Toropets, Staritsa, Babaevo and Kologriv (south -taiga subzone), as well as Trubchevsk (coniferous-broad-leaved subzone).

Comparing the course of isolines for two periods, one can trace how the coverage of agricultural areas, exposed to an increasing risk of very sharp waterlogging of crops, expanded. In 1945-1980, the frequency of years with conditions of very sharp waterlogging, exceeding 6%, occurred only for the southern part of the zone of drained lands. In 1981-2017, regions belonging to the northern part of the southern taiga subzone and the middle non-taiga subzone were added to the territories with such frequency of years.

In general, in the spatial configuration of territories vulnerable to waterlogging, a shift in boundaries in the northeast direction was observed. That is, in recent decades, areas where areas with waterlogged soils and wetlands accounted for a significant proportion of the total land area have been subject to an increasing risk of very sharp waterlogging.

Thus, along with the peculiarities of atmospheric circulation (increased cyclonic activity), the thermal factor in combination with the properties of the underlying surface (the degree of its moisture content) makes an increasingly significant contribution to the aggravation of waterlogging. An important role in the manifestation of the effects of atmospheric waterlogging in relation to the state of crops is also played by soil texture, on which the degree of permeability of atmospheric moisture into soil layers depends.

The assessment of the vulnerability of field farming to the effects of waterlogging for 2021-2030 [30] was based on the results of simulation of time series of air temperature, precipitation and HTC values for July using transitive atmospheric and oceanic general circulation models (AOGCMs). Models developed in Central Europe [31, 32] and Canada [33] were used. The scenario of controlled release of greenhouse gases into the atmosphere was considered as an emission scenario.

Since the models differed in the size of the regular grid cell and in the detail of the description of the physical mechanisms of feedback within the model blocks, this led to a discrepancy in the values of the modeled variables. However, through all selected models, the mid-taiga subzone was identified as more vulnerable to excess atmospheric moisture. At the same time, for this subzone, better comparability was achieved between the real and model-estimated percentage of years with the effects of sudden waterlogging, with a predicted increase in the frequency of such effects for 2021-2030. Thus, in agroclimatic conditions simulated using the MPI-ESM1-2-HR model (Max Planck Institute Earth System Model), the frequency of years with conditions of severe waterlogging ($2.5 \leq HTC_{VII} \leq 3.5$) in Vytegra was 35%. The frequency of such years in Petrozavodsk according to the EC Earth 3 (European community Earth-System Model) model was estimated at 20%. The Can ESM5 model (The Canadian Center for Climate Modeling and Analysis) estimated 18% years for Kotlas. This model also revealed the frequency

of years with conditions of very sharp waterlogging ($HTC_{VII} > 3.5$), which in Kotlas for 2021-2030 amounted to 2%.

Since models are constantly being improved, we also mention the regional model HARMONIE-Climate (the Rossby Center) for Fennoscandinavia, which takes into account convection at the meso-orographic level [34]. Estimates for the near and distant future indicate that the greatest increase in precipitation intensity is expected during the summer season in the northern part of the subregion.

In this regard, adaptation of field farming to climate change acts as a set of measures aimed at effectively managing changes in the thermal regime of the active layer of the atmosphere and the components of the water balance of the underlying surface, including surface layers of soil.

As is known, managing the risks of atmospheric waterlogging of crops is achieved by regulating surface and subsoil runoff through the removal of excess water while simultaneously providing the cultivated soil layers with sufficient moisture to replenish its losses due to evaporation. Therefore, in a changing climate with increasing amounts of heavy rainfall, the management of such risks consists of optimizing reclamation technological and agrotechnical methods, taking into account the characteristics of soils and topography.

In the middle taiga subzone of the European Non-Black Earth Region with frequent soils of heavy mechanical composition, trench drainage should be used, which provides a runoff volume that is on average two times greater than the runoff volume of trenchless drainage [35, 36]. In the lowlands of the southern taiga subzone, which are characterized by a slight slope towards river beds, it is recommended to drain excess rain and storm water over the surface by optimally selecting the width and frequency of furrows [37]. In the coniferous-deciduous subzone, due to the presence of highly dissected topography, measures are needed to prevent increasing water erosion [38]. In floodplains of unregulated watercourses, optimization of the area of diked lands is required to eliminate the risk of flooding of agricultural land and washing out of the alluvial layer as a result of a sharp rise in water levels caused by heavy rains during the summer low-water period [39]. It is also necessary to improve the designs of closed drainage used in all subzones and for all soils based on selecting the optimal distance between drains and increasing their throughput [40].

It should be noted that under conditions of increasing surface and subsurface runoff, the removal of nutrients from agricultural fields increases. To reduce the risk of their entry into water bodies, it is necessary to increase the efficiency of the treatment modules of drainage systems and, if necessary, carry out partial reconstruction of the latter [35].

Managing the productivity of agricultural land and planting crops in changing climatic conditions comes down to proactive adaptation measures aimed at both mitigating the negative effects of atmospheric waterlogging [41] and taking advantage of the increased temperature background [42-44].

Leveling the surface of fields and using lightweight propellers in lowland meadows ensures uniform absorption of moisture into the soil, while simultaneously improving aeration and heat exchange between soil layers [45]. Due to the increasing supply of atmospheric moisture, which is a very weak acidic solution, fertilizer doses and rates of lime application for crops on acidic soils are optimized [46]. Achievements of genetic engineering, selection and seed production are being introduced into agricultural production, including the sowing of high-quality elite seeds to increase the proportion of varieties in crops that are highly resistant to lodging and highly resistant to pathogens that become active in excessively wet conditions [47, 48].

Climate changing promotes more heat-demanding and moisture-loving crops and varieties further north of their traditional areas (for example, buckwheat

crops in the middle part of the southern taiga subzone) and allows the inclusion of more productive plants in forage and grass crop rotations due to an increase in heat supply.

Draining low-lying swamps, the soils of which are rich in organic matter, for the creation of cultivated hayfields and pastures with an expansion of the species composition of high-yielding sown grasses can lead to an increase in productivity, potential for the use of agricultural land and reduces the negative effects of atmospheric waterlogging due to weakened convection.

The intensification of economic activity has an increasing impact not only on the components of the radiation balance of the atmosphere-surface of the Earth system, but also leads to an increasing release of pollutants into the atmosphere in the form of chemical compounds and suspended particles of technogenic origin [49, 50]. They are aerosols, either concentrated solutions or solid particles suspended in the atmosphere. Aerosols have a surface that is necessary for water vapor to condense. Some of them serve as so-called nuclei of water vapor condensation with the subsequent formation of clouds and precipitation. Compliance with environmental standards creates the possibility of targeted control of the quantity and quality of moisture coming from the atmosphere at the regional and intraregional levels.

Better land management and improved internal combustion engines can reduce precipitation. In this case, the entry into the atmosphere of suspended particles of technogenic origin (particles of dust, clay and soot), on which water vapor condenses to form cloud droplets, which through coagulation are converted into droplets of water and fall as rain from water clouds.

Improved recycling technologies provide a reduction in the amount of aerosols in the atmosphere consisting of sulfur and nitrogen oxides, which cause the deposition of acidic compounds, leading to a decrease in soil fertility, damage to the leaf surface of crops, disruption of transpiration and photosynthesis, and weakening of crop immunity to harmful organisms.

It should be noted that the regional adaptation measures we have outlined largely correspond to those used in foreign agricultural regions. For example, such a correspondence in a changing climate occurs for agricultural landscapes similar to the European Non-Black Earth in the middle and northern parts of the Canadian provinces of Quebec and Ontario [51] and northern European countries [52, 53], as well as for excessively humid areas in Central Europe [54-56]. This can serve as the basis for expanding opportunities for adaptation through optimal management decisions.

So, changes in the thermal conditions and the nature of atmospheric moisture are having an increasingly noticeable impact on field cultivation in the zone of drained lands of the European Non-Black Earth Region. An increase in the sum of active temperatures that satisfy the thermal needs of plants contributes to the expansion of more productive varieties of hay and silage crops, as well as the expansion of some moisture-loving forages towards the northern territories. However, the increasing loss of abundant and super-abundant amounts of atmospheric moisture due to an increase in the precipitation of advective and convective origin leads to a sharp waterlogging of crops. Particularly, the crop losses occure which in excessively wet years exceed losses in dry years with a simultaneous reduce in the quality of crop products. In the context of increasing anthropogenic influence on the climate, there is an expansion of agricultural areas that are at risk of sudden waterlogging. The isolines on the map with frequency of years with definite HTC values, indicating the effects of very sharp waterlogging, shows that with ongoing climate changes, vulnerable territories are expanding due to the inclusion of regions located in the northern part of the southern taiga subzone and in the middle

taiga subzone. In the future, the most sensitive to the effects of sudden waterlogging will be the northernmost part of the zone of drained lands, the territories lying north of 60°N latitude and belonging to the middle taiga subzone. This fact can be explained by the presence of spaces with waterlogged soils and wetlands. Increased frequency of high temperatures leads to increased evaporation from large areas, followed by the formation of convective thunderclouds and heavy rainfall.

REFERENCES

1. Core Writing Team. *Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. R.K. Pachauri, L.A. Meyer (eds.). IPCC, Geneva, Switzerland, 2014.
2. *Climate extremes and their implications for impact and risk assessment*. J. Sillman, S. Sippel, S. Russo (eds.). Elsevier, Amsterdam, 2020.
3. Vogel E., Meyer R. Chapter 3. Climate change, climate extremes, and global food production — adaptation in the agricultural sector. In: *Resilience. The science of adaptation to climate change*. Z. Zommers, K. Alverson (eds.). Elsevier, 2018: 31-49 (doi: 10.1016/b978-0-12-811891-7.00003-7).
4. Regoto P., Dereszynski C., Chou S.C., Bazzanela A.C. Observed changes in air temperature and precipitation extremes over Brazil. *International Journal of Climatology*, 2021, 41(11): 5125-5142 (doi: 10.1002/joc.7119).
5. Dong Z., Wang L., Sun Y., Hu T., Limsakul A., Singhruck P., Pimonsree S. Heatwaves in Southeast Asia and their changes in a warmer world. *Earth's Future*, 2021, 9(7): e2021EF001992 (doi: 10.1029/2021EF001992).
6. Walsh K.J.E., Camargo S.J., Knutson T.R., Kossin J., Lee T.-C., Murakami H., Patricola C. Tropical cyclones and climate change. *Tropical Cyclone Research and Review*, 2019, 8(4): 240-250 (doi: 10.1016/j.tcrr.2020.01.004).
7. Rosa M.B., Satyamurty P.I., Ferreira N.J., Silva L.T. A comparative study of intense surface cyclones off the coasts of southeastern Brazil and Mozambique. *International Journal of Climatology*, 2019, 39(8): 3523-3542 (doi: 10.1002/joc.6036).
8. Burke C., Stott R. Impact of anthropogenic climate change on the East Asian summer monsoon. *Journal of Climate*, 2017, 30(14): 5205-5220 (doi: 10.1175/JCLI-D-16-0892.1).
9. Kjellström E., Hansen F., Belušić D. Contributions from changing large-scale atmospheric conditions to changes in scandinavian temperature and precipitation between two climate normals. *Tellus A: Dynamic Meteorology and Oceanography*, 2022, 74(1): 204-221 (doi: 10.16993/tellusa.49205).
10. Pepler A.S., Dowdy A.J., Hope P. The differing role of weather systems in southern Australian rainfall between 1979-1996 and 1997-2015. *Climate Dynamics*, 2021, 56: 289-2302 (doi: 10.1007/s00382-020-05588-6).
11. *Landshaftnaya karta SSSR* /Pod redaktsiey A.G. Isachenko [Landscape map of the USSR. A.G. Isachenko(ed.)]. Moscow, 1988 (in Russ.).
12. *Pochvennaya karta RSFSR* /Pod redaktsiey V.M. Fridland [Soil map of the RSFSR. V.M. Fridland (ed.)]. Moscow, 1988 (in Russ.).
13. Kiryushin V.I. *Teoriya adaptivno-landshaftnogo zemledeliya i proektirovanie agrolandshaftov* [Theoretical basis of adaptive landscape agriculture and design of agricultural landscapes]. Moscow, 2011 (in Russ.).
14. Kiryushin V.I. *Kontseptsiya razvitiya zemledeliya v Nechernozem'e* [The concept of the development of agriculture in the Non-Chernozem region]. St. Petersburg, 2020 (in Russ.).
15. Selyaninov G.T. V sbornike: *Voprosy agroklimaticheskogo rayonirovaniya SSSR* [In: Agro-climatic zoning of the USSR]. Moscow, 1958: 7-13 (in Russ.).
16. Nikolaev M.V. *Izvestiya Russkogo geograficheskogo obshchestva*, 2018, 150(6): 1-14 (doi: 10.1134/S0869607118060010) (in Russ.).
17. Wibig J. Heat waves in Poland in the period 1951-2015: trends, patterns and driving factors. *Meteorology, Hydrology and Water Management*, 2018, 6(1): 37-45 (doi: 10.26491/mhwm/78420).
18. Murav'ev A.V., Kulikova I.A. *Meteorologiya i gidrologiya*, 2011, 5: 5-16 (in Russ.).
19. *European Climate Assessment & Dataset KNMI Climate Explorer (climatic indices)*. Available: https://www.ecad.eu/. No date.
20. Franzke C., Feldstein S.B. The continuum and dynamics of Northern Hemisphere teleconnection patterns. *Journal of Atmospheric Science*, 2005, 62(9): 3250-3267 (doi: 10.1175/JAS3536.1).
21. Nesterov E.S. *Meteorologiya i gidrologiya*, 2009, 12: 32-40 (in Russ.).
22. Nesterov E.S. *Severoatlanticheskoe kolebanie: atmosfera i okean* [North Atlantic oscillation: atmosphere and ocean]. Moscow, 2013 (in Russ.).
23. National Aeronautics and Space Administration Goddard Institute for Space Studies. *GISS surface temperature analysis* (GISTEMP v4). Available: http:/data.giss.nasa.gov/gistemp. No date.

24. European Environment Agency. *Wet and dry — heavy precipitation and river floods*. Available: https://www.eea.europa.eu/publications/europes-changing-climate-hazards-1/wet-and-dry-1/wet-and-dry-heavy. No date.
25. Fischer E.M., Knutti R. Observed heavy precipitation increase confirms theory and early models. *Nature Climate Change*, 2016: 986-991 (doi: 10.1038/nclimate3110).
26. Hawcroft M., Walsh E., Hodges K., Zappa G. Significantly increased extreme precipitation expected in Europe and North America from extratropical cyclones. *Environmental Research Letters*, 2018, 13(12): 124006 (doi: 10.1088/1748-9326/aaed59).
27. Rajczak J., Schär C. Projections of future precipitation extremes over Europe: a multimodel assessment of climate simulations. *Journal of Geophysical Research: Atmospheres*, 2017, 122(20): 773-800 (doi: 10.1002/2017JD027176).
28. Gringof I.G., Kleshchenko A.D. *Osnovy sel'skokhozyaystvennoy meteorologii. Tom 1* [Fundamentals of agricultural meteorology. Volume 1]. Obninsk, 2011: 601-609 (in Russ.).
29. Selyaninov G.T. V sbornike: *Rastenievodstvo SSSR. Tom1* [In: Crop production of the USSR. Volume 1]. Moscow, 1933: 1-15 (in Russ.).
30. Nikolaev M.V. *Izvestiya Russkogo geograficheskogo obshchestva*, 2021, 153(4): 47-67 (doi: 10.31857/S0869607121040030) (in Russ.).
31. Massonnet F., Ménégoz M., Acosta M., Yepes-Arbós X., Exarchou E., Doblas-Reyes F.J. Replicability of the EC-Earth3 Earth system model under a change in computing environment. *Geosci. Model Dev.*, 2020, 13: 1165-1178 (doi: 10.5194/gmd-13-1165-2020).
32. Müller W.A., Jungclaus J.H., Mauritsen T., Baehr J., Bittner M., Budich R., Bunzel F., Esch M., Ghosh R., Haak H., Ilyina T., Kleine T., Kornblueh L., Li H., Modali K., Notz D., Pohlmann H., Roeckner E., Stemmler I., Tian F., Marotzke J. A higher-resolution version of the Max Planck Institute Earth System Model (MPI-ESM1.2-HR). *Journal of Advances in Modeling Earth Systems*, 2018, 10(7): 1383-1413 (doi: 10.1029/2017MS001217)
33. Swart N.C., Cole J.N.S., Kharin V.V., Lazare M., Scinocca J.F., Gillett N.P., Anstey J., Arora V., Christian J.R., Hanna S., Jiao Y., Lee W.G., Majaess F., Saenko O.A., Seiler C., Seinen C., Shao A., Sigmond M., Solheim L., von Salzen K., Yang D., Winter B. The Canadian Earth System Model version 5 (Can ESM5.03) *Geosci. Model Dev.*, 2019, 12(11): 4823-4873 (doi: 10.5194/gmd-12-4823-2019).
34. Lind P., Belušić D., Médus E., Dobler A., Pedersen R.A., Wang F., Matte D., Kjellström E., Landgren O., Lindstedt D., Christensen O.B., Christensen J.H. Climate change information over Fenno-Scandinavia produced with a convection-permitting climate model. *Climate Dynamic*s, 2023 (doi: 10.1007/s00382-022-06589-3).
35. Shtykov V.I., Yanko Yu.G., Uskov I.B., Nikolaev M.V. *Sbornik materialov XVIII Mezhdunarodnogo ekologicheskogo foruma «Den' Baltiyskogo morya»* [Proceedinfs of the XVIII International Environmental Forum "Baltic Sea Day"]. St. Petersburg, 2017: 63-64 (in Russ.).
36. Harris L.I., Roulet N.T., Moore T.R. Drainage reduces the resilience of a boreal peatland. *Environ. Res. Commun.*, 2020, 2: 065001 (doi: 10.1088/2515-7620/ab9895).
37. Uskov I.B., Nikolaev M.V., Yanko Yu.G. *Sbornik materialov XVIII Mezhdunarodnogo ekologicheskogo foruma «Den' Baltiyskogo morya»* [Proceedinfs of the XVIII International Environmental Forum "Baltic Sea Day"]. St. Petersburg, 2016: 49-51 (in Russ.).
38. Clubb F.J., Mudd S.M., Attal M., Milodowski D.T., Grieve S.W.D. The relationship between drainage density, erosion rate, and hilltop curvature: implications for sediment transport processes. *J. Geophys. Res. Earth Surf.*, 2016, 121(10): 1724-1745 (doi: 10.1002/2015JF003747).
39. Markov B.S. *Melioratsiya poym Nechernozemnoy zony* [Reclamation of the floodplains of the Non-Chernozem zone]. Moscow, 1973 (in Russ.).
40. Abendroth L.J., Chighladze G., Frankenberger J.R., Bowling L.C., Helmers M.J., Herzmann D.E., Jia X., Kjaersgaard J., Pease L.A., Reinhart B.D., Strock J., Youssef M. Paired field and water measurements from drainage management practices in row-crop agriculture. *Sci. Data.*, 2022, 9: 257 (doi: 10.1038/s41597-022-01358-7).
41. Castellano M.J., Archontoulis S.V., Helmers M.J., Poffenbarger H.J., Six J. Sustainable intensification of agricultural drainage. *Nat. Sustain.*, 2019, 2: 914-921 (doi: 10.1038/s41893-019-0393-0).
42. Ceglar A., Zampieri M., Toreti A., Dentener F. Observed northward migration of agro-climate zones in Europe will further accelerate under climate change. *Earth's Future*, 2019, 7(9): 1088-1101 (doi: 10.1029/2019EF001178).
43. Peltonen-Sainio P., Jauhiainen L. Large zonal and temporal shifts in crops and cultivars coincide with warmer growing seasons in Finland. *Regional Environmental Change*, 2020, 20: 89 (doi: 10.1007/s10113-020-01682-x).
44. Trnka M., Balek J., Brázdil R., Dubrovský M., Eitzinger J., Hlavinka P., Chuchma F., Možný M., Prášil I., Růžek P., Semerádová D., Štěpánek P., Zahradníček P., Žalud Z. Observed changes in the agroclimatic zones in the Czech Republic between 1961 and 2019. *Plant Soil Environ.*, 2021, 67(3): 154-163 (doi: 10.17221/327/2020-PSE).
45. Aryal J.P., Sapkota T.B., Khurana R., Khatri-Chhetri A., Rahut D.B., Jat M.L. Climate change and agriculture in South Asia: adaptation options in smallholder production systems. *Environ.*

Dev. Sustain., 2020, 22: 5045-5075 (doi: 10.1007/s10668-019-00414-4).
46. Yakushev V.P., Osipov A.I. *Izvestiya Sankt-Peterburgskogo gosudarstvennogo agrarnogo universiteta*, 2013, 30: 68-72 (in Russ.).
47. Chesnokov Yu.V., Kocherina N.V., Kosolapov V.M. *Molekulyarnye markery v populyatsionnoy genetike i selektsii* [Molecular markers in population genetics and breeding]. Moscow, 2019 (in Russ.).
48. Arkhipov M.V., Gusakova L.P., Velikanov L.P., Vilichko A.K., Zheludkov A.G., Alferov V.B. *Metodika kompleksnoy otsenki biologicheskoy i khozyaystvennoy prigodnosti semennogo materiala (metodicheskie ukazaniya)* [A comprehensive assessment of the biological and economic suitability of seed material (guidelines)]. St. Petersburg, 2013 (in Russ.).
49. Sonwani S., Maurya V. Chapter 7. Impact of air pollution on the environment and economy. In: *Air pollution: sources, impacts and controls*. P. Saxena, V. Naik (eds.). CABI Publisher, Oxford, U.K., 2019: 113-135 (doi: 10.1079/9781786393890.0113).
50. Walker T.R. Effectiveness of the national pollutant release inventory as a policy tool to curb atmospheric industrial emissions in Canada. *Pollutants*, 2022, 2(3): 289-305 (doi: 10.3390/pollutants2030019).
51. *Canada in a changing climate: national issues report*. F.J. Warren, N. Lulham (eds.). New York, 2021 (doi: 10.4095/328384).
52. Woods B.A., Nielsen J., Pedersen A.B., Kristofersen N. Farmers' perceptions of climate change and their likely responses in Danish agriculture. *Land Use Policy*, 2017, 65: 109-120 (doi: 10.1016/j.landusepol.2017.04.007).
53. Ibrahim M.A., Johansson M. Attitudes to climate change adaptation in agriculture – A case study of Öland, Sweden. *Journal of Rural Studies*, 2021, 86: 1-15 (doi: 10.1016/j.jrurstud.2021.05.024).
54. Dalla Marta A., Eitzinger J., Kersebaum K.C., Todorovic M., Altobelli F. Assessment and monitoring of crop water use and productivity in response to climate change. *The Journal of Agricultural Science*, 2018, 156(5): 575-576 (doi: 10.1017/S002185961800076X).
55. Pohanková E., Hlavinka P., Kersebaum K.-C., Rodríguez A., Balek J., Bednařík M., Dubrovský M., Gobin A., Hoogenboom G., Moriondo M., Nendel C., Olesen J.E., Rötter R.P., Ruiz-Ramos M., Shelia V., Stella T., Hoffmann M.P., Takáč J., Eitzinger J., Dibari C., Ferrise R., Bláhová M., Trnka M. Expected effects of climate change on the production and water use of crop rotation management reproduced by crop model ensemble for Czech Republic sites. *European Journal of Agronomy*, 2022, 134: 126446 (doi: 10.1016/j.eja.2021.126446).
56. Lalić B., Firanj Sremac A., Dekić L, Eitzinger J., Perišić D. Seasonal forecasting of green water components and crop yields of winter wheat in Serbia and Austria. *The Journal of Agricultural Science*, 2018, 156(5): 645-657 (doi: 10.1017/S0021859617000788).

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 75-86
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.12:631.671:581.113:58.1/.2

doi: 10.15389/agrobiology.2023.1.75eng
doi: 10.15389/agrobiology.2023.1.75rus

LEAF WATER-USE EFFICIENCY PARAMETERS OF *Fagopyrum esculentum* Moench PLANTS AS INFLUENCED BY ENDOGENOUS AND EXOGENOUS FACTORS

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The authors declare no conflict of interests
Acknowledgements:
Supported financially from the Russian Science Foundation, the grant No. 22-26-00041, https://rscf.ru/project/22-26-00041/ within the framework of the thematic plan of the Central Collective Use Center of the Oryol State Agrarian University "Plant genetic resources and their use" under a joint program with breeders of the Federal Scientific Center of Legumes and Cereals
Final revision received May 31, 2022 g
Accepted July 11, 2022

Abstract

Sustainable development of contemporary agriculture is hampered by a number of facotors, one of which is the increasing aridization of the planet's climate. In this regard, the water use efficiency (WUE) of plants is of great importance. In this paper the data of long-term field experiments are submitted which illustrate the influence of photosynthesis rate, transpiration rate, stomatal conductance and, also, growing conditions on the buckwheat plant WUE. The purpose of the study is to identify the specific peculiarities of WUE of common buckwheat (*Fagopyrum esculentum* Moench) plants with regard to photosynthesis and production processes. A total of 22 varieties of common buckwheat (K-406 and K-1709 — local populations; Kalininskaya, Bogatyr, Shatilovskaya 5 — old varieties; Dikul, Dozhdik, Demetra, Devyatka, Design — modern commercial varieties; Bashkirskaya krasnostebelnaya, Batyr, Usha, Chatyr-Tau, Inzerskaya, Design 2, P 66, P 69, P 70, P 84, P 85, SPR 52 — varieties which are perspective for different conditions) were examined. Plants were grown on the experimental field of buckwheat breeding lab of FSC of Legumes and Groats Crops (Orel District, Orel Province) in 2010-2015. A plot area was 10 m^2. The plots' locations were random, with fourfold replication. Photosynthesis rate (PI), transpiration intensity (TI), and stomatal conductance were measured according to the original method of Heinz Walz GmbH (Germany) using a GFS-3000 FL portable gas analyzer. The assessment was conducted on 5-7 plants typical for the genotype, growing in the middle of the plot, which leaves were not damaged by pests and diseases. The measurements were carried out in real time at the main growth phases (branching, flowering + 10 days, flowering + 20 days, flowering + 30 days) from 7 AM to 7 PM with a frequency of 3 hours. In the measuring chamber of the device the light intensity was maintained at 1000 μmol photons $\cdot m^{-2} \cdot s^{-1}$, the air temperature was 25 °C. The measurements were performed on the 3rd leaves from the top. WUE was calculated as the ratio of the values of the photosynthesis and transpiration intensities. The grain yield from each plot was evaluated both by direct weighing and by structural analysis of plants. As a result of the research, it was found that the buckwheat WUE values significantly depend on both growing conditions and hereditary characteristics. Depending on the weather conditions of the growing season, the WUE varied from 1.03 to 2.08 μmol CO_2/mmol H_2O. Its highest value (2.08 μmol CO_2/mmol H_2O) was noted in 2012 when the weather was relatively favorable for plant growth and development. In ontogeny, the maximum efficiency of water use for the photosynthesis was recorded at branching (2.43 μmol CO_2/mmol H_2O on average) and mass filling of seeds (1.78 μmol CO_2/mmol H2O/m2s), and the lowest WUE values were during budding and flowering (1.17 μmol CO_2/mmol H_2O on average). In the daytime, the most CO_2 molecules per unit of evaporated water were assimilated from 9 AM to 11 AM when the highest intensity of leaf photosynthesis and moderate transpiration activity were observed. The correlation coefficient between WUE and the intensity of leaf photosynthesis was

positive ($r = 0.69$, $p < 0.05$), and it was negative between WUE and the intensity of transpiration ($r = -0.89$, $p < 0.05$). Buckwheat varieties significantly differ in terms of WUE values. As a result of breeding, the value of WUE increased (P = 0,95) by 20.5 % on average, which was due to an increase in PI by 29.0 %, TI by 7.9 %, and stomatal conductance by 18.1 % on average.

Keywords: *Fagopyrum esculentum*, buckwheat, rate of photosynthesis, rate of transpiration, water-use efficiency, crop yield

Modern agriculture is low sustainable, including due to increasing climate aridization [1, 2]. In this regard, the evaporation of water by plants [3-5], which depend on both exogenous and endogenous factors of growth and development [6-8], are important. The moisture regime and air temperature as exogenous factors [9] and photosynthesis and leaf transpiration as endogenous factors, due to their physiological position in the production process and the plant's defense system [10, 11], play the determining role. Many researchers point to the importance of studying the efficiency of water use by plant leaves [12, 13].

Water use efficiency (WUE) is usually assessed using three indicators. This is the assimilation-transpiration ratio (ATR), i.e., the ratio of the current values of the intensity of photosynthesis to transpiration; transpiration productivity (TP), i.e., the dry biomass formed per unit of water transpired by the plant; isotope discrimination of carbon ($\Delta^{13}C$) that characterizes ATR in a leaf [10]. Despite certain differences, the basis of all these indicators is the conjugacy of gas and water exchange processes at different levels of plant organization.

When studying the relationships between the parameters that determine WUE and drought resistance, QTL (quantitative trait loci) associated with water use efficiency were established in 120 F_2 wheat hybrids, and it was shown that biomass accumulation is positively correlated with ATR, leaf specific surface density (SSD) and trancpiration intencity (TI), but negatively with $\Delta^{13}C$ [9].

The study of these issues is also relevant for such a popular crop on the world food market as buckwheat which acreage expansion is largely hampered by the low plant adaptability [14]. It is known that buckwheat consumes 2-3 times more water than millet and other crops [15-17]. Moreover, during selection, plant resistance to moisture limitation not only does not increase, but has a pronounced tendency to decrease [18]. In modern buckwheat varieties, grain yield sharply decreases in dry and hot weather [19].

In this work, we for the first time obtained data on the efficiency of water use by the leaves of buckwheat plants depending on the growth phase, time of day and weather conditions of the growing season, and also described the nature of its relationship with the intensity of photosynthesis, transpiration and seed productivity.

The purpose of the study is to identify species-specific characteristics of water consumption during photosynthesis and the production process in common buckwheat (*Fagopyrum esculentum* Moench) plants.

Materials and methods. The objects of study were 22 varieties of common buckwheat. Of these, 10 were created in the course of selection from local populations to the best modern zoned varieties. These are K-406 and K-1709 (local populations); Kalininskaya, Bogatyr, Shatilovskaya 5 (old varieties); Dikul, Dozhdik, Demeter, Devyatka, Design (modern zoned varieties). Other 12 varieties were studied as promising for different conditions: Bashkir Krasnostebelnaya, Batyr, Usha, Chatyr-Tau, Inzerskaya, Design 2, R 66, R 69, R 70, R 84, R 85, SPR 52.

The studied varieties were grown in the selection crop rotation of the Federal Research Center for Leguminous and Cereal Crops (Oryol Province, Oryol District) in 2010-2015 on 10 m^2 plots in 4 repetitions using a randomized design. Crop care and harvesting were carried out according to methodological recommendations for the region.

Additionally, for five buckwheat varieties (K-406, K-1709, Bogatyr, Dikul, Dozhdik), two series of model pot experiments were conducted to study the daily dynamics of photosynthesis intensity, transpiration intensity and water use efficiency. For this purpose, plants were grown in special 9-liter pots (5 plants typical for the variety in each pot, 4-fold repetition) at soil moisture of 30 and 70% of the full moisture capacity.

In the pot tests, the analyzed indicators were determined for each variety sample. In small-plot experiments, 5-7 plants typical for the genotype and not damaged by pests or diseases were examined. Measurements were carried out in real time during the main growth phases (branching, flowering + 10 days, flowering + 20 days, flowering + 30 days) on the physiologically mature 3rd leaf from the top of of the main stem.

The photosynthesis intensity (PI), the transpiration intensity (IT) and stomatal conductance (SC) were assessed by the original method of Heinz Walz GmbH (Germany) using a portable gas analyzer GFS-3000 FL. Measurements were carried out from 7AM to 7PM, with 3-hour interval, in the pot test, and from 8AM to 11AM in the small-plot experiment. In the measuring chamber of the device, the light intensity was maintained at 1000 μmol photons $\cdot m^{-2} \cdot s^{-1}$, the air temperature at 25 °C.

WUE was determined by calculating the ratio of the current values of photosynthesis intensity to the values of transpiration intensity [10, 20].

Grain yield was assessed for each plot of the variety by weighing (VK-600 scales, ZAO Massa-K, Russia) and by structural analysis of 15 plants in 4 repetitions.

Statistical processing (variance and correlation analysis) was carried out using the Microsoft Excel 2013 and Statistica v. 10.0 software packages (StatSoft, Inc., USA). The significance of differences was determined by Student's *t*-test at P = 0.95. Mean values (*M*) and standard deviations (±SD) were calculated.

Results. The years of investigations differed in weather conditions. The year 2010 was the most extreme, throughout of growing season, there was 54.9% less precipitation, and the average monthly air temperature was 5.5 °C higher than the long-term average. The conditions in 2012 turned out to be the most comfortable for buckwheat plants: the amount of precipitation and the average monthly temperature during the growing season were close to the long-term averages. The weather conditions in 2011 and 2013-2015 during certain growth phases were harsh, but not extreme.

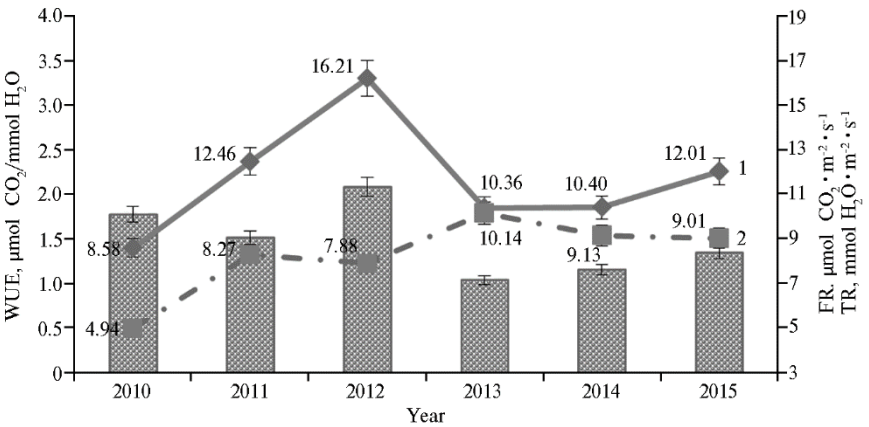

Fig. 1. Water use efficiency (WUE) (diagram)**, photosynthesis rate** (FR) (1) **and transpiration rate** (TR) (2) **in the leaves of common buckwheat (*Fagopyrum esculentum* Moench) in different years during the flowering phase + 10 days** (average of 22 variety samples, for each variety sample $n = 5$, $M \pm SD$; Federal Research Center of Leguminous and Cereal Crops, Oryol Province, Oryol District). For descriptions of variety samples, see the Materials and methods section.

It has been found out that in buckwheat plants, as in other agricultural crops [11, 21, 22], the efficiency of water use is closely related to weather conditions. Its value during the years of research varied from 1.03 to 2.08 μmol CO_2/mmol H_2O. The highest efficiency of water use for photosynthesis was observed in 2012, the lowest in 2013-2015. In 2012, the WUE value was higher (at $P = 0.95$) by an average of 14.9% compared to 2010, by 27.4% vs. 2011, by 50.5% vs. 2013, by 44.7% vs. 2014, and by 35.6% vs. 2015. The influence of weather conditions during the growing season on the efficiency of water use was mediated through the impact on the processes of transpiration and photosynthesis (Fig. 1).

The correlation coefficient between WUE and the leaf photosynthesis intensity was positive ($r = +0.69$, $p < 0.05$), and between WUE and the transpiration intensity negative ($r = -0.89$, $p < 0.05$). Therefore, in 2012, high water use efficiency was due to increased photosynthesis intensity and moderate transpiration activity. There were favorable weather conditions for the growing of buckwheat plants, so there was no particular need to spend a large amount of energy on water evaporation, organic matter was mainly formed and the yield was the highest, the 3.5 t/ha [18, 19].

In the more severe weather conditions of 2013-2015, plants needed protection from overheating, which was ensured by increased transpiration to the detriment of photosynthesis: the efficiency of water use to maintain photosynthesis in these years was reduced compared to more favorable conditions on average by 34.6%, and seed productivity by 25.4% ($P = 0.95$).

However, in stressful situations, the plant physiological responses was somewhat different. In 2010, under pronounced soil and air drought (hydrothermal coefficient HTC = 0.36), in order to avoid cell dehydration and maintain their viability, plants were forced to use water and energy very sparingly. This was achieved due to the low rate of transpiration and photosynthesis, which resulted in a decrease in the efficiency of the production process as a whole. In model pot tests, when soil moisture decreased from 70 to 30% of the total moisture capacity, the photosynthesis intensity of buckwheat plants decreased 4.4-fold, the transpiration intensity by more than 35%, and productivity by 43.6% ($P = 0.95$).

As a result, in 2010, the efficiency of water use in plants of all varieties was above average, and the total and seed productivity was less on average by 66.6% ($P = 0.95$) compared to less extreme growing conditions (Table 1).

1. Water use efficiency (WUE, μmol CO_2/mmol H_2O)**, seed weight and dry weight of above-ground organs in 11 varieties of common buckwheat (*Fagopyrum esculentum* Moench) in different years** (M±SD; Federal Research Center for Leguminous and Cereal Crops, Oryol Province, Oryol District)

Paramter	Year					
	2010	2011	2012	2013	2014	2015
WUE	1.77±0.24	1.51±0.12	2.08±0.26	1.03±0.08	1.15±0.10	1.34±0.14
Dry mass of aboveground organs, g/plant	3.20±0.40	5.10±0.65	6.70±0.13	6.04±0.55	5.09±0.46	5.38±0.49
Seed weight, g/plant	0.52±0.17	1.41±0.45	1.77±0.29	1.60±0.48	1.54±0.37	1.51±0.35

N o t e. The WUE values are given for the period of fruits formation (flowering phase + 10 days); for their calculation, we used data obtained from 8.00 a.m. to 11.00 a.m. (for each variety $n = 5$). The dry weight of above-ground organs was determined at the stage of harvest ripeness (for each variety, 15 plants were analyzed in 4 replicates). For descriptions of variety samples, see the Materials and methods section.

A sharp decrease in the intensity of photosynthesis during drought and, as a consequence, productivity can be caused by both an insufficient supply of water and nutrients to the leaves due to low transpiration activity, and a disruption in the functioning of the reaction centers of photosystems [23].

The efficiency of water use by buckwheat plants varied during ontogenesis from 1.17 to 2.43 μmol CO_2/mmol H_2O. The value was highest at branching stage

and during mass filling of seeds. WUE during vegetative growth period was on average 52 and 34% higher compared to the stages flowering + 10 days and flowering + 30 days. The variability of the indicator was closely related to the photosynthetic and transpiration activities of the leaves.

An increase in the intensity of photosynthesis was observed mainly before the flowering phase + 20 days, and then sharply decreased, while the transpiration rate was the highest during the flowering phase + 10 days, and during the period of vegetative growth and mass filling of seeds (flowering phase + 20 days) it was minimal (4.31 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$) or moderate (5.92 mmol $H_2O \cdot m^{-2} \cdot s^{-1}$), which correspondingly affected the efficiency of water use by plants (Fig. 2).

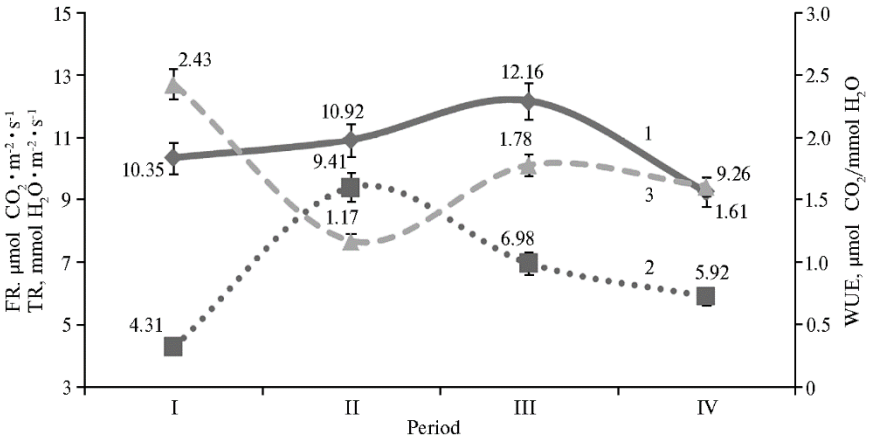

Fig. 2. Photosynthesis rate (FR) (1)**, transpiration rate** (TR) (2) **and water use efficiency** (WUE) (3) **in the leaves of common buckwheat (*Fagopyrum esculentum* Moench) accessions K-406, K-1709, Kalininskaya, Bogatyr, Shatilovskaya 5, Demeter, Dozhdik, Dikul, Inzerskaya, Devyatka, Design in different phases of growth and development:** I — vegetative growth, II — flowering + 10 days, III — flowering + 20 days, IV — flowering + 30 days (average of 11 variety samples, for each variety sample for each variety sample $n = 5$, $M \pm SD$; Federal Research Center of Leguminous and Cereal Crops, Oryol Province, Oryol District, 2013-2015). For descriptions of variety samples, see the Materials and methods section.

The manifestation of a certain disconnect between the intensity of photosynthesis and transpiration is explained by the fact that CO_2 and H_2O molecules use one route into the leaf, through the stomata. However, the diffuse gradient driving transpiration is approximately 50 times greater than that driving carbon dioxide uptake, which is reflected in the efficiency of water use by plants during ontogeny [10, 24). In crops, it is typically low because plants lose nearly 100 times more water than they assimilate equivalent units of carbon through photosynthesis. To increase crop production, it is important to pay attention to increasing WUE in cultivated plant species [11]. An analysis of the water use efficiency of soybean plants showed that a 1% increase in WUE in leaf blades at the field scale leads to an increase in WUE by approximately 10% [25]. It is proposed to use this indicator as a secondary criterion for selecting crop genotypes based on seed yield [26]. It has been established that, in absolute value, the closest to the WUE indicator in field conditions is the so-called instantaneous (i) measured WUE (WUEi) of the leaf calculated from the respiration value [27].

During daylight hours, the highest rate of CO^2 assimilation per unit amount of water evaporated by a unit of leaf surface in buckwheat plants was observed in the pre-lunch time (from 9AM to 11AM), with an increase in solar radiation, when the air temperature is still low (20-25 °C). During this period, there was a peak in photosynthetic activity and moderate transpiration. After 11AM, WUE values decreased noticeably, primarily due to a pronounced drop in

photosynthetic activity and increased transpiration due to high air temperature and insolation (Fig. 3). Obviously, in the afternoon, the transpiration process in the leaves is aimed to a greater extent at preventing overheating of the above-ground organs, which reduces the possibilities of photosynthesis. A decrease in WUE at midday was also observed in plants of such a heat-loving crop as soybean, which is also explained by increased transpiration rates and an increase in PAR (photosynthetically active radiation) [27].

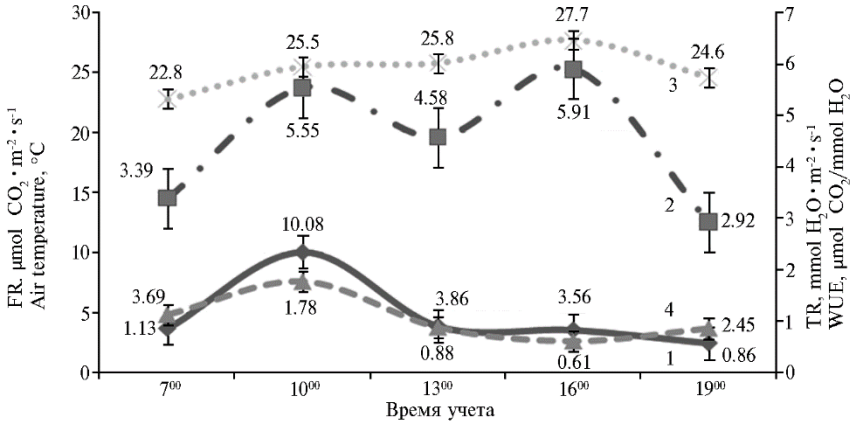

Fig. 3. Daily dynamics of photosynthesis rate (PR) (1)**, transpiration rate** (TR) (2)**, air temperature** (3) **and water use efficiency** (WUE) (4) in the leaves of common buckwheat (*Fagopyrum esculentum* Moench) varieties K-406, K-1709, Bogatyr, Dikul, Dozhdik during the flowering phase + 20 days according to two series of vegetation tests (n = 5, M±SD; Federal Research Center of Leguminous and Cereal Crops, Oryol Province, Oryol District, 2014-2015).

2. Photosynthesis rate, transpiration rate, stomatal conductivity and water use efficiency in 10 local populations and varieties of common buckwheat (*Fagopyrum esculentum* Moench) bred during different periods at lowering + 20 days (for each sample n = 5, M±SD; Federal Scientific Center for Grain Legumes and Cereal Crops, Oryol Province, Oryol District, 2013-2015)

Variety sample	Photosynthesis rate, μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$	Transpiration rate, mmol $H_2O \cdot m^{-2} \cdot s^{-1}$	Stomatal conductance, mol $H_2O \cdot m^{-2} \cdot s^{-1}$	Water use efficiency, μmol CO_2/mmol H_2O
Local populationsи (Oryol Province)				
K-406	9.55±0.30	6.20±0.21	0.452±0.025	1.54±0.28
K-1709	11.53±0.29	7.28±0.20	0.441±0.023	1.58±0.30
Varieties bred in 1930-1960				
Kalininskaya	11.76±0.31	7.61±0.22	0.447±0.024	1.55±0.24
Bogatyr	12.35±0.27	7.37±0.19	0.465±0.025	1.68±0.10
Shatilovskaya 5	9.76±0.25	6.47±0.17	0.472±0.027	1.51±0.31
Varieties bred in 1990-2010				
Demeter	13.97±0.29	8.07±0.17	0.551±0.027	1.73±0.27
Rain	13.28±0.27	7.36±0.19	0.564±0.026	1.80±0.28
Dikul	15.58±0.28	7.84±0.21	0.553±0.025	1.99±0.44
Devyatka	13.22±0.30	6.49±0.20	0.480±0.024	2.04±0.51
Design	11.96±0.31	6.58±0.22	0.492±0.026	1.82±0.36
LSD_{05}	0.31	0.22	0.027	0.52

It is important to note that as a result of selection, the efficiency of water use by buckwheat plants has increased significantly, primarily due to an increase in the intensity of photosynthesis during the formation and mass filling of seeds. During the period of selection of buckwheat crops from local populations to the best modern zoned varieties, the values of WUE of plants became higher (P = 0.95) on average by 20.5%, PI by 29.0%, TI by 7.9%, which was significantly due to increased stomatal conductance of leaves (Table 2). Similar changes are observed in the selection of durum and soft wheat, during the transition from old varieties

to modern ones [28, 29].

This is to a certain extent consistent with evolutionary changes in plants, accompanied by an increase [30, 31] or optimization of water use [31, 32]. The strategy is to open the stomata enough to allow the required amount of CO_2 molecules to be absorbed during photosynthesis while avoiding tissue dehydration during transpiration. In this case, the ratio of PI to SC remains fairly constant over a wide range of factors [32].

We have identified largely similar trends in the nature of manifestation in other cultivated plant species [22, 33], despite certain differences. For example, in spring and winter wheat, under the same weather and agrotechnical growing conditions, the efficiency of water use by plants was on average 23.4 and 35.1% higher than that of buckwheat (Fig. 4).

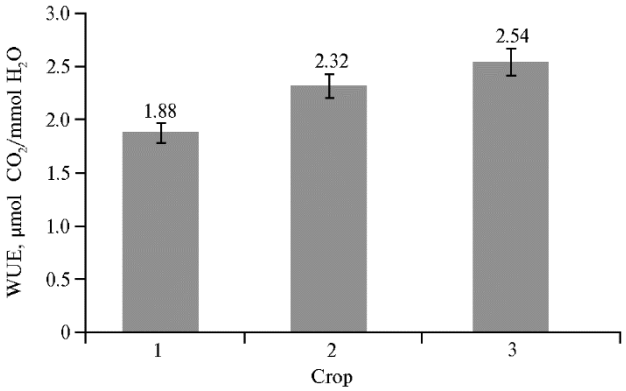

Fig. 4. Water use efficiency during grain formation in different agricultural crops: 1 — buckwheat *Fagopyrum esculentum* Moench of modern zoned varieties Demetra, Dozhdik, Dikul, Devyatka, Design (for each variety $n = 5$, $M \pm SD$, Federal Research Center for Leguminous and Cereal Crops, Oryol Province, Oryol district, 2013-2015); 2 — spring wheat *Triticum aestivum* L. varieties Burlak, Voronezhskaya 13, Voronezhskaya 18, Rima, Ulyanovskaya 105, Chernozemnouralskaya 2, Voronezhskaya 20, Liza, Tulaikovskaya Nadezhda, Khutoryanka, Yubileinaya 80, Arsea, Al Varis, Zlata, Radmira, *Triticum durum* Desf. varieties Donela M, Melodiya Dona, Bezenchukskaya Niva, Bezenchukskaya 210, Donskaya Elegiya); 3 — winter wheat *Triticum aestivum* L. varieties Moskovskaya 40, Moskovskaya 39, Nemchinovskaya 17, Asket, Povolzhskaya Niva, Angelina, Morozko, Moskovskaya 56, Biryuza, Oktava 15, Chernozemka 130, Nemchinovskaya 57, Yuka, Ariadna, Donera ($n = 5$, $M \pm SD$) [33] (Federal Research Center for Leguminous and Cereal Crops, Oryol Province, Oryol District, 2017-2019).

Over the years of research, WUE changed in spring wheat plants from 1.63 to 2.73, in winter wheat plamts from 1.88 to 4.19 μmol CO_2/mmol H_2O with a significant impact on the yield, mainly in harsh weather conditions of the growing season. In the dry year of 2018, according to this indicator, high-yielding varieties of spring wheat outperformed medium- and low-yielding varieties by an average of 70% ($P = 0.95$) [22]. In winter wheat, a close relationship between WUE and the content of protein and gluten in the grain was revealed [33].

In *Fagopyrum esculentum* plants, high efficiency of water use by plants and seed yield were observed mainly in favorable weather conditions during the growing season. This indicates their lower adaptive capabilities, especially under drought conditions, obviously due to the formation of a larger (by 40.8%) evaporative leaf surface, increased (by 21.2%) transpiration intensity and decreased (by 13.9%) photosynthetic activity compared to spring and winter wheat. The identified species characteristics of cultivated plants are apparently due to different conditions of their evolution. Buckwheat, as is known, is a subtropical crop, originated from Southern China, under conditions of increased humidity and high air temperatures [34], while wheat, according to N.I. Vavilov [35], originated from South-

West Asia, the places with an arid climate.

The correctness of this assumption is also evidenced by the results of research by D.A. Ronzhina et al. [36]. Studying the structural and functional parameters of leaves in 21 plant species growing in different water supply conditions, they came to the conclusion that with a decrease in the degree of hydrophilicity in the helophyte-hygrophyte series, a structural restructuring of the leaf occurs in the direction of increasing its density and dry matter content, which is accompanied by a decrease in speed transpiration and a natural increase in water use efficiency [36).

The efficiency of water use by plants can be increased using both agrotechnical and breeding methods. According to E.I. Koshkin [9], the simplest way for C3 grain crops is to combine the period of accumulation of maximum biomass with the coolest weather conditions, for which it is necessary to change the sowing time. In this case, selection should be aimed at activating the processes of initial growth, which will lead to an increase in the leaf surface index, PAR absorption and, as a consequence, an increase in WUE [9].

To achieve this goal in buckwheat, in our opinion, it is important to improve the light transmission capacity of the agrocenosis by regulating the seeding rate and optimizing plant architecture. It is advisable to select forms with a smaller leaf surface but dense leaf blades, that is, to select for xeromorphism. New varieties should also have increased photosynthetic activity with moderate transpiration. Work in this direction may well be successful, since the buckwheat gene pool is characterized by wide polymorphism in parameters of photosynthetic and transpiration activity [37, 38].

To summarize, we note that, unlike spring and winter wheat, in buckwheat *Fagopyrum esculentum* Moench, high efficiency of water use and seed yield were observed mainly under favorable weather conditions during the growing season. This indicates lower adaptive capabilities (especially when exposed to drought) due to the formation of a larger (by 40.8%) evaporative leaf surface, increased (by 21.2%) transpiration intensity and decreased (by 13.9%) photosynthetic activity . The identified differences between cultivated plant species are largely due to the different conditions of their evolutionary formation. Buckwheat, as is known, is a subtropical crop, the development of which took place in Southern China, with increased moisture and high temperatures.

Thus, the water use efficiency (WUE) of common buckwheat plants is significantly influenced by hereditary characteristics and weather conditions of growth. At elevated air temperatures and limited precipitation, WUE decreases, and under favorable weather conditions it increases. Of the endogenous factors, the intensity of leaf photosynthesis has a positive effect on plant WUE ($r = +0.69$, $p < 0.05$), while the intensity of transpiration has a negative effect ($r = -0.89$, $p < 0.05$). As a result of selection, the value of WUE increased ($P = 0.95$) by an average of 20.5%, which was due to an increase in the intensity of photosynthesis by 29.0%, the intensity of transpiration by 7.9%, including due to an increase in stomatal conductance of leaves on average by 18.1%. This had a positive effect on the growth of crop yields only under optimal growing conditions, because the plants' resistance to air and soil drought decreased. Selection of forms with increased photosynthetic activity and moderate transpiration can be considered as one of the priority areas of crop selection.

REFERENCES

1. Kulikov A.I., Ubugunov L.L., Mangataev A.Ts. *Aridnye ekosistemy*, 2014, 3(60): 5-13 (in Russ.).
2. Stuart D., Schewe R.L. Constrained choice and climate change mitigation in US agriculture: structural barriers to a climate change ethic. *Journal of Agricultural and Environmental Ethics*,

2016, 29: 369-385 (doi: 10.1007/s10806-016-9605-z).
3. Zhang Y., Yu X., Chen L., Jia G. Whole-plant instantaneous and short-term water-use efficiency in response to soil water content and CO_2 concentration. *Plant Soil*, 2019, 444: 281-298 (doi: 10.1007/s11104-019-04277-6).
4. Tränkner M., Jákli B., Tavakol E., Geilfus C.-M., Cakmak I., Dittert K., Senbayram M. Magnesium deficiency decreases biomass water-use efficiency and increases leaf water-use efficiency and oxidative stress in barley plants. *Plant Soil*, 2016, 406: 409-423 (doi: 10.1007/s11104-016-2886-1).
5. Karthika G., Kholova J., Alimagham S., Ganesan M., Chadalavada K., Kumari R., Vadez V. Measurement of transpiration restriction under high vapor pressure deficit for sorghum mapping population parents. *Plant Physiology Reports*, 2019, 24: 74-85 (doi: 10.1007/s40502-019-0432-x).
6. Li Y., Li H., Li Y., Zhang S. Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in drought resistant wheat. *The Crop Journal*, 2017, 5(3): 231-239 (doi: 10.1016/j.cj.2017.01.001).
7. Zhu X., Yu G., Wang Q., Hu Z., Han S., Yan J., Wang Y., Zhao L. Seasonal dynamics of water use efficiency of typical forest and grassland ecosystems in China. *Journal of Forest Research*, 2014, 19(1): 70-76 (doi: 10.1007/s10310-013-0390-5).
8. Damour G., Simonneau T., Cochard H., Urban L. An overview of models of stomatal conductance at leaf level. *Plant, Cell & Environment*, 2010, 33(9): 1419-1438 (doi: 10.1111/j.1365-3040.2010.02181.x).
9. Koshkin E.I. Fiziologicheskie *osnovy selektsii rasteniy* [Physiological basis of plant breeding]. Moscow, 2014 (in Russ.).
10. Koshkin E.I. *Fiziologiya ustoychivosti sel'skokhozyaystvennykh kul'tur* [Physiology of crop resistance]. Moscow, 2010 (in Russ.).
11. Bramley H., Turner N.C., Siddique K.H.M. Water use efficiency. In: *Genomics and breeding for climate-resilient crops* /C. Kole (ed.). Springer, Berlin, Heidelberg, 2013: 225-268 (doi: 10.1007/978-3-642-37048-9_6).
12. Zhou L., Meng X., Zhang Z., Wu Q. Association analysis of growth characteristics, WUE, and RUE of rice in cold region under different irrigation patterns. *Journal of The Institution of Engineers (India): Series A*, 2020, 101: 421-431 (doi: 10.1007/s40030-020-00452-6).
13. Moradgholi A., Mobasser H., Ganjali H., Fanaie H., Mehraban A. WUE, protein and grain yield of wheat under the interaction of biological and chemical fertilizers and different moisture regimes. *Cereal Research Communications*, 2022, 50: 147-155 (doi: 10.1007/s42976-021-00145-1).
14. Fesenko A.N., Fesenko I.N. *Zemledelie*, 2017, 3: 24-26 (in Russ.).
15. Vazhov V.M. *Grechikha na polyakh Altaya* [Buckwheat in the fields of Altai]. Moscow, 2013 (in Russ.).
16. Vavilov P.P., Gritsenko V.V., Kuznetsov V.S. *Rastenievodstvo* [Crop production]. Moscow, 1986 (in Russ.).
17. Lakhanov A.P., Kolomeychenko V.V., Fesenko N.V., Napolova G.V., Muzalevskaya R.S., Savkin V.I., Fesenko A.N. *Morfofiziologiya i produktsionnyy protsess grechikhi* [Morphophysiology and production process of buckwheat]. Orel, 2004 (in Russ.).
18. Amelin A.V., Fesenko A.N., Chekalin E.I., Zaikin V.V. Adaptiveness of productivity and photosynthesis in buckwheat (*Fagopyrum esculentum* Moench) landraces and varieties produced at different periods. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2016, 51(1): 79-88 (doi: 10.15389/agrobiology.2016.1.79eng).
19. Amelin A.V., Fesenko A.N., Zaikin V.V., Boyko T.V. *Agrarnyy nauchnyy zhurnal*, 2014, 11(23): 3-6 (in Russ.).
20. Polley W.H. Implications of atmospheric and climate change for crop yield and water use efficiency. *Crop Sciense*, 2002, 42(1): 131-140 (doi: 10.2135/cropsci2002.1310).
21. Ruggiero A., Punzo P., Landi S., Costa A., Van Oosten M.J., Grillo S. Improving plant water use efficiency through molecular genetics. *Horticulturae*, 2017, 3(2): 31 (doi: 10.3390/horticulturae3020031).
22. Amelin A.V., Chekalin E.I., Zaikin V.V., Ikusov R.A., Shishkin A.S. *Vestnik Kurskoy GSKhA*, 2022, 6: 6-12 (in Russ.).
23. Udovenko G.V. V sbornike: *Fiziologicheskie osnovy selektsii rasteniy* [In: Physiological basis of plant breeding]. St. Petersburg, 1995, 2: 293-352 (in Russ.).
24. Alekhina N.D, Balnokin Yu.V., Gavrilenko V.F., Zhigalova T.V. *Fiziologiya rasteniy* [Plant physiology]. Moscow, 2007 (in Russ.).
25. Gorthi A. *Quantifying water use efficiency at leaf and field-scales for soybean, miscanthus and switchgrass. Doctoral Thesis.* Purdue University, 2017.
26. Amoanimaa-Dede H., Su C., Yeboah A., Zhou H., Zheng D., Zhu H. Growth regulators promote soybean productivity: a review. *PeerJ*, 2022, 10: e12556 (doi: 10.7717/peerj.12556).
27. Kharchuk O.A., Kirillov A.F., Budak A.B. *Evraziyskiy Soyuz Uchenykh*, 2018, 11-1(56): 42-46 (in Russ.).

28. Li L.H., Chen S.B. Study on root function efficiency of spring wheat under different moisture condition. *Sci. Agric. Sin.*, 2002, 35: 867-871 (in Chinese).
29. Fan X.-W., Li F.-M., Xiong Y.-C., An L.-Z., Long R.-J. The cooperative relation between non-hydraulic root signals and osmotic adjustment under water stress improves grain formation for springwheat varieties. *Physiologia Plantarum*, 2008, 132(3): 283-292 (doi: 10.1111/j.1399-3054.2007.01007.x).
30. Li Y.Y., Zhang S.Q., Shao M.A. Interrelationship between water use efficiency and nitrogen use efficiency of different wheat evolution materials. *Chinese Journal of Applied Ecology*, 2003, 14(9): 1478-1480 (in Chinese).
31. Song L., Li F.M., Fan X.W., Xiong Y.C., Wang W.Q., Wu X.B., Turner N.C. Soil water availability and plant competition affect the yield of spring wheat. *European Journal of Agronomy*, 2009, 31(1): 51-60 (doi: 10.1016/j.eja.2009.03.003).
32. Bacon M. *Water use efficiency in plant biology*. Armsterdam, 2004.
33. Amelin A.V., Chekalin E.I., Zaikin V.V., Mazalov V.I., Ikusov R.A. Biochemical grain quality indicators and photosynthetic rate of leaves in modern varieties of winter wheat. *IOP Conference Series: Earth and Environmental Science*, 2021, 848: 012096 (doi: 10.1088/1755-1315/848/1/012096).
34. Ohnishi O. Distribution and classification of wild buckwheat species. 1. Cymosum group. *Fagopyrum,* 2010, 27: 1-8.
35. Vavilov N.I. *Trudy po prikladnoy botanike i selektsii*, 1926, tom 16, vyp. 2 (in Russ.).
36. Ronzhina D.A., Rupyshev Yu.A., Ivanova L.A., Migalina S.V., Ivanov L.A. *Problemy botaniki Yuzhnoy Sibiri i Mongolii*, 2022, 21(2): 171-174 (doi: 10.14258/pbssm.2022077) (in Russ.).
37. Amelin A.V., Fesenko A.N., Zaikin V.V. *Vestnik OrelGAU*, 2015, 6(57): 18-22 (doi: 10.15217/issn1990-3618.2015.6.18) (in Russ.).
38. Amelin A.V., Fesenko A.N., Zaikin V.V., Chekalin E.I., Ikusov R.A. Transpiration activity of leaves in buckwheat varieties of different breeding periods. *BIO Web of Conferences*, 2022, 47: 01002 (doi: 10.1051/bioconf/20224701002).

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 87-99
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Symbiogenetics

UDC 631.52:581.557:575

doi: 10.15389/agrobiology.2023.1.87eng
doi: 10.15389/agrobiology.2023.1.87rus

BIODIVERSITY OF THE SYMBIOTIC SYSTEMS FORMED BY NODULE BACTERIA *Rhizobium leguminosarum* WITH THE LEGUMINOUS PLANTS OF GALEGOID COMPLEX

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The authors declare no conflict of interests
Acknowledgements:
The authors are grateful to Ya.V. Pukhalsky for help in measuring nitrogenase activity.
Supported financially by the Russian Science Foundation under grant No. 19-16-00081П
Final revision received December 28, 2022
Accepted January 17, 2023

Abstract

Nodule bacteria of the species *Rhizobium leguminosarum* are differentiated into two biovars (bv.) that form N_2-fixing symbioses with leguminous plants of the galegoid complex, tribes *Fabeae* (genera *Lathyrus, Lens, Pisum, Vavilovia, Vicia*, symbiont — *R. leguminosarum* bv. *viciae*) and *Trifolieae* (genus *Trifolium*, symbiont — *R. leguminosarum* bv. *trifolii*) (J. Sprent et al., 2017). It was previously assumed that cross-inoculation between these biovars is impossible or rare, while data on the control of host specificity of *R. leguminosarum* were limited by interactions between pea (*P. sativum*) lines with different alleles of *Sym2* gene and bv. *viciae* strains that differ in the presence of *nod*X gene (T.A. Lie, 1978). The aim of our work was to analyze the variability of *R. leguminosarum* bv. *viciae* strains from ancestral (A) and evolutionarily advanced (D) genomic groups in terms of host specificity and N_2-fixing activity, aimed at the functional characterization of ancestral genome elements, which were previously identified by the comparative genetic analysis of strains isolated from representatives of the *Fabeae* tribe that differ in phylogenetic affiliation. In accordance with the previously proposed genotyping technique, strains were assigned to group A if they contained the *nod*X and *fix*W genes, did not contain a chromosomal copy of the *fix*NOPQ operon, and the *nod*T gene was outside the *nod* cluster. In the absence of at least one of these features, the strains were assigned to group D (E. Chirak et al., 2019). Group A strains were isolated from the relict legume *Vavilovia formosa* and from wild-growing Afghan lines of *P. sativum*, group D strains were isolated from cultivated European lines of *P. sativum*, from *Vicia sativa* and *V. alpestris.* In experiments on the analysis of cross-inoculation of two *R. leguminosarum* biovars we used bv. *viciae* strains isolated from nodules of *Vavilovia formosa, Vicia sativa, V. subrotunda*, European lines of *Pisum sativum*, Afghan lines of *P. sativum*, as well as bv. *trifolii* strains from clover (*Trifolium pratense, T. ambiguum, T. montanum*) nodules. In microvegetative experiments, plants inoculated with rhizobia were grown under gnotobiotic conditions on vermiculite. N_2-fixing activity was determined using the acetylene method based on the use of C_2H_2 as a substrate for nitrogenase. Based on the results obtained, the following symbiotic phenotypes were identified: Fix^+ — N_2-fixing (large, pink) nodules; Fix^- — non-fixing N_2 (small, white, but morphologically normal) nodules; $Fix^{+/-}$ — nodules not fixing N_2, but similar to Fix^+ nodules (large, pink); Ndv^- — non-fixing N_2, tumor-like nodules; Nod^- — nodules were absent. It turned out that 9 out of 11 strains of the ancestral group formed on clover nodules of Fix^- phenotype, and 2 strains formed nodules of Ndv^- phenotype. Among 8 strains of the evolutionarily advanced group, the Fix^- and Ndv^- phenotypes were detected in 4 and 2 strains, respectively, and 2 strains did not form nodules on clover (Nod^-), indicating a narrowing of the host specificity of rhizobia during coevolution of bv. *viciae* with host plants. Therefore, we have shown for the first time that during the transition of bv. *viciae* strains to symbiosis with evolutionarily young representatives of the tribe *Fabeae* (transition from the A- to the D-group), bacteria lose the ability to form symbiosis with a heterologous host (*Trifolium*). Among 6

strains of clover rhizobia, 4 strains showed the ability to inoculate vetch forming Fix⁻ nodules. In experiments to control the absence of contamination, DNA was isolated from nodules using the NucleoSpin™ Soil (Macherey-Nagel GmbH & Co. KG, Germany), the *nod*A gene fragment was amplified using universal primers for *R. leguminosarum* biovars (ndARL302_F YTDGGMATCGCHCACT/ndARL518_R RDACGAGBACRTCTTCRGT). The data obtained showed that under the conditions of sterile microvegetation experiments there is no contamination and the majority of strains are able for cross-inoculation: bv. *viciae* strains form nodules on clover and bv. *trifolii* form nodules on vetch. However, this ability is limited by the formation of non-fixing N_2 nodules in heterologous hosts, including morphologically abnormal (tumor-like) nodules. The study of symbioses formed by 9 species of leguminous plants of tribe *Fabeae* (*Pisum sativum, Vicia sativa, V. villosa, V. alpestris, Vavilovia formosa, Lens culinaris, L. nigricans, Lathyrus pratensis, L. sylvestris*) with 6 *R. leguminosarum* bv. *viciae* strains, demonstrated a pronounced specificity of N_2-fixing symbiosis formation, which depends mostly on the bacteria origin. Strains isolated from the same legume species (*V. formosa* or *P. sativum*) are more similar in host specificity than strains from different hosts. A hypothetical scheme of *R. leguminosarum* evolution is proposed, according to which: a) divergent evolution of bv. *viciae* is determined by host plant speciation in tribe *Fabeae*; b) the closest relative of a common ancestor of *R. leguminosarum* is represented by bv. *trifolii*, which display an evolutionary primitive *sym* gene organization and possibly originated from the ancestral bv. *viciae* strains that changed their host specificity. The rhizobia isolated from *V. formosa* may be considered as the close relatives of these ancestral strains since they exceed the pea and vetch isolates in the ability to form morphologically normal nodules with the heterologous host, clover. The data obtained show the possibility of constructing rhizobia strains with an increased symbiotic activity by editing the ancestral components of their genomes.

Keywords: nodule bacteria, leguminous plants, symbiotic N_2-fixation, host specificity, biodiversity, *Rhizobium leguminosarum*, genomic groups, evolution of symbiosis

Symbioses of leguminous plants with N_2-fixing nodule bacteria (rhizobia) are very diverse in their structural and functional organization and the specificity of the interaction of partners [1]. The most studied symbioses are those formed by bacteria of the family. *Rhizobiaceae* (genera *Rhizobium*, *Sinorhizobium*, *Neorhizobium*) with plants of the galegoid complex, including the tribes *Fabeae*, *Galegae* and *Trifolieae*. Rhizobia infect these plants through root hairs where infection threads arise. Infection threads are tubular structures that allow rhizobia to enter intracellular symbiosomes with subsequent transformation into irreversibly differentiated N_2-fixing bacteroids [2].

Symbioses formed by galegoid legumes are highly specific interactions of partners within separated cross-inoculation groups (CIG) [3]. For example, the species *Rhizobium leguminosarum* is divided into two biovars that form different CIGs. Their hosts are plants of the IRLC group of the galegoid complex, whose plastid DNA does not carry the 25-kb inverted repeat found in most galegoid legumes. Plant symbionts of the tribe *Fabeae* (genera *Lathyrus*, *Lens*, *Pisum*, *Vavilovia*, and *Vicia*) are classified as biovar (bv.) *viciae*, and symbionts of the genus *Trifolium* (tribe *Trifolieae*) are classified as bv. *trifolii*. Analysis of the genetic diversity of *R. leguminosarum* strains showed that the biovars *viciae* and *trifolii* differ in the structure of the symbiosis *sym* genes of the accessory part of the genome much more strongly than in the genes of its core part which determine housekeeping functions [4, 5].

We have previously shown [6] that strains of *R. leguminosarum* bv. *viciae* are divided into two genomic groups, the ancestral (A) and evolutionarily advanced (D). The characteristic features of group A strains from the nodules of *Vavilovia formosa*, the putative closest relative of the common ancestor of the tribe *Fabeae* [7], and wild (Afghan) forms of the common pea *P. sativum* are i) the presence of the *nod*X gene for broad host specificity control [8] and the gene *fix*W encoding an enzyme that breaks down disulfide bonds in proteins [9], ii) absence of a chromosomal copy of the f*ix*NOPQ operon, encoding cytochrome oxidase with high affinity for O_2 [10], iii) more than 90 kb plasmid cluster of *sym* genes, and iv) location of the *nod*T gene outside this cluster. Group D strains lack the *nod*X and *fix*W genes, a chromosomal copy of the *fix*NOPQ operon is detected, the *sym* gene

cluster is less than 60 kb in size, and *nod*T is included in the *nod* gene cluster. It is assumed that the transition from the A- to the D-group, which occurred due to the coevolution of rhizobia and their hosts, led to an increase in the adaptability of bacteria to plant-soil systems associated with the activity of symbiosis [11].

This work shows for the first time that during the transition of bv. *viciae* to symbiosis with evolutionarily young representatives of the tribe *Fabeae* (transition from A- to D-group), the bacteria lose the ability to form symbiosis with a heterologous host (*Trifolium*). Under conditions of sterile micro-pot tests, most bv. *viciae* strains which form nodules on clover and bv. *trifolii* strains which form nodules on vetches exhibit the ability to cross-inoculate. However, this ability is limited by the formation of nodules in heterologous hosts that do not fix N_2, including those with abnormal morphology (tumor-like). A hypothetical scheme for the evolution of the species *R. leguminosarum* has been proposed according to which the divergence of bv. *viciae* is determined by the increasing diversity of plants of the tribe *Fabeae*. The bv. *trifolii* is the closest to the common ancestor of this species, it retained the evolutionarily primitive organization of *sym* genes and possibly arose through changes in host specificity in ancestral strains of bv. *viciae*, the closest relatives of which can be considered the symbionts of *V. formosa*.

The purpose of our work was to analyze the variability of *Rhizobium leguminosarum* bv. *viciae* ancestral (A) and evolutionarily advanced (D) genomic groups in terms of host specificity and N_2-fixing activity, aimed at the functional characterization of ancestral elements of the genome, which were previously identified during a comparative genetic analysis of strains isolated from the tribe *Fabeae* representatives that differ in phylogenetic position.

Materials and methods. The experiments used strains of *R. leguminosarum* bv. *viciae* from the collection of the All-Russian Research Institute of Agricultural Microbiology, isolated from the nodules of *Vavilovia formosa* (Steven) Fed. (Vaf10, Vaf12, Vaf13, Vaf35, Vaf25, Vaf01, Vaf46, Vaf96, Vaf108, 1B2, 1G1), *Vicia sativa* L. (Vst35-4, Vst36-3, 1-32), *V. subrotunda* (Maxim.) Czefr. (Vs35-4, Vs36-3, Vs37-3, 2S1, 3S1, 2-1k, 3Vsb, 1Vsd, L1-1, L2-1), European lines of *Pisum sativum* L. (1079, CIAM1026, Wp1, Wp3, Wp40, Wp19, Wp24, Wp60), Afghan lines of *P. sativum* L. (A1, TOM), as well as strains of *R. leguminosarum* bv. *trifolii* from clover nodules (*Trifolium pretens*e L., *T. ambiguum* M. Bieb., *T. montanum* L.) (Tp73-4, Tr11, Tm2, Ta6, Ka1, Ka5). Strains of alfalfa rhizobia *Sinorhizobium meliloti* (AK57, A18) and goat's rue *Neorhizobium galegae* (Gr12/7, Gr1025), which do not form nodules on host plants of the species *R. leguminosarum*, were used as negative controls in cross-inoculation experiments.

Seeds of *Vicia alpestris* Steven (I-0146902), *V. sativa* L. (variety Nikolskaya, K-36638), *V. villosa* Roth. (variety Lugovskaya 2, K-37019), *Lens culinaris* Medikus (variety Pikantnaya, K-3051), *L. nigricans* (M.Bieb.) Godr. (ILWL37), *Lathyrus pratensis* L. (N094275), *L. sylvestris* L. (K 1959), *T. pratense* L. (cultivar Suydinets, K-34600) were obtained from the collection of the Vavilov All-Russian Research Institute of Plant Resources, seeds of *P. sativum* L. (Afghan line NGB2150, European lines Frisson and SGE) derived from the collection of the All-Russian Research Institute for Agricultural Microbiology, seeds of *V. formosa* (Steven) Fed. were collected from wild plants in the Caucasus [12].

In micro-pot experiments, plants inoculated with rhizobia were grown under gnotobiotic conditions on vermiculite with nitrogen-free Krasilnikov-Korenyako medium in 60 ml test tubes (*Trifolium*, *Lathyrus*, *Vicia*) or in 1000 ml glass cylinders (*Lens*, *Pisum*, *Vavilovia*). N_2-fixing activity was determined using the acetylene method with C_2H_2 as a substrate for nitrogenase [13]. Roots with nodules were placed in hermetically sealed 50 ml bottles, into which 2.5 ml of C_2H_2 (5% volume) was introduced and incubated for 1 hour. In this case, the reduction of N_2 is blocked and the formation of C_2H_4 occurs, the amount of which was

determined on a gas chromatograph GC-2010 (Shimadzu, Japan). Based on the results of micro-pot tests, the following symbiotic phenotypes were identified: Fix^+ — N_2-fixing (large, pink) nodules; Fix^- — non-fixing N_2 (small, white, but morphologically normal) nodules; $Fix^{+/-}$ — nodules that do not fix N_2 and are similar in appearance to Fix+ nodules (large, pink); Ndv — non-N_2 fixing, tumor-like nodules; Nod^- — without nodules.

DNA was isolated from nodules using the NucleoSpin™ Soil kit (Macherey-Nagel GmbH & Co. KG, Germany), the *nod*A gene fragment was amplified at 30 s, 95 °C, 30 s, 50 °C, 30 s, 72 °C (35 cycles) using universal primers for *R. leguminosarum* biovars (ndARL302_F YTDGGMATCGCHCACT/ndARL518_R RDACGAGBACRTCTTCRGT). Sequencing of amplicon libraries was carried out using Illumina technology (an Illumina MiSeq instrument, Illumina, Inc., USA) with a MiSeq® ReagentKit v3 (600 cycle) with double-sided reading (2×300 nt). The obtained sequences were processed using Illumina software.

In accordance with the previously proposed genotyping technique [6], strains were assigned to the ancestral genomic group A if they contained the *nod*X and *fix*W genes, did not contain a chromosomal copy of the *fix*NOPQ operon, and the *nod*T gene was located outside the *nod* cluster. In the absence of at least one of these characters, the strains were assigned to the evolutionarily advanced group D.

Statistical processing of the results was carried out using analysis of variance and Student's *t*-test, the values of which (t_{St}) were used to assess the probability of the null hypothesis (P0) about the absence of differences between the studied samples of strains [14]. Strains were considered N_2-fixing (Fix^+) if the amount of C_2H_4 in the experimental sample was significantly higher than in the control without inoculation. To carry out cluster analysis, symbiotic phenotypes were given numerical values (0 — Nod^-, 1 — Fix^-, 2 — $Fix^{+/-}$, 3 — Fix^+), which were used to calculate Euclidean distances between strain phenotypes, to construct a dendrogram using the UPGM method and to statistically support clusters bootstrap using PAST program [15].

Results. Firstly, we analyzed the cross-inoculation of biovars *viciae* and *trifolii* with their host plants (tribe *Fabeae* and genus *Trifolium*). The results of micro-pot tests showed (Table 1, Fig. 1) that among the 33 tested *R. leguminosarum* bv. *viciae* strains isolated from peas, vetch and Vavilovia, 28 strains formed on meadow clover (*T. pratense*) nodules that did not fix N_2 (23 strains formed morphologically normal nodules of the Fix^- phenotype, 5 strains formed tumor-like nodules of the Ndv^- phenotype), and 5 strains did not formed nodules (Nod^- phenotype). It is important to note that Fix^- nodules on clover were formed by 91.6% of strains isolated from Vavilovia, and only 57.1% of strains from vetch and peas ($t_{St} = 2.57$; $P0 < 0.05$). In addition, on vetch plants, Fix^+ nodules were formed by only 41.7% of strains isolated from Vavilovia, and 100% of strains from vetch and peas ($t_{St} = 2.93$; $P0 < 0.05$), which indicated the specialization of Vavilovia symbionts to their to the host.

To compare the phenotypic variation of the studied strains of *R. leguminosarum* bv. *viciae* with their genomic organization, using the previously proposed method [6], we identified among them the ancestral (A) and evolutionarily advanced (D) groups (see Table 1). It turned out that 9 out of 11 strains of group A exhibited the Fix^- phenotype on clover, and 2 strains exhibited the Ndv^- phenotype. Among the 8 strains of group D, the Fix^- and Ndv^- phenotypes were detected in 4 and 2 strains, respectively, and 2 strains did not form nodules on clover (Nod^- phenotype). Of the 6 strains of clover rhizobia (*R. leguminosarum* bv. *trifolii*) we studied, 4 strains exhibited the Fix^- phenotype on hairy vetch (*V. villosa*), and 2 strains exhibited the Nod^- phenotype (see Table 1). When clover and vetch were inoculated with symbionts of alfalfa (*Sinorhizobium meliloti*) and goat's

rue (*Neorhizobium galegae*), as in controls without inoculation, nodules were absent.

1. Cross-inoculation of *Rhizobium leguminosarum* bv. *viciae* and *R. leguminosarum* bv. *trifolii* of host plants depending on the strain origin and genomic structure

Plants from which strains were isolated	Number of strains studied (number of strains having the gene *nod*X)	Ratio of genomic groups A:D	Symbiotic phenotypes of the strains on plants	
			Vicia villosa Roth.	*Trifolium pratense* L.
R. leguminosarum bv. *viciae*				
Vavilovia formosa (Steven) Fed.	12 (12)	7:0	5 Fix^+, 7 Fix^-	11 Fix^-, 1 Nod^-
Vicia sativa L.	3 (3)	0:3	3 Fix^+	3 Fix^-
V. subrotunda (Maxim.) Czefr.	10 (8)	2:1	10 Fix^+	6 Fix^-, 2 Ndv^-, 2 Nod^-
Pisum sativum L. (European lines)	6 (0)	0:4	6 Fix^+	3 Fix^-, 1 Ndv^-, 2 Nod^-
P. sativum L. (Afghan lines)	2 (2)	2:0	2 Fix^+	2 Ndv^-
R. leguminosarum bv. *trifolii*				
Trifolium spp.	6 (6)	Not tested	4 Fix^-, 2 Nod^-	6 Fix^+

N o t e. For bv. *viciae* the ratio of strains belonging to the ancestral (A) and evolutionarily advanced (D) genomic groups is indicated. Fix^+ — N_2-fixing (large, pink) nodules, Fix^- — not N_2-fixing (small, white, but morphologically normal) nodules, Ndv^- — not N_2-fixing, tumor-like nodules, Nod^- — no nodules.

Fig. 1. Phenotypes of nodules formed on the roots of *Trifolium pratense* L. by various strains of *Rhizobium leguminosarum*: a — Vaf12 bv. *viciae* (tumor-like nodules that do not fix N_2, Ndv^-), b — Vaf108 bv. *viciae* (morphologically normal nodules that do not fix N_2, Fix^-, c — Ta6 bv. *trifolii* (N_2-fixing nodules, Fix^+).

Genotyping showed that the ability of *R. leguminosarum* biovars to form nodules on plants from heterologous cross-inoculation groups is not associated with the presence of the nodX gene. Indeed, the Fix^- phenotype on clover was demonstrated by *nod*X-containing bv. *viciae* strains isolated from Vavilovia and strains from European pea lines lacking this gene. Despite the contrasting variation of bv. *trifolii* in terms of their ability to form nodules on vetch, they all had the *nod*X gene.

To confirm the phenomenon of cross-inoculation between rhizobia of different biovars, excluding contamination of bv. *viciae* suspensions used to inoculate clover, with "spontaneous" strains of bv. *trifolii*, as well as suspensions of bv. *trifolii* used for vetch inoculation with bv. *viciae*, we isolated total DNA from the formed Fix^- nodules, from which amplicon libraries were prepared for the *nod*A gene, which has clear differences in the compared rhizobial biovars [16]. Deep sequencing of these libraries using over 10 thousand *nod*A gene sequences from Fix^- nodules formed by strains Vaf12 and Vaf108 on clover, as well as strain Tr11 on vetch did not reveal contaminants.

At the second stage of work, strains of *R. leguminosarum* bv. *viciae*, representing different genomic groups, were used to inoculate 9 legume species from all 5 genera of the tribe *Fabeae*. Fix^+ nodules were formed in only 19 of 60 genotypic combinations of partners, indicating the high specificity of the formation of N_2-fixing symbiosis (Table 2). Among 3 broadly specific strains that formed Fix+ nodules with 5-8 plant species, 2 strains (Vaf-12 and A1) represented genomic group A (*nod*X is present), and 1 strain (1079) represented group D (*nod*X is absent). Strains Vaf-10, Vaf-46 and Vaf-108 included in group A formed nodules that did not fix N_2 with all the legumes studied, but in the original host, Vavilovia,

they were similar to Fix^+ nodules (large size, pink color) and were isolated into a separate phenotype ($Fix^{+/-}$). It is important to note that all plant species studied formed Fix^+ nodules with at least one of the tested rhizobia strains. Thereof, the specificity of the formation of N_2-fixing symbiosis depended more significantly on the bacterial strain than on the plant species.

2. Specificity of interaction between different strains of *Rhizobium leguminosarum* bv. *viciae* and plants of the tribe *Fabeae*

Strain	Host plants										
	Pisum sativum		*Vicia*			*Vavilovia*	*Lens*		*Lathyrus*		total
	Afghan	European	*alpestris*	*sativa*	*villosa*	*formosa*	*culinaris*	*nigricans*	*pratensis*	*sylvestris*	Fix^+
Vaf-10 (A)	Fix^-	Fix^-	Fix^-	Fix^-	Fix^-	$Fix^{+/-}$	Fix^-	Fix^-	Fix^-	Fix^-	0
Vaf-12 (A)	Fix^-	Fix^-	Fix^+	Fix^+	Fix^+	$Fix^{+/-}$	Fix^+	Fix^+	Fix^-	Fix^-	5
Vaf-46 (A)	Fix^-	Fix^-	Fix^-	Fix^-	Fix^-	Fix^-	Fix^-	Fix^-	Fix^-	Fix^-	0
Vaf-108 (A)	Fix^-	Fix^-	Fix^-	Fix^-	Fix^-	$Fix^{+/-}$	Fix^-	Fix^-	Fix^-	Fix^-	0
A1 (A)	Fix^+	Fix^+	Fix^-	Fix^+	Fix^+	Fix^+	Fix^+	Fix^+	Fix^+	Fix^-	8
1079 (D)	Nod^-	Fix^+	Nod^-	Fix^+	Fix^+	Fix^-	Fix^+	Fix^+	Fix^-	Fix^+	6
Total Fix^+	1	2	1	3	3	1	3	3	1	1	19

N o t e. The belonging of strains to genomic groups is indicated in brackets: A — ancestral, D — evolutionarily advanced. Fix^+ — N_2-fixing (large, pink) nodules, Fix^- — not N_2-fixing (small, white, but morphologically normal) nodules, $Fix^{+/-}$ — not N_2-fixing nodules, similar to Fix^+ nodules (large, pink), Nod^- — no nodules.

To study the relationship between host specificity of *R. leguminosarum* bv. *viciae* and their origin, we analyzed the similarity of these strains in terms of symbiotic phenotypes manifested during interaction with 10 plant forms (see Table 2). It turned out that when comparing strains isolated from the same legume species (Vavilovia or pea), the average number of matches for the Fix^+ phenotype is 6 ± 0.97, and among strains from different legume species it is only 2 ± 0.68 ($t_{St} = 3.39$; $P0 < 0.01$). Cluster analysis of the obtained data revealed the phenotypic similarity of the Vavilovia strains Vaf10, Vaf46 and Vaf108, forming a compact cluster with a bootstrap support level of 99%, as well as a cluster with a much lower level of support (50%), uniting strains Vaf12 from Vavilovia, A1 from Afghan peas and 1079 from European peas (Fig. 2).

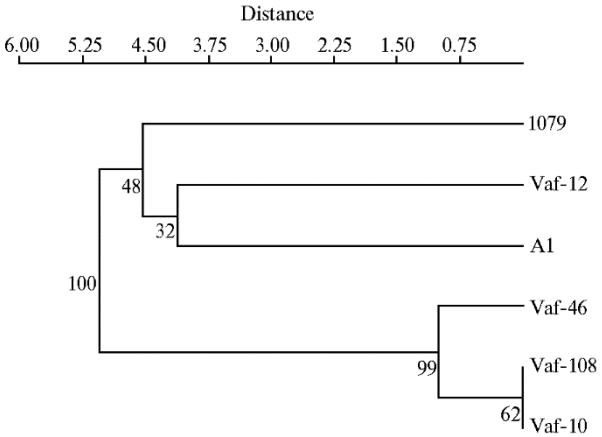

Fig. 2. Cluster analysis of *Rhizobium leguminosarum* bv. *viciae* according to the phenotype manifested in symbiosis with various legume species of the tribe *Fabeae* (based on data in Table 2; Euclidean distances and boot-straps for clusters are indicated).

Symbioses formed by nodule bacteria (rhizobia) and legumes are one of the most developed models for the genetic analysis of interactions between prokaryotes and eukaryotes, as well as for the development of methods for constructing microbial-plant systems for agricultural purposes [17]. Previously proposed approaches to solve this problem include the production of mutants and recombinants with increased N_2-fixing activity, symbiotic efficiency (the ability to increase

the productivity of host plants), as well as the ability to compete with native strains of rhizobia for the formation of nodules in plants.

It has been shown that an increase in the N_2-fixing activity of rhizobia can be achieved through amplification of genes that ensure the formation of the nitrogenase complex and its energy supply [18]. An even more promising approach turned out to be inactivation of negative regulators of symbiosis, including genes that control the conversion of plant-derived C-compounds into storage nutrients poly-beta-hydroxybutyrate [19-21] and glycogen [22], as well as sugar transport [23], high respiration intensity [24] and the formation of surface polysaccharides that protect bacteria from edaphic (osmotic, temperature, water) stresses [25].

Similar approaches can be used to improve the competitiveness of rhizobia. They are based on the amplification of positive regulators of this trait - genes that activate the synthesis of surface polysaccharides [26] and proline dehydrogenase [27], nodule formation [28], as well as on the inactivation of negative regulators of competitiveness, suppressing the expression of nodule formation genes [29], synthesis NADP-dependent dehydrogenase [30] and biofilm formation [31].

Considering that during the evolution of the legume-rhizobium symbiosis its ecological efficiency increased [1], editing the ancestral elements of the rhizobial genome, which are involved in the control of symbiotic properties, seems promising. Previously, to identify these elements in the polytypic (consisting of several biovars differing in host specificity) species *R. leguminosarum*, we used an approach that was based on a comparative genetic analysis of strains isolated from representatives of the tribe *Fabeae* differing in phylogenetic position [11]. Among the strains of *R. leguminosarum* that are promising for identifying ancestral elements of the genome, the most interesting are the symbionts of *Vavilovia formosa*, a relict species of the tribe *Fabeae*, which can be considered the closest relative of its common ancestor [7]. Based on data on the distribution of Vavilovia in the high mountainous regions of the Caucasus and Central Asia, as well as on the peculiarities of the genomic organization of its symbionts, they were determined to be close to the common ancestor of bv. *viciae*, and possibly the entire species *R. leguminosarum* [5, 6].

This article characterizes the variability of *R. leguminosarum* strains that differ in the composition of ancestral elements of the genome, according to their effect on host specificity and N_2-fixing activity, which allows us to assess the possibility of using these elements in biotechnological programs. We studied the host specificity of *R. leguminosarum* by analyzing cross-inoculation of *viciae* and *trifolii* biovars and interactions of bv. *viciae* strains with various species of the tribe *Fabeae*. Previously, the cross-inoculation groups formed by biovars *viciae* and *trifolii* were thought to be completely separate from each other [3]. However, we have shown that most strains of bv. *viciae* form nodules on clover, and most strains of bv. *trifolii* on vetch (see Table 1). Although the cross-inoculation of biovars that we identified was limited to the formation of nodules that did not fix N_2, including those with anomalous morphology, the data obtained indicate an incomplete symbiotic divergence of the biovars *viciae* and *trifolii*, which may be associated with their recent separation from a common ancestor that had broad host specificity.

Previously, based on an analysis of the genomic diversity of the ancestral (A) and evolutionarily advanced (D) groups of strains, we suggested [11] that the transition from group A to group D causes an increase in the adaptive potential of the species *R. leguminosarum* due to the Nod factor transport intensification associated with migration of the *nod*T gene into the *nod* cluster, bacterial respiration in the soil because of acquisition of a chromosomal copy of the *fix*NOPQ operon and deep differentiation of bacteroids determined by the loss of the *fix*W gene.

In this work, it was established that 9 out of 11 strains of group A formed

on clover nodules with normal morphology that did not fix N_2 (Fix^- phenotype). Two strains formed tumor-like nodules, in which, as it was shown by the study of rhizobia mutants defective in synthesis of lipo- and exopolysaccharides [32], the penetration of bacteria into root tissue was impaired (Ndv^- phenotype). In group D, the occurrence of the Fix^- phenotype was lower than among strains of group A. These data indicate that, during the transition from ancestral to evolutionarily developed genome organization, the bv. *viciae* has lost the ability to inoculate clover plants.

Importantly, the ability of the compared biovars of *R. leguminosarum* to form nodules on alien hosts is not associated with the presence of the *nod*X gene, which was previously considered the main determinant of the host specificity of this rhizobial species. This gene, first identified in bv. *viciae* from Afghan pea lines [8], was later found in strains isolated from Vavilovia and some other plants of the tribe *Fabeae* [6], as well as in all bv. *trifolii* strains studied [33]. The submitted data indicate that the loss of the *nod*X gene, characteristic of the D group, apparently was not the direct cause of the diversification of the *R. leguminosarum* species in terms of host specificity, although *nod*X can be considered as a marker of this process.

We assessed the host specificity of bv. *viciae*, which is determined by the formation of N_2-fixing nodules in 9 species of legumes, representing all 5 genera of the tribe *Fabeae* (see Table 2). This specificity is more dependent on bacteria than on plants and is evident when comparing strains isolated from *P. sativum* and its evolutionary predecessor *V. formosa* (see Fig. 2).

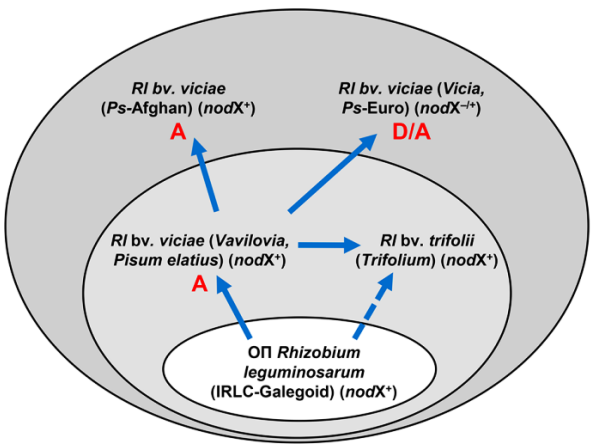

Fig. 3. Divergent evolution of the species *Rhizobium leguminosarum* (*Rl*), which led to the formation of biovars *viciae* and *trifolii*. *Vavilovia* is the closest relative of the common ancestor of the legume tribe *Fabeae*, the hosts of *R. leguminosarum* bv. *viciae*. *Pisum elatius* Bieb. is a wild pea species whose range overlaps with the Mediterranean center of origin of the genus *Trifolium*. *Ps*-Afghan and *Ps*-Euro are wild (Afghan) and cultivated (European) lines of pea (*P. sativum*). The white oval represents the hypothetical common ancestor (CA) of the species *Rhizobium leguminosarum*, a symbiont of the common ancestor of the IRLC group of legumes of the halegoid complex; in the light gray oval markes ancestral forms of bv. *viciae* with the *nod*X gene (symbionts of *Vavilovia formosa* Steven Fed. and *P. elatius* Bieb. Of the genomic group A) and bv. *trifolii*; in the dark gray oval are evolutionarily advanced forms of bv. *viciae* (most *Ps*-Euro and *Vicia* symbionts belong to genomic group D, which is characterized by the loss of the *nod*X gene). The most probable directions of evolution are shown by solid arrows.

The data obtained allowed us to propose a hypothetical scheme for the evolution of the species *R. leguminosarum* (Fig. 3), according to which its common ancestor (protosymbiont) was able to form symbiosis with ancestral representatives of the IRLC group of legumes of the galegoid complex. The evolution of protosymbionts may have begun with divergence based on host specificity, which

led to the emergence of biovars *viciae* and *trifolii* capable of N_2-fixing symbiosis with plants of only one tribe — *Fabeae* or *Trifolieae*.

In this case, the bv. *trifolii* could arise either directly from the protosymbiont, or through a change in the host specificity of ancestral strains of bv. *viciae*. The second mode is supported by the fact that the distribution area of one of the wild pea species (*P. elatius*) overlaps with the Mediterranean center of origin of the genus *Trifolium* [34, 35], and most strains of bv. *viciae* and bv. *trifolii* exhibit the ability to cross-inoculate with the formation of non-N_2-fixing nodules. It can be assumed that bv. *viciae*, which appears to have evolved faster than bv. *trifolii* due to wider taxonomic diversification of the corresponding host plants, with specialization to newly emerging legume species, the ability to form nodules on clover was lost or replaced by the formation of tumor-like structures in which bacterial infection of plant tissues was impaired.

The data obtained suggest that the symbiotic properties of the common ancestor bv. *viciae* were most preserved by strains isolated from *V. formosa*, 91.6% of which were able to form morphologically normal (Fix^-) nodules on clover, while among strains from vetch and pea only 57.1% showed this ability. In addition, only 41.7% of strains from Vavilovia exhibited the Fix^+ phenotype on vetch, which is shown for all strains from vetch and pea. Hence, strains from Vavilovia which to the greatest extent retained broad host specificity towards different tribes of legumes, have not yet fully acquired the ability to symbiose with evolutionarily young representatives of the tribe *Fabeae*. This confirms the expediency of the previously expressed [4] proposal to assign Vavilovia strains into a separate symbiovar included in bv. *viciae*.

Thus, when studying the host specificity of the species *Rhizobium leguminosarum*, we showed cross-inoculation of biovars *viciae* and *trifolii*, which leads to the formation of nodules that do not fix N_2, having either a normal or an abnormal (tumor-like) structure, and in the second case, infection of plant tissues by bacteria is blocked. Based on the data obtained, a hypothetical scheme of the evolution of the species *R. leguminosarum* was constructed and for the first time a hypothesis is proposed about the emergence of the biovar *trifolii* (which retaines primitive features of the genome organization of this species) through changes in host specificity of ancestral bv. *viciae* strains. The ancestral strains of the bv. *viciae* isolated from the nodules of the relict legume Vavilovia exhibit the highest cross-inoculation activity with clover, indicating that broad host specificity, which in rhizobia is usually combined with low N_2-fixing activity, may be a property that significantly limits the agronomic potential of production strains. Since a significant number of ancestral genetic elements were previously identified in symbionts of the evolutionarily advanced legume tribe *Fabeae*, it is relevant to identiy these elements in the genomes of economically valuable strains. The presence of the *fix*W gene which prevents the full development of bacteroids, limiting their N_2-fixing activity, requires special attention. Since changes in the organization of *sym* genes that occurred during the transition from the ancestral genomic group A to the evolutionarily advanced group D are associated with an increase in the symbiotic activity of bacteria, it is obvious that introducing similar changes into the genomes of commercial strains is of great biotechnological interest. It is obvious that targeted modifications of the genomes of practically valuable *R. leguminosarum* bv. *viciae* strains by editing ancestral genomic elements will help improve the effectiveness of biologicals based on these bacteria.

REFERENCES

1. Sprent J.I., Ardley J., James E.K. Biogeography of nodulated legumes and their nitrogen-fixing symbionts. *New Phytol.*, 2017, 215(1): 40-56 (doi: 10.1111/nph.14474).
2. Haag A.F., Arnold M.F., Myka K.K., Kerscher B., Dall'Angelo S., Zanda M., Mergaert P.,

Ferguson G.P. Molecular insights into bacteroid development during *Rhizobium*-legume symbiosis. *FEMS Microbiology Reviews*, 2013, 37(3): 364-383 (doi: 10.1111/1574-6976.12003).
3. Provorov N.A. The interdependence between taxonomy of legumes and specificity of their interaction with rhizobia in relation to evolution of the symbiosis. *Symbiosis*, 1994, 17: 183-200.
4. Kimeklis A.K., Kuznetsova I.G., Sazanova A.L., Safronova V.I., Belimov A.A., Onishchuk O.P., Kurchak O.N., Aksenova T.S., Pinaev A.G., Musaev A.M., Andronov E.E., Provorov N.A. *Genetika*, 2018, 54(7): 851-855 (in Russ.).
5. Kimeklis A.K., Chirak E.R., Kuznetsova I.G., Sazanova A.L., Safronova V.I., Belimov A.A., Onishchuk O.P., Kurchak O.N., Aksenova T.S., Pinaev A.G., Andronov E.E. Provorov N.A. Rhizobia isolated from the relict legume *Vavilovia formosa* represent a genetically specific group within *Rhizobium leguminosarum* bv. *viciae*. *Genes*, 2019, 10(12): 991 (doi: 10.3390/genes10120991).
6. Chirak E.R., Kimeklis A.K., Karasev E.S., Kopat V.V., Safronova V.I., Belimov A.A., Aksenova T.S., Kabilov M.R., Provorov N.A., Andronov E.E. Search for ancestral features in genomes of *Rhizobium leguminosarum* bv. *viciae* strains isolated from the relict legume *Vavilovia formosa*. *Genes*, 2019, 10(12): 990 (doi: 10.3390/genes10120990).
7. Vishnyakova M., Burlyaeva M., Akopian J., Murtazaliev R., Mikič A. Reviewing and updating the detected locations of beautiful vavilovia (*Vavilovia formosa*) on the Caucasus *sensu stricto*. *Genet. Resour. Crop Evol.*, 2016, 63: 1085-1102 (doi: 10.1007/s10722-016-0440-x).
8. Lie T.A. Symbiotic specialization in pea plants: the requirement of specific *Rhizobium* strains for peas from Afghanistan. *Ann. Appl. Biol.*, 1978, 88(3): 462-465.
9. Abicht H.K., Schärer M.A., Quade N., Ledermann R., Mohorko E., Capitani G., Hennecke H., Glockshuber R. How periplasmic thioredoxin TlpA reduces bacterial copper chaperone ScoI and cytochrome oxidase subunit II (CoxB) prior to metallation. *The Journal of Biological Chemistry*, 2014, 289(47): 32431-32444 (doi: 10.1074/jbc.M114.607127).
10. Kopat' V.V., Chirak E.R., Kimeklis A.K., Safronova V.I., Belimov A.A., Kabilov M.R., Andronov E.E., Provorov N.A. *Genetika*, 2017, 53(7): 795-804 (doi: 10.7868/S0016675817070062) (in Russ.).
11. Provorov N.A., Andronov E.E., Kimeklis A.K., Onishchuk O.P., Igolkina A.A., Karasev E.S. Microevolution, speciation and macroevolution in rhizobia: genomic mechanisms and selective patterns. *Front. Plant Sci.*, 2022, 13: 1026943 (doi: 10.3389/fpls.2022.1026943).
12. Kimeklis A.K., Safronova V.I., Kuznetsova I.G., Sazanova A.L., Belimov A.A., Pinaev A.G., Chizhevskaya E.P., Pukhaev A.R., Popov K.P., Andronov E.E., Provorov N.A. Phylogenetic analysis of *Rhizobium* strains, isolated from nodules of *Vavilovia formosa* (Stev.) Fed. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2015, 50(5): 655-664 (doi: 10.15389/agrobiology.2015.5.655eng).
13. Rumyantseva M.L., Simarov B.V., Onishchuk O.P., Andronov E.E., Chizhevskaya E.P., Belova V.S., Kurchak O.N., Muntyan A.N., Rumyantseva T.B., Zatovskaya T.V. *Biologicheskoe raznoobrazie kluben'kovykh bakteriy v ekosistemakh i agrotsenozakh. Teoreticheskie osnovy i metody* /Pod redaktsiey M.L. Rumyantsevoy, B.V. Simarova [Biological diversity of nodule bacteria in ecosystems and agrocenoses. Theoretical foundations and methods. M.L. Rumyantseva, B.V. Simarov (eds.),]. St. Petersburg, 2011 (in Russ.).
14. Lakin G.F. *Biometriya* [Biometrics]. Moscow, 1990 (in Russ.).
15. Hammer Ø., Harper D.A.T., Ryan P.D. PAST: Paleontological statistics software package for education and data analysis. *Palaeontologia Electronica*, 2001, 4(1): 1-9.
16. Andronov E.E., Onishchuk O.P., Kurchak O.N., Provorov N.A. *Mikrobiologiya*, 2014, 83(4): 500-508 (doi: 10.7868/S0026365614030033) (in Russ.).
17. Provorov N.A., Tikhonovich I.A. *Rekonstruktsiya organellogeneza* [Reconstruction of organellogenesis]. St. Petersburg, 2022 (in Russ.).
18. Onishchuk O.P., Provorov N.A., Vorob'ev N.I., Simarov B.V. Estimation of phenotypic presentations of bacterial genes, controlling the efficiency of nitrogen-fixing symbiosis with plants. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2011, 1: 32-42 (in Eng.).
19. Cevallos M.A., Encarnacion S., Leija A., Mora Y., Mora J. Genetic and physiological characterization of a *Rhizobium etli* mutant strain unable to synthesize poly-β-hydroxybutyrate. *Journal of Bacteriology*, 1996, 178(6): 1646-1654 (doi: 10.1128/jb.178.6.1646-1654.1996).
20. Mandon K., Michel-Reydellet N., Encarnacion S., Kaminski P.A., Leija A., Cevallos M.A., Elmerich C., Mora J. Poly-β-hydroxybutyrate turnover in *Azorhizobium caulinodans* is required for growth and affects *nifA* expression. *Journal of Bacteriology*, 1998, 180(19): 5070-5076 (doi: 10.1128/JB.180.19.5070-5076.1998).
21. Zatovskaya T.V. *Poluchenie i analiz Tn5-mutantov Sinorhizobium meliloti s izmenennymi poverkhnostnymi polisakharidami. Avtoreferat kandidatskoy dissertatsii* [Construction and characterization of S*inorhizobium meliloti* Tn5 mutants with altered surface polysaccharides. PhD thesis]. St. Petersburg, 2012 (in Russ.).
22. Marroqui S., Zorreguieta A., Santamaria C., Temprano F., Soberon M., Megías M., Downie A.J. Enhanced symbiotic performance by *Rhizobium tropici* glycogen synthase mutants. *Journal of Bacteriology*, 2001, 183(3): 854−864 (doi: 10.1128/JB.183.3.854-864.2001).
23. Sharypova L.A., Onishchuk O.P., Chesnokova O.N., Fomina-Eshchenko J.G., Simarov B.V. Isolation and characterization of *Rhizobium meliloti* Tn5 mutants showing enhanced symbiotic

effectiveness. *Microbiology*, 1994, 140(3): 463-470 (doi: 10.1099/00221287-140-3-463).
24. Yurgel' S.N., Sharypova L.A., Simarov B.V. *Genetika*, 1998, 34(6): 737-741 (in Russ.).
25. Sharypova L.A., Yurgel S.N., Keller M., Simarov B.V., Pühler A., Becker A. The *eff*-482 locus of *Sinorhizobium meliloti* CXM1-105 that influences symbiotic effectiveness consists of three genes encoding an endoglucanase, a transcriptional regulator and an adenylate cyclase. *Mol. Gen. Genet.*, 1999, 261(6): 1032-1044 (doi: 10.1007/s004380051052).
26. Janczarek M., Jaroszuk-Ściseł J., Skorupska A. Multiple copies of *rosR* and *pssA* genes enhance exopolysaccharide production, symbiotic competitiveness and clover nodulation in *Rhizobium leguminosarum* bv. *trifolii*. *Antonie van Leewenhoek*, 2009, 96(4): 471-486 (doi: 10.1007/s10482-009-9362-3).
27. Van Dillewijn P., Soto M., Villadas P., Toro N. Construction and environmental release of a *Sinorhizobium meliloti* strain genetically modified to be more competitive for alfalfa nodulation. *Applied and Environmental Microbiology*, 2001, 67(9): 3860-3865 (doi: 10.1128/AEM.67.9.3860-3865.2001).
28. Mavingui P., Flores M., Romero D., Martinez-Romero E., Palacios R. Generation of *Rhizobium* strains with improved symbiotic properties by random DNA amplification (RDA). *Nat. Biotechnol.*, 1997, 15: 564-569 (doi: 10.1038/nbt0697-564).
29. Sugawara M., Sadowsky M.J. Enhanced nodulation and nodule development by *nol*R mutants of *Sinorhizobium medicae* on specific *Medicago* host genotypes. *Molecular Plant-Microbe Interactions*, 2014, 27(4): 328-335 (doi: 10.1094/MPMI-10-13-0312-R).
30. Ampomah O.Y., Jensen J.B., Bhuvaneswari T.W. Lack of tregalose catabolism in *Sinorhizobium* species increases their nodulation competitiveness on certain host genotypes. *New Phytologist*, 2008, 179(2): 495-504 (doi: 10.1111/j.1469-8137.2008.02460.x).
31. Frederix M., Edwards A., Swiderska A., Stanger S., Karunakaran R., Williams A., Abbruscato P., Sanchez-Contreras M., Poole P.S., Downie J.A. Mutation of *praR* in *Rhizobium leguminosarum* enhances root biofilms, improving nodulation competitiveness by increased expression of attachment proteins. *Molecular Microbiology*, 2014, 93(3): 464-478 (doi: 10.1111/mmi.12670).
32. Pellock B.J., Cheng H.-P., Walker G.C. Alfalfa root nodule invasion efficiency is dependent on *Sinorhizobium meliloti* polysaccharides. *Journal of Bacteriology*, 2000, 182(15): 4310-4318 (doi: 10.1128/JB.182.15.4310-4318.2000).
33. Aksenova T.S., Chirak E.R., Onishchuk O.P., Kurchak O.N., Afonin A.M., Pinaev A.G., Andronov E.E., Provorov N.A. Identification of the ancestral characteristics in the genome of *Rhizobium leguminosarum* bv. *trifolii*. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2020, 55(3): 489-498 (doi: 10.15389/agrobiology.2020.3.489eng).
34. Govorov L.I. V knige: *Kul'turnaya Flora SSSR. Tom IV. Zernovye bobovye* [In: Cultural flora of the USSR. Volume IV. Cereal legumes]. Moscow, 1937: 229-336 (in Russ.).
35. Lukjanová E., Řepková J. Chromosome and genome diversity in the genus *Trifolium* (*Fabaceae*). *Plants*, 2021, 10(11): 2518 (doi: 10.3390/plants10112518).
36. Pariyskaya A.N. *Uspekhi mikrobiologii*, 1975, 10: 189-200 (in Russ.).

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 100-113
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Non traditional crops and agrotechnologies

UDC 633.91:633.913.322:581.192:577.344.3:577.355

doi: 10.15389/agrobiology.2023.1.100eng
doi: 10.15389/agrobiology.2023.1.100rus

BIOSYNTHESIS OF RUBBER AND INULIN DEPENDING ON THE SPECTRAL COMPOSITION OF LIGHT AND ACTIVITY OF THE PHOTOSYNTHETIC APPARATUS DURING AEROPONIC CULTIVATION OF *Taraxacum kok-saghys* E. Rodin

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The authors declare no conflict of interests
Acknowledgements:
Partly supported financially with the grant from the Russian Foundation for Basic Research, project No. 20-316-90032\20
Final revision received December 14, 2022
Accepted December 20, 2022

Abstract

Due to the intensive development of industry and new technologies, the demand for natural rubber is increasing. The synthetic rubber cannot replace this biopolymer due to its unique consumer and operational characteristics. Along with the traditional source of natural rubber production from the latex of Brazilian Hevea *Hevea brasiliensis* (Willd. ex A.Juss.) Müll. Arg., work is underway to obtain it from kok-saghyz plants *Taraxacum kok-saghys* E. Rodin which can be grown both in natural and controlled conditions. The determination of the most favorable light conditions, taking into account the physiological state of plants, is important to obtain a high yield of target products. In this study, we have shown for the first time an increase in the rate of rubber biosynthesis when irradiating kok-saghyz plants with light with a greater proportion of blue spectrum. The paper also describes the changes in light and dark reactions of the photosynthetic apparatus, and in sucrose and glucose accumulation in plants when changing the light regime for several hours. The aim of the work was to study the influence of light conditions on the physiological and biochemical processes and biosynthesis of rubber and inulin in kok-saghyz plants grown under controlled phytotron conditions. Kok-saghyz seeds (a collection form 391 from the VIR collection of the Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg) were germinated under sterile conditions. From days 19-20, when 3-4 true leaves appeared, the plants were grown in the aeroponic phytotron with full-spectrum light-emitting diodes (LED) of photosynthetic active radiation $PAR_{400\text{-}700\ nm}$ of 400±28 μmol photons · m^{-2} · s^{-1}. In the first chamber of the phytotron, there was a 255.6 μmol photons · m^{-2} · s^{-1} exposure for $PAR_{400\text{-}700\ nm}$ blue spectrum (BS, λ_{max} = 460 nm) and 75.6 μmol photons · m^{-2} · s^{-1} for $PAR_{600\text{-}700\ nm}$ red spectrum (RS, λ_{max} = 660 nm), with the RS/BS ratio of 0.30. In the second chamber, the RS irradiation intensity

($PAR_{600\text{-}700\ nm}$) was 259.6 μmol photons · m^{-2} · s^{-1}, the BS irradiation intensity ($PAR_{400\text{-}500\ nm}$) was 71.8 μmol photons · m^{-2} · s^{-1}, with the RS/BS ratio of 3.6. The revealed parameters of growth, photosynthetic activity, and accumulation of glucose and sucrose in leaves and rubber and inulin in roots under various spectral modes during long-term growth and with changing the irradiation mode draw us to the following conclusions. When growing plants for 28 days in phytotron chambers with an increased proportion of RS in the spectrum, the content of rubber increased 3-fold, of inulin 4.1-fold compared to the initial values. With an increase in the BS proportion in PAR, the levels of rubber and inulin rose 5.4 times and 4.6 times, respectively. Ultimately, when irradiated with light with a higher proportion of BL, plants accumulated 1.75 times more rubber. The change in the irradiation spectrum from BS to RS led to a short-term increase in the concentration of glucose and sucrose in the leaves compared to the initial values. This dependence persisted for 2 hours, after which the sucrose content did not change, but there was a decrease in glucose content. When the irradiation mode changed from BS to RS, the activity of the photosynthetic apparatus decreased, i.e., the rate of photosynthesis from 26.7 to 15.2 μmol CO_2 · m^{-2} · s^{-1} at light saturation, the rate of dark respiration from 2.80 to 2.38 μmol CO_2 · m^{-2} · s^{-1}, and the quantum yield of photosynthesis from 0.066 to 0.055. Switching from RS to BS led to opposite results. It follows from the obtained data that the change in the concentration of soluble carbohydrates in plants is associated with a change in the spectral composition of irradiation and, as a consequence, with a change in the activity of the photosynthetic apparatus. When the irradiation changed from RS to BS, there was an increase in the rate of photosynthesis and activity of photosystem II, but a decrease in the accumulation of glucose and sucrose during the first 2 hours with a return to the initial values after 3 hours.

Keywords: *Taraxacum kok-saghyz*, kok-saghyz, growth, rate of photosynthesis, dark transpiration, quantum yield of photosynthesis, rubber, inulin, LED light sources, phytotron, aeroponics

Secondary plant metabolites perform many important functions and are widely used by humans. Well-known compounds are alkaloids, polyphenols, including flavonoids and terpenoids, inulin, and rubber. Many of them are irreplaceable (artificial synthesis is impossible) and are used for medical, nutraceutical purposes, as well as in industry [1, 2]. Studying the influence of various factors on the synthesis, accumulation and localization of substances of secondary metabolism remains one of the pressing problems of modern plant physiology [3-5].

Currently, all over the world, due to the intensive development of industry and new technologies, the demand for natural rubber (NR, the 1-4-cis-polyisoprene) is increasing. NR is 91-96% polyisoprene, contains proteins, amino acids and fatty acids and cannot always be replaced with synthetic analogues. NR has unique consumer and operational characteristics (impact resistance, wear resistance, effective heat dissipation) [6-9], is highly elastic and, even under the influence of low forces, has a reversible tensile deformation of up to 1000%. The elasticity of rubber is maintained over a wide temperature range; this is its characteristic property, therefore, for example, in the automotive industry, tires are used in the production of which the composition of the materials includes up to 20% of natural rubber, while in aircraft tires its share is up to 100% [10]. NR is used in tire casings where high strength is required, and synthetic rubbers are used in tread materials to provide tire traction [11].

The main economically significant source of industrial production of NR was and remains latex from *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. [12]. However, the increasing demand for the product is becoming an incentive to search for alternative raw materials for the production of natural rubber [13, 14)] and to study the biochemical and molecular genetic aspects of its biosynthesis [15, 16]. One of the promising ones may be the rubber plant kok-saghys (*Taraxacum kok-saghys* E. Rodin), which was widely grown in the USSR in the 1930-1950s [17]. In addition to rubber, inulin can be obtained from kok-sagyz, which makes up up to 35% of the dry weight of the roots [18]. In field conditions, kok-sagyz is cultivated for 2 years. Under phytotron conditions, the period of collecting root biomass begins after 2 months of growing plants and lasts for quite a long time, during which part of the root system is periodically cut off. After each such procedure, the plants regenerate the root system to its previous size within 28-30 days,

maintaining the functions of active biosynthesis of rubber and inulin. Therefore, along with growing plants in open ground conditions, it seems possible to obtain a high yield of the target product year-round, under controlled conditions using artificial irradiation sources [19].

In kok-sagyz plants, rubber is synthesized in specialized structures, the laticifers. Part of the NR accumulates in the milky sap, mainly in the roots. By the end of the growing season, the amount of rubber in the roots increases significantly. On average, from 7 to 15% of rubber accumulates in plant roots, and in some collection samples up to 25% of the dry weight of the roots [20, 21]. In this case, the inulin content reaches 30-35% of the dry weight of the root.

To date, the light regime (intensity, spectral composition, duration of the photoperiod) for growing kok-saghyz remains unstudied, and the determination of the most favorable light conditions, taking into account the physiological state of plants, is considered as an important element in increasing the rate of biosynthesis of rubber and inulin.

The results presented in this work provide initial information about the influence of the spectral composition of irradiation on the accumulation of rubber and inulin, as well as on the activity of the photosynthetic apparatus during long-term cultivation of kok-saghyz when irradiated with light with different spectral composition. For the first time, it is shown that the rate of rubber biosynthesis increases under irradiation with a greater proportion of the blue part of the spectrum. We also revealed changes which occurred in the speed of light and dark reactions of the photosynthetic apparatus and the concentrations of sucrose and glucose when modulating the light regime during several hours.

The purpose of the work is to study the influence of light conditions on physiological and biochemical processes and the biosynthesis of rubber and inulin in kok-saghyz plants grown under controlled phytotron conditions.

Materials and methods. The studies were carried out in phytotrons with controlled light irradiation, temperature, humidity, mineral nutrition, and gas composition. Kok-sagyz plants (k-391, VIR collection, the Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg), grown from seeds under sterile conditions, on days 19-20 (3-4 true leaves) were planted in aeroponic phytotrons, in two chambers with full spectral LED irradiation in the region of photosynthetically active radiation ($PAR_{400\text{-}700\ nm}$) 400±28 μmol photons · m^{-2} · s^{-1}. In the first chamber, irradiation in the $PAR_{400\text{-}500\ nm}$ of the blue spectrum (λ_{max} = 460 nm, LED BS) was 255.6 μmol photons · m^{-2} · s^{-1}, in the $PAR_{600\text{-}700\ nm}$ of the red spectrum (λ_{max} = 660 nm, LED RS) 75.6 μmol photons · m^{-2} · s^{-1}. In the second chamber, the RS intensity of $PAR_{600\text{-}700\ nm}$ was 259.6 μmol photons · m^{-2} · s^{-1}, the BS intensity of $PAR_{400\text{-}500\ nm}$ was 71.8 μmol photons · m^{-2} · s^{-1}.

Air temperature, humidity, and CO_2 concentration were monitored using a wireless sensor E+E EE244 (E+E Elektronik, Austria) integrated into the control system in the phytotron. The CO_2 concentration in both chambers was 418±15 μmol/mol, air humidity was 60-80%, temperature was 22±1 °C. Spectral regimes of irradiation were determined using an ASENSEtek PG100N spectrometer (UPRtek Corp., Taiwan). Measurements were carried out in 0, 1, 2, and 3 hours under a stationary light regime with predominant blue or red light, as well as after changing one predominant light to another.

The rate of photosynthesis was studied using an infrared gas analyzer CPro+ (ADC BioScientific Ltd., UK) to assess CO_2 exchange directly in chambers under given light conditions for growing plants. The dependence of the rate of CO_2 exchange on light intensity was determined for 0 to 1600 μmol photons · m^{-2} · s^{-1} at the air CO_2 concentration 418±15 μmol/mol upon successively increasing the light intensity. To describe the light curve of photosynthesis, we

used the equation of an irregular hyperbola [22].

The activity of photosystem II (PSII) was determined by the variable fluorescence method using a compact minifluorimeter JUNIOR-PAM (Heinz Walz GmbH, Germany). Leaves of kok-saghyz plants were left in the dark for 15 min, then illuminated with flashes of light [23, 24]. The functional state of PSII, the maximum quantum yield of PSII, the effective quantum yield of PSII at the irradiation intensity duirng the fluorescence measurement, non-photochemical fluorescence quenching which estimates the part of the energy that is used by the plant for non-photochemical reactions, the relative rate of electron transport as an indirect indicator of the rate of photosynthesis were determined.

For quantitative analysis of glucose and sucrose, the middle parts (0.5 g) of fully developed kok-sagyz leaves (without the central vein) were homogenized manually in a mortar with 4.5 ml of water, and then centrifuged for 20 min at 6000 rpm (FC5718, OHAUS Corporation, USA). The resulting extract was used to quantify water-soluble low molecular weight sugars. It was previously shown that there are no low molecular weight carbohydrates in the sediment.

To quantify the glucose concentration, the standard Glucose-Novo kit (Vector-Best, Russia) for the analysis of liquid substrates was used. After adding reagents to the extract, it was incubated for 20 minutes at 50 °C. The optical density (OD) of the solution was measured at $\lambda = 508$ nm and $\lambda = 343$ nm (a spectrophotometer UV2600, Shimadzu, Japan) and the glucose concentration was calculated. The GOPOD (glucose oxidase peroxidase) method was used based on measurement of the color resulting from the reaction of the chromogen with hydrogen peroxide, which, in turn, serves as a product of glucose oxidation with atmospheric oxygen in the presence of glucoperoxidase [25].

When determining the amount of sucrose, the enzyme invertase (Available Carbohydrates Assay Kit, Megazyme cat. no. K-AVCHO, Megazyme, Ltd., Ireland) was added to the extract to hydrolyze sucrose into glucose and fructose. Then the sample was incubated for 30 min at 30 °C and pH 6.5. After the hydrolysis reaction was completed, free glucose and glucose formed during the enzymatic hydrolysis of sucrose were specifically stained. To determine the sucrose concentration, the difference between the obtained concentration and the previously determined free glucose concentration was calculated [26].

The rubber content was assessed gravimetrically. The rubber was isolated as described by D.A. Ramirez-Cadavid et al. [27]. Freshly collected roots were washed with water, dried for 24 hours at 65 °C, ground in a mortar, and the powder was sifted on a 1×1 mm sieve. A sample of root powder (300 mg) was used for extraction. Extraction with chloroform (40-45 ml, grade CP, OOO TD HIMMED, Russia) was carried out (a Soxhlet extractor, OOO MLS Klin, Russia) for 6 hours. The resulting extract was concentrated by evaporation at 75 °C to a residual volume of 3-5 ml. Rubber from an aliquot of the extract was precipitated with a triple volume of ethanol at 4 °C for 15-16 hours in a tared microtube. The precipitate was separated by centrifugation for 20 min at 14,500 rpm (Eppendorf 5424, Eppendorf, Germany). Associated substances were removed from the rubber sediment by sequentially washing the sediment with distilled water and then with acetonitrile (Sigma, USA) followed by centrifugation. Residues of water and acetonitrile were removed by washing the precipitate three times with ethyl alcohol, followed by centrifugation. The purified rubber was dried by blowing with argon. Based on the dry root biomass, the percentage of rubber from the mass of the sediment was calculated.

To determine the amount of inulin, the roots were washed with water, dried for 24 hours at 65 °C, ground in a mortar, and the powder was sifted on a 1×1 mm sieve. A water bath was used for aqueous extraction of inulin (85-90 °C

for 60 minutes) from a 200 mg sample of root powder. The suspension was centrifuged at 14,500 rpm for 5 min or filtered through a glass fiber membrane with a pore size of 1 μm. Acid hydrolysis of inulin was carried out with 10% HCl in a 1:1 ratio (sample:acid) for 60 minutes in a boiling (100 °C) water bath. The Nelson-Somogyi method was used (GOST R 54905-2012. M., 2013) to determine reducing sugars before and after hydrolysis (a UV-1202 spectrophotometer, Shimadzu, Japan) [28].

Experiments were performed in 4-5-fold analytical and 3-fold biological replicates. The general pattern did not change depending on the experimental variants; therefore, the results are presented based on data from one biological replicate. Statistical processing of the results was carried out using the Microsoft Excel software package. The figures and table show the arithmetic mean values (*M*) with standard error (±SEM). The significance of differences was determined by Student's *t*-test at $P = 0.95$.

Results. Light is a key factor in the growth and development of plants whose photoreceptors evaluate the quality (spectral composition) and quantity (intensity) of light and adapt to thse parameters. Light signals, sensed primarily by phytochromes and cryptochromes, regulate plant growth, metabolic, and morphogenetic responses [29].

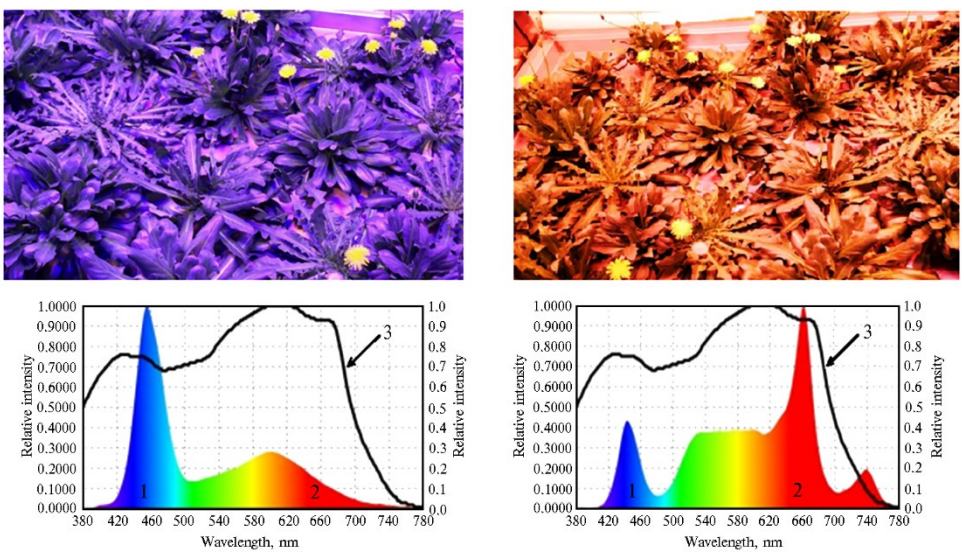

Fig. 1. Spectral characteristics of LED irradiators during long-term aeroponics cultivation of kok-saghys plants (*Taraxacum kok-saghys* E. Rodin) in the chambers of an aeroponic phytotron: 1 — $PAR_{400\text{-}500\ nm}$ of the blue spectrum, 2 — $PAR_{600\text{-}700\ nm}$ of the red spectrum, 3 — curve dependence of the intensity of photosynthesis on the wavelength of incident light (action spectrum of photosynthesis according to K.J. McCree). The radiation intensity was measured at the landing field level.

An increase in the proportion of blue light (Fig. 1, on the left) or red light (see Fig. 1, on the right) when growing kok-saghyz plants in the chambers of the aeroponic phytotron led to a change in the RS/BS ratio in the irradiation spectrum. The RS/BS ratio in the first cultivation chamber was 0.30, in the second chamber 3.60. There were practically no differences in the rate of photosynthesis per unit leaf area, 9.4 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ at the initial RS (Fig. 2, A, 0 RS) and 10.9 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ at the initial BS (see Fig. 2, B, 0 BS), respectively.

Changing the light regime from a predominantly red spectrum (RS) to a predominantly blue spectrum (BS) in 1 hour after the switch led to a decrease in the rate of photosynthesis, and in 2 hours and onwards to its increase compared to the initial values (see Fig. 2, A). With the opposite change in the light regime

(from BS to RS), the changes differed. A slight increase in the rate of photosynthesis was followed by a noticeable decrease, but in 3 and 4 hours, the rate of photosymthesis increased, although not as notable as when the spectral mode changed from RS to BS (see Fig. 2, B).

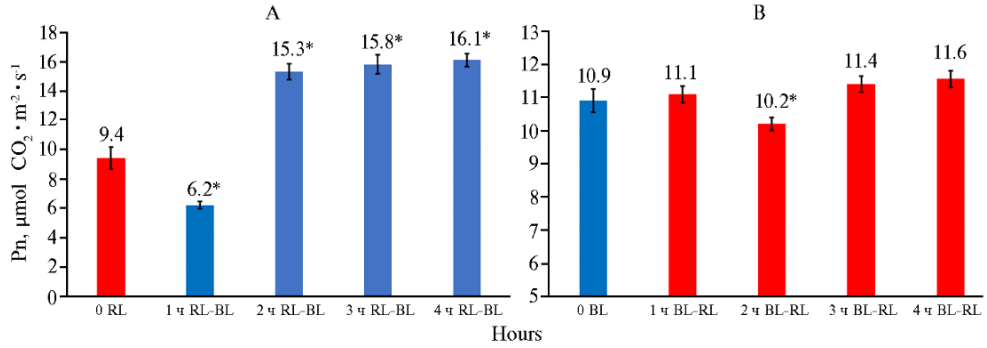

Fig. 2. The rate of photosynthesis in the leaves of kok-saghys plants (*Taraxacum kok-saghys* E. Rodin) during long-term cultivation by aeroponics in the chambers of an aeroponic phytotron at a light intensity of 400 μmol photons · m^{-2} · s^{-1} with a predominance of red light (RS) (A) **or blue light** (BS) (B) **in the irradiation spectrum, as well as when the irradiation spectrum changes from RS to BS** (A) **and from BS to RS** (B) (*M*±SEM, *n* = 5).
* Differences from RS (A) and BS (B) are statistically significant at P = 0.95.

To clarify in more detail the nature of the influence of a change in the spectral regime on the activity of the photosynthetic apparatus, light curves of the rate of plant photosynthesis were measures.

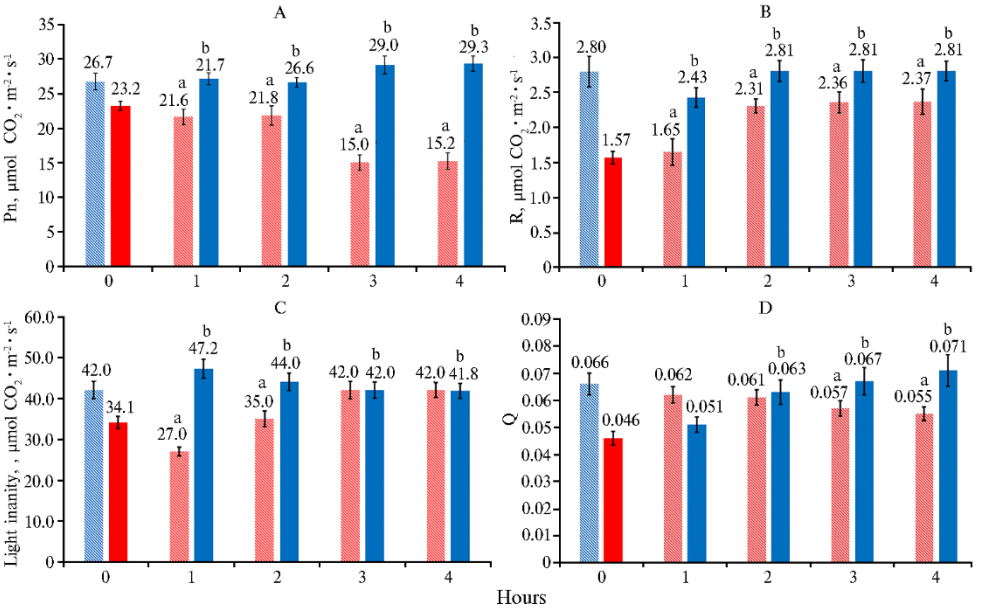

Fig. 3. The rate of photosynthesis on the plateau of the light curve (A)**, the rate of respiration** (B)**, the light compensation point** (C) **and the quantum yield of photosynthesis** (D) **in the leaves of kok-saghys (*Taraxacum kok-saghys E. Rodin*) plants during long-term aeroponic cultivation in chambers of an aeroponic phytotron at a light intensity of 400 μmol photons · m^{-2} · s^{-1} with a predominance of blue light** (BS, left columns of the leftmost pair) **or red light** (RS, right columns of the leftmost pair)**, and when the irradiation spectrum changes from BS to RS** (left bars of the remaining pairs) **and from RS to BS** (right bars of the remaining pairs) (*M*±SEM, *n* = 6).
[a, b] The differences from the RS option at the starting point and from the BS option at the starting point, respectively, are statistically significant at P = 0.95.

When changing the irradiation mode from BS to RS, the rate of photo-

synthesis decreased from 26.7 to 15.2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ at the plateau of the light curve (at light saturation) (Fig. 3, A). When switching from BS to RS, there was also a decrease in the rate of dark respiration from 2.80 to 2.38 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ (see Fig. 3, B) and in the quantum yield of photosynthesis from 0.066 to 0.055 (see Fig. 3, D). Changing the light regime from RS to BS increased the rate of photosynthesis at the photosynthesis light curve saturation, the rate of dark respiration, the quantum yield of photosynthesis, and the light compensation point.

The observed changes in the activity of the photosynthetic apparatus may be associated with the activity of the light stage of photosynthesis. Thus, changing the regime from RS to BS initially led to a decrease in the maximum quantum yield of PSII, the effective quantum yield, and the electron transport rate, but non-photochemical fluorescence quenching increased (Fig. 4). After 3 hours, the values were comparable to those in plants irradiated predominantly with RS before changing the light regime.

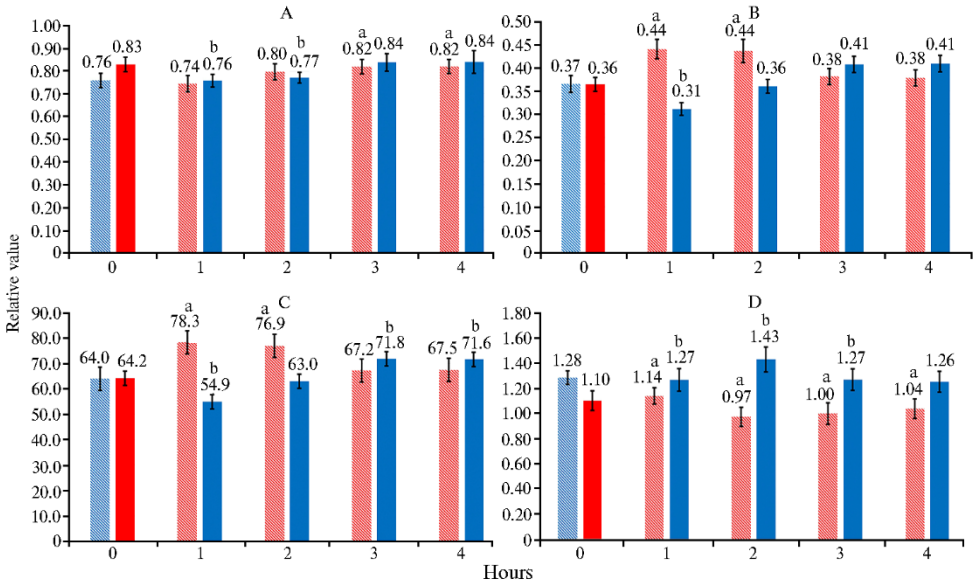

Fig. 4. Maximum quantum yield of PSII (A)**, effective quantum yield** (B)**, electron transport rate** (C) **and non-photochemical quenching** (D) **in leaves of kok-saghys (*Taraxacum kok-saghys* E. Rodin) plants during long-term aeroponic cultivation in chambers of an aeroponic phytotron at a light intensity of 400 μmol photons $\cdot m^{-2} \cdot s^{-1}$ with a predominance of blue light** (BS, left columns of the leftmost pair) **or red light** (RS, right columns of the leftmost pair) **and when the irradiation spectrum changes from BS to RS** (left bars of the remaining pairs) **and fro**m RS to BS (right bars of the remaining pairs) (*M*±SEM, *n* = 6)**.**

[a, b] The differences from the RS option at the starting point and from the BS option at the starting point, respectively, are statistically significant at P = 0.95.

Changing the spectral mode from BS to RS led to a gradual increase in the maximum quantum yield of PSII, an increase in the effective quantum yield and electron transport rate during the first 2 hours, and a decrease in non-photochemical fluorescence quenching. After 3 hours, the values were the same or lower (e.g., for non-photochemical quenching) compared to those for plants irradiated predominantly with BS before changing the light regime.

The mechanisms by which certain parts of the spectrum and light irradiation regimes affect the primary and secondary metabolism of plants differ. The influence of the irradiation spectrum when growing plants is manifested both in changes in the operation of the photosynthetic apparatus and in the direction of secondary metabolism reactions. Thus, a change in the irradiation spectrum of

plants from BS to RS led to an increase in the concentration of glucose and sucrose compared to the initial values. This dependence persisted for 2 hours, then the concentration of sucrose remained at the same level, and the glucose content decreased. When switching from RS to BS, the opposite effect occurred. At first, the concentrations of glucose and sucrose decreased and then increased (Fig. 5).

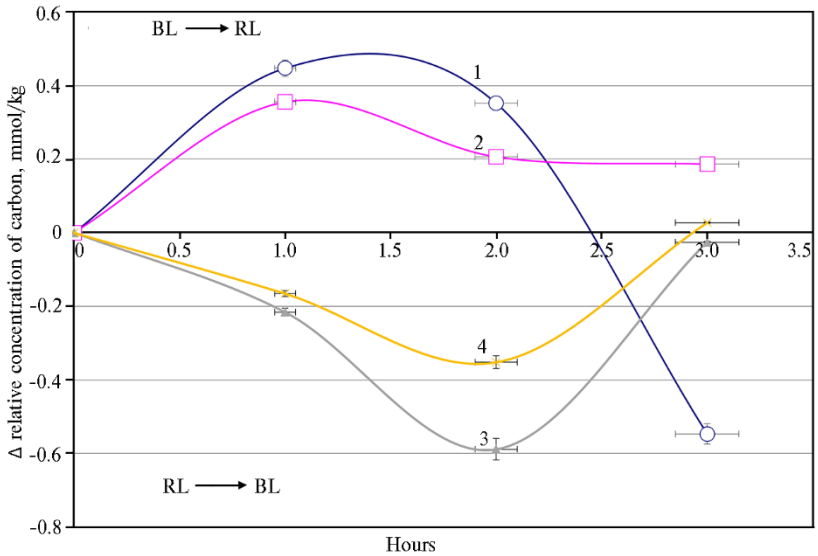

Fig. 5. Changes in the concentration of glucose (1, 3) **and sucrose** (2, 4) **in the leaves of kok-saghys (*Taraxacum kok-saghys* E. Rodin) plants during long-term aeroponic cultivation in the chambers of an aeroponic phytotron at a light intensity of 400 μmol photons · m^{-2} · s^{-1} with a predominance of red light** (RS) **or blue light (BL) after changing the irradiation spectrum from BS to RS** (1, 2) **and from RS to BS** (3, 4) (*M*±SEM, *n* = 8).

Soluble carbohydrates are plant metabolic substrates important for various physiological and biochemical processes, regulating carbon transport and incorporation into metabolism. In plant leaves, about half of the fixed carbon is transported in the form of sucrose or glucose to the stem and roots where they are converted into polysaccharides, and in kok-saghyz mainly into inulin. Along with the accumulation of inulin, the synthesis and accumulation of isopentenyl pyrophosphate (IPP), a rubber monomer, occurs. Plants use two pathways for PPI biosynthesis, through mevalonate (MVA) and methylerythritol (MEP). The mevalonate pathway occurs in the cytosol of the cell, the methylerythritol pathway in the plastids. Isopentyl pyrophosphate biosynthetic enzymes in both pathways convert intermediates generated from the metabolism of sucrose via pyruvate and glyceraldehyde-3-phosphate or via acetyl-CoA, respectively, for the MEP and MVA pathways [30, 31]. In the cytoplasmic MVA pathway, the main substrate is cytosolic acetyl-CoA, derived from either sucrose or glucose and fructose. Changing the biosynthesis of these products [32, 33] can be an effective lever for regulating the growth and accumulation of biomass by kok-saghyz plants, and ultimately the synthesis and accumulation of inulin and rubber.

Active accumulation of these compounds occurred in the roots of kok-sagyz plants grown in the chambers of an aeroponic phytotron for 28 days (Table). When plants were irradiated with a higher proportion of RS, there was an increase in the rubber and inulin contents by 3.0 and 4.1 times, resprctively, with a higher proportion of BS, by 5.4 and 4.6 times compared to the initial values before planting in the phytotron. The effect of the spectral composition on day 28 was reliably expressed in an increase in the rubber content in plants grown under a higher proportion of blue light.

The content of rubber and inulin in the roots of kok-saghys (*Taraxacum kok-saghy*s E. Rodin) plants during long-term aeroponic cultivation in the chambers of an aeroponic phytotron at a light intensity of 400 μmol photons · m^{-2} · s^{-1} with a predominance of red light (RS) **or blue light** (BS) ($M \pm$SEM, $n = 5$)

Light intensity in phytotron	Time	Rubber, %	Инулин, %
400 μmol photons · m^{-2} · s^{-1} with a larger proportion of RS in the irradiation spectrum	Planting in a phytotron	2.0±0.1	3.8±0.1
	28 days	5.9±0.2*	15.6±1.1*
400 μmol photons · m^{-2} · s^{-1} with a larger proportion of BS in the irradiation spectrum	Planting in a phytotron	1.9±0.1	4.0±0.1
	28 days	10.3±0.3*	18.3±1.2*

* Differences from values at planting are statistically significant at P = 0.95.

The aeroponic phytotronic technologies under defined cultivation parameters (temperature, humidity, CO_2 content, light intensity and spectral composition, mineral nutrition, etc.) create a strategy for controlling plant metabolism depending on the growth stage and/or the feasibility of choosing a priority pathway for the directed biosynthesis of secondary metabolites, rubber and inulin.

According to our results, light with a predominance of red or blue components affects the biosynthesis of soluble sugars, rubber and inulin in kok-saghyz plants. An increase in rubber biosynthesis under the influence of BS may be associated with an adjustment in the metabolic pathways of carbohydrate metabolism due to the specific effect of BS on the photosynthetic apparatus.

As it was shoen, an increase in the synthesis of soluble carbohydrates or proteins when plants are irradiated predominantly in the red or blue spectrum [34] causes a change in the rate of appearance and growth of new leaves, and in the ratio of the biomass of the above-ground parts of plants and the root system [35]. When plants are irradiated with BS, photosynthesis products are not used for rapid leaf growth, therefore, sucrose or glucose flow into the roots where, after a series of biochemical reactions in the cytoplasmic MVA pathway, secondary metabolic products are synthesized. In our experiments, when BS predominated in the radiation spectrum, a greater accumulation of rubber in the roots occurred compared to the RS predominance.

Along with long-term exposure of plants to RS or BS, periodic changes in the irradiation spectrum can also occur [36], which affects the rate of synthesis and the accumulation and transport of photosynthetic products. In our tests, a change in the spectral mode of irradiation of kok-sagyz plants for several hours from BS to RS did not cause a decrease in the rate of photosynthesis (see Fig. 2), however, there was an increase in the rate of electron transport and the real quantum yield, as well as a decrease in non-photochemical quenching of fluorescence. More efficient PSII operation increases the concentration of glucose and sucrose in the leaves.

When switching from RS to BS, the rate of photosynthesis sharply decreased within 1 hour, after which (after 2-3 hours) there was an increase in the rate of CO_2 absorption, a decrease in the quantum yield of PSII and the rate of electron transport. Non-photochemical quenching also increased at the very beginning of the transition from RS to BS, then, the values returned to its original figures. Under these conditions, a decrease in the concentration of glucose and sucrose in the leaves was noted during the first 2 hours, followed by a return to initial values after 3 hours (see Fig. 5).

A change in the activity of the photosynthetic apparatus in response to modulation of the spectral composition of irradiation develops over several hours, and this technique can be used to regulate the rate of metabolic processes and the yield of final products. A similar approach can also be used to increase the accumulation of products of secondary metabolism of kok-saghyz plants, primarily rubber. However, further research is required to understand the key factors influencing the biosynthesis of target substances, as well as the study of gene expression and protein synthesis involved in rubber biosynthesis. In combination

with genetic engineering methods for improving the productivity of rubber plants, this will significantly increase the yield of rubber and inulin to economically acceptable values. The comprehensive processing of all grown plant biomass to obtain various valuable products will undoubtedly play a major role in the commercialization of aeroponic cultivation of kok-saghyz.

Thus, changes in the light regime during long-term cultivation of kok-saghyz plants in the controlled conditions of the phytotron influenced the activity of light processes of the photosynthetic apparatus, i.e., the maximum and effective quantum yield, the rate of electron transport, and the rate of carbon dioxide absorption. An increase in the proportion of red light (RS) in the irradiation spectrum of plants grown under the predominance of blue light (BS) led to an increase in the rate of electron transport and effective quantum yield, and to a decrease in non-photochemical quenching of fluorescence after 3 hours. The data obtained indicate that photosynthetic apparatus can utilize red light more effectively. When switching from RS to BS, an increase in the rate of photosynthesis and respiration occurs. When changing the irradiation regime from BS to RS, on the contrary, the rate of photosynthesis decreased from 26.7 to 15.2 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$ (at light saturation), the rate of dark respiration decreased from 2.80 to 2.38 μmol $CO_2 \cdot m^{-2} \cdot s^{-1}$, quantum yield of photosynthesis from 0.066 to 0.055. Higher proportion of BS in the irradiation spectrum increases the accumulation of rubber in the kok-sagyz roots 1.75-fold compared to plants grown under irradiation with greater level of the RS. In addition, a 1.17-fold increase in inulin accumulation occurs. The kok-saghyz growing technology based on these effects seems very promising to ensures the biosynthesis of rubber and inulin under controlled phytotron conditions with the optimization of all growth factors.

REFERENCES

1. Evstaf'ev S.N., Tiguntseva N.P. *Izvestiya vuzov. Prikladnaya khimiya i biotekhnologiya*, 2014, 1(6) (in Russ.).
2. Tikhomirova L.I., Bazarnova N.G., Il'icheva T.N., Martirosyan Yu.Ts., Afanasenkova I.V. *Khimiya rastitel'nogo syr'ya*, 2018, 4: 235-245 (in Russ.).
3. Verma N., Shukla S. Impact of various factors responsible for fluctuation in plant secondary metabolites. *Journal of Applied Research on Medicinal and Aromatic Plants*, 2015, 2(4): 105-113 (doi: 10.1016/j.jarmap.2015.09.002).
4. Yang L., Wen K.-S., Ruan X., Zhao Y.-X., Wei F., Wang Q. Response of plant secondary metabolites to environmental factors. *Molecules*, 2018, 23: 762 (doi: 10.3390/molecules23040762).
5. Jan R., Asaf S., Numan M., Kim K.M. Plant secondary metabolite biosynthesis and transcriptional regulation in response to biotic and abiotic stress conditions. *Agronomy*, 2021, 11(5): 968 (doi: 10.3390/agronomy11050968).
6. Wei Y.-C., Liu G.-X., Zhang L., Zhao F., Liao S., Luo M.-C. Exploring the unique characteristics of natural rubber induced by coordination interaction between proteins and Zn^{2+}. *Polymer*, 2020, 193: 122357 (doi: 10.1016/j.polymer.2020.122357).
7. Gronover S.C., Wahler D., Prufer D. Natural rubber biosynthesis and physic-chemical studies of plant derived latex. In: *Biotechnology of biopolymers*. M. Elnashar (ed.). InTech, Rijeka, 2011: 75-88 (doi: 10.5772/17144).
8. Cornish K. Biochemistry of natural rubber, a vital raw material, emphasizing biosynthetic rate, molecular weight and compartmentalization, in evolutionarily divergent plant species. *Natural Product Report*, 2001, 18(2): 182-189 (doi: 10.1039/a902191d).
9. Cornish K. Alternative natural rubber crops: why should we care? *Technology and Innovation*, 2017, 18: 245-256 (doi: 10.21300/18.4.2017.245).
10. Yager T.J., McCarty J.L. Friction characteristics of three 30 × 11.5-14.5, Type VIII, aircraft tires with various tread groove patterns and rubber compounds. *NASA Technical Paper*, 1977, 100: 1.
11. Araujo-Morera J., Verdejo R., López-Manchado M.A., Santana M.H. Sustainable mobility: the route of tires through the circular economy model. *Waste Management*, 2021, 126: 309-322 (doi: 10.1016/j.wasman.2021.03.025).
12. Ahrends A., Hollingsworth P.M., Ziegler A.D., Fox J.M., Chen H., Su Y., Xu J. Current trends of rubber plantation expansion may threaten biodiversity and livelihoods. *Global Environmental Change*, 2015, 34: 48-58.

13. Amerik A.Yu., Martirosyan L.Yu., Martirosyan V.V., Martirosyan Yu.Ts. *Parthenium argentatum* A. Gray, *Taraxacum kok-saghyz* L.E. Rodin, and *Scorzonera tau-saghyz* Lipsch. et Bosse as alternative sources of natural rubber: do we really need them? *Sel'skokhozyaistvennaya Biologiya* [*Agricultural Biology*], 2022, 57(1): 3-26 (doi: 10.15389/agrobiology.2022.1.3eng).
14. Amerik A.Yu., Martirosyan Yu.Tc., Gachok I.V. Regulation of natural rubber biosynthesis by proteins associated with rubber particles. *Russian Journal of Bioorganic Chemistr*y, 2018, 44(2): 140-149 (doi: 10.1134/S106816201801003X).
15. Amerik A.Y., Martirosyan Y.T., Martirosyan L.Y., Goldberg V.M., Uteulin K.R., Varfolomeev S.D. Molecular genetic analysis of natural rubber biosynthesis. *Russian Journal of Plant Physiology*, 2021, 68(1): 31-45 (doi: 10.1134/S1021443721010039).
16. Ramirez-Cadavid D.A., Cornish K., Michel Jr F.C. *Taraxacum kok-saghyz* (TK): Compositional analysis of a feedstock for natural rubber and other bioproducts. *Industrial Crops and Products*, 2017, 107: 624-640 (doi: 10.1016/j.indcrop.2017.05.043).
17. Prokof'ev A.A. *Sovetskie kauchuki*, 1934, 3: 4 (in Russ.).
18. Cherian S., Ryu S.B., Cornish K. Natural rubber biosynthesis in plants, the rubber transferase complex, and metabolic engineering progress and prospects. *Plant Biotechnology Journal*, 2019, 17(11): 2041-2061 (doi: 10.1111/pbi.13181).
19. Martirosyan L.Yu., Martirosyan Yu.Ts., Varfolomeev S.D., Gol'dberg V.M. *Sposob aeroponnogo vyrashchivaniya kauchukonosnogo rasteniya kok-sagyz Taraxacum kok-saghyz R. Patent RF № 2779988. Opubl. 16.09.2022. Byul. № 26* [The method of aeroponic cultivation of the rubber plant kok-saghyz *Taraxacum kok-saghyz* R. Patent of the Russian Federation No. 2779988. Publ. 09/16/2022. Bull. No. 26] (in Russ.).
20. Kutuzova S.N., Petrosyan I.A. *Trudy po prikladnoy botanike, genetike i selektsii*, 2011, 167: 125-132 (in Russ.).
21. Kutuzova S.N., Brach N.B., Kon'kova N.G., Gavrilova V.A. *Mezhdistsiplinarnyy nauchnyy i prikladnoy zhurnal «Biosfera»*, 2015, 7(3): 391-401 (in Russ.).
22. Prioul J.L., Chartier P. Partitioning of transfer and carboxylation components of intracellular resistance to photosynthetic CO_2 fixation: a critical analysis of the methods used. *Annals of Botany,* 1977, 41(4): 789-800 (doi: 10.1093/oxfordjournals.aob.a085354).
23. Junior-PAM chlorophyll fluorometer: operator's guide. E. Pfündel (ed.). Germany, Heinz Walz GmbH, 2007.
24. Gol'tsev V.N., Kaladzhi Kh.M., Kuzmanova M.A. Allakhverdiev S.I. *Peremennaya i zamedlennaya fluorestsentsiya khlorofilla a — teoreticheskie osnovy i prakticheskoe prilozhenie v issledovanii rasteniy* [Variable and delayed fluorescence of chlorophyll a — theoretical foundations and practical application in plant research]. Izhevsk-Moscow, 2014 (in Russ.).
25. Vorob'ev V.N., Yakushenkova T.P., Vorob'ev G.V. *Praktikum po fiziologii i biokhimii rasteniy. Fotosintez: uchebno-metodicheskoe posobie* [Workshop on physiology and biochemistry of plants. Photosynthesis]. Kazan', 2013 (in Russ.).
26. McCleary B.V., McLoughlin C. Measurement of available carbohydrates in cereal and cereal products, dairy products, vegetables, fruit and related food products and animal feeds. *Journal of AOAC INTERNATIONAL*, 2021, 104(6): 1465-1478 (doi: 10.1093/jaoacint/qsab019).
27. Ramirez-Cadavid D.A., Valles-Ramirez S., Cornish K., Michel Jr. F.C. Simultaneous quantification of rubber, inulin, and resins in *Taraxacum kok-saghyz* (TK) roots by sequential solvent extraction. *Industrial Crops and Products*, 2018, 122: 647-656 (doi: 10.1016/j.indcrop.2018.06.008).
28. Nelson N. A photometric adaptation of the somogyi method for determination of glucose. *J. Biol. Chem.*, 1944, 153: 375-380.
29. Voytsekhovskaya O.V. *Fiziologiya rasteniy*, 2019, 66(3): 163-177 (doi: 10.1134/S0015330319030151) (in Russ.).
30. Whitted M.G., Geher J.F., Benko A.D., Crvin A.M., Chotani K.G., Mcauliffe C.J., LaDuca J.R., Ben-Shoshan A.E., Sanford J.K. Technology update: development of a gas-phase bioprocess for isoprene-monomer production using metabolic pathway engineering. *Industrial Biotechnology*, 2010, 6(3): 152-163 (doi: 10.1089/ind.2010.6.152).
31. Lange I., Poirier B.C., Herron B.K., Lange B.M. Comprehensive assessment of transcriptional regulation facilitates metabolic engineering of isoprenoid accumulation in *Arabidopsis. Plant Physiology*, 2015, 169(3): 1595-1606 (doi: 10.1104/pp.15.00573).
32. Lastdrager J., Hanson J., Smeekens S. Sugar signals and the control of plant growth and development. *Journal of Experimental Botany*, 2014, 65(3): 799-807 (doi: 10.1093/jxb/ert474).
33. Ruan Y.L. Sucrose metabolism: gateway to diverse carbon use and sugar signaling. *Annual Review of Plant Biology*, 2014, 65: 33-67 (doi: 10.1146/annurev-arplant-050213-040251).
34. Voskresenskaya N.P. V knige: *Fiziologiya fotosinteza* [In: Physiology of photosynthesis]. Moscow, 1982: 203-220 (in Russ.).
35. Spalholz H., Hernández R. Transplant lettuce response to different blue:red photon flux ratios in indoor LED sole-source lighting production. *Acta Horticulturae*, 2018, 1227: 555-562 (doi: 10.17660/ActaHortic.2018.1227.70).
36. Martirosyan Yu.Ts., Martirosyan L.Yu., Kosobryukhov A.A. Dynamic regulation of photosynthetic processes under variable spectral led irradiation of plants. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2019, 54(1): 130-139 (doi: 10.15389/agrobiology.2019.1.130eng).

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 114-141
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.8:58.144:58.01:57.017.6:574.45:581.192

doi: 10.15389/agrobiology.2023.1.114eng
doi: 10.15389/agrobiology.2023.1.114rus

EXPERIENCE OF *Rhaponticum carthamoides* (Willd.) Iliin CULTIVATION AS A NATURAL SOURCE OF ECDYSTERONE UNDER THE CONDITIONS OF THE ARKHANGELSK REGION

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Final revision received May 15, 2022
Accepted July 7, 2022

Abstract

In 2021, the global market for ecdysterone-containing substances amounted to $100 billion and is expected to grow significantly in the next 5 years. The aboveground and underground parts of *Rhaponticum carthamoides* (Willd.) Iliin are primarily suitable to obtain ecdysterone-containing products of pharmaceutical quality. However, both in Russia and abroad, common technologies for *R. carthamoides* cultivation and study are not focused on the quality of the medicinal raw materials. In this paper, we have implemented for the first time an alternative technology for the production of ecdysterone-containing substance from the leaf parts of *R. carthamoides*. The technology is simple and can be scaled up in agropopulations of the Northern European Russia and meets the key requirements for industrial raw materials and end ecdysterone products set by international experts. Our goal was to summarize 32 years of experience in growing *R. carthamoides* on a plantation located in the European Northeast of Russia (Arkhangelsk Province). We assessed the potential for longevity and productivity of the agricultural population by life cycle stages and age, the regularities of ecdysterone accumulation in annually harvested aboveground plant parts, and the quality of obtained plant raw material for the content of standardized substances. The study was performed in the southeast of the Arkhangelsk Province (the Middle Taiga subzone, Kotlass District; 61°20′N, 47°E) in an agropopulation of *R. carthamoides* (the field area of 1 ha) in 1989-2022. The seeds obtained from the Botanical Garden of the Komi Scientific Center, UB RAS (Syktyvkar) were initially originated from the Altai natural population (first collected in 1956). Autumn sowing was performed in mid-October after the beginning of autumn frosts (row spacing of 70 cm, seed embedding depth of 2-3 cm, seed rate of 2.7 kg/ha; 58 % field germination of seeds). Mineral fertilizers (NPK_{60-90}) were applied during the first three years after sowing followed by organic farming without use of mineral and organic fertilizers, chemical pesticides, and plant growth regulators. The aboveground parts were annually harvested during budding. A set of population, agrochemical, morphoanatomical, biochemical and statistical methods were used to asses parameters of the plant population (age states in ontogenesis, population density, gross production of above-ground and under-ground organs, seed yield) and individual plants (growth, development, morphological structure, productivity of roots, leaves, and seeds). Samples were collected at optimal phases of plant development, the aboveground phytomass during budding (I-II decade of June), rhizomes in autumn, after the end of vegetation (October), or in early spring, before the beginning of vegetation (April). Shortened vegetative (rosette) and stem generative (reproductive) shoots with inflorescences were morphologically heterogeneous organs separated in the aboveground part. Industrial harvesting of plant raw materials was carried out in late May–early June, during budding. Plant material (organs, elements and fractions) was dried at 23-25 to 35-40 °C and relative humidity of 25-40 % in accordance with the procedure for harvesting and drying medicinal raw materials. The samples of air-dry raw materials for further determination of the content of primary and secondary metabolites were formed by quartering method. The amount of ecdysterone in dry samples was determined by reverse phase high performance liquid chromatography with the internal standards (a liquid microcolumn chromatograph Milichrom-5, column 80×2 mm, Nucleosil C18 sorbent with a particle size of 5 μm; LLC «Medicant», Russia). Potentially dangerous substances were also assessed. Duration of agropopulation ontogenesis was close to parameters of natural populations in subalpine meadows and is more than 30 years without transition to senile age on the 33rd year of life, plant density reached optimum values of 28-23 thousand pcs/ha, starting from the 3-4 years of life. Diseases and pests did not affect vegetative aboveground mass and roots with rhizomes. The average root mass with rhizomes during the period of relatively stable mass production (from the 5th to the 32nd year of life) was 246.3 g/plant, aboveground

g

mass was 223.4 g/plant. Calculated annual productivity of the agropopulation during the same period averaged approximately 5300 kg/ha for aboveground parts, and approximately 6100 kg/ha for underground parts. Vegetative type of reproduction was most pronounced for subsenile age state (from the 13th to the 32nd year and onwards), when seed production was extremely low, 1.3 kg/ha. The seed type of reproduction was characteristic of the generative period from years 4-5 (8-30 kg/ha) with a peak during years 6-7 (108 and 78 kg/ha). In general, the percent of generative shoots in total plant biomass was insignificant throughout the life cycle. Ecdysterone biosynthesis and accumulation in the leaves of rosellate shoots were directly related to vegetative reproduction, namely to the aboveground mass value (R^2 = 0,768, or close to 80 %). More than 90 % of annually synthesized ecdysterone (22 kg/ha) was concentrated in the aboveground part of plants at optimal harvesting age (from year 5 to year 32), or about 600 kg of ecdysterone for 27 years of operation. Qualitative indicators of medicinal raw materials from leaves of *R. carthamoides* were high and met the requirements for the manufacture of pharmaceuticals with a relative purity of ecdysterone 97 %. The plant material met all the regulatory requirements of the supervisory authorities. The levels of heavy metals (Hg, Cd, As, Zn; Ni, Cu, Cr) did no exceeded the permissible level, there were no prohibited organochlorine and phosphorus compounds, the content of radionuclides ^{90}Sr and ^{137}Cs and nitrites were below the permissible limit. Ecdysterone from the dried flour of the *R. carthamoides* leaves was well extracted into aqueous and alcohol solutions and was well preserved (up to 93-98 % within 24 hours) without preservatives. Its use significantly improved animal health (a 1.6-2.5-fold decrease in mortality), had an anabolic and economic effects (i.e., a 24-33 % higher daily weight gain and a decrease in feed consumption by 11-17 %).

Keywords: phytoecdysteroids, 20-hydroxyecdysone, *Rhaponticum carthamoides*, Leuzea safflower, maral root, feed additives, anabolic substances

Leuzea safflower *Rhaponticum carthamoides* (Willd.) Iliin, 1933 is a perennial plant of the Asteraceae family (genus *Rhaponticum*, subgenus *Fornicium*) [1, 2], synthesizing the biologically active substance ecdysterone (syn.: 20-hydroxyecdysone, 20E) of ecdysteroids (ES). This is the only ecdysterone-containing adaptogen plant (syn.: *Leuzea carthamoides* DC; *Stemmacantha carthamoides*, rapontic-um safflower, maral grass, maral root), included in the official pharmacopoeia of the Russian Federation (IX-XIV editions, since 1961) [3], and also Republic of Belarus [4]. It is non-toxic and has no contraindications for use [5]. This species is currently absent from the pharmacopoeia of other countries in Europe, Asia and America [6, 7].

The main pharmacotherapeutic effects of *R. carthamoides* are adaptogenic, anabolic, anti-stress, anticoagulant, antioxidant, hemorheological, hypoglycemic, nootropic, and ergogenic [5]. In recent years, a series of scientific reviews have been published on the emerging opportunities and prospects for the practical use of ecdysterone-containing substances in official pharmacology and medicine for the treatment of cardiovascular, neurodegenerative and metabolic diseases [5, 8, 9]; for protection and recovery from complications of coronavirus infection (COVID-19) [10-12]; for the prevention and adjuvant therapy of malignant neoplasms, in particular breast cancer [13-15]; for preventing degenerative changes in the body associated with long-term stress and human aging [6, 16]; for overcoming heavy physical and mental stress in a healthy person, including high-performance sports where ecdysterone is not a prohibited substance [17-21]. It can also ne used as a phytobiotic and anabolic substance in feed additives for the health of farm animals and a significant increase in their average daily gain and productivity [22-25]. In this regard, it is interesting to note the recent discovery of a relatively high content of ecdysterone and its metabolites coming from wild plant seeds in the bloodstream of 20 species of birds of the family *Passeridae* (*Aves*: *Passeriformes*), which contributes to protection from harmful environmental factors and a high rate of metabolic processes of growth and development [26].

The global market for ecdysterone-containing substances in 2021 was estimated at $100 billion and, according to an analytical report and forecast by the global research and consulting company Quince Market Insights (India), the need will increase significantly in the next 5 years [27]. The market for ecdysterone

drugs is largely rigged due to a sharp shortage of raw materials, since it is not controlled by regulatory authorities. Products with ecdysterone declared for sale via the Internet do not meet quality and safety standards, as well as labeling [28, 29], mainly this is an extract from *Cyanotis arachnoidea* C.B. Clarke from Asian countries, prohibited for sale due to for the content of toxic substances, in particular carcinogenic aristolochic acid [30, 31].

Highly purified isolated ecdysterone is too expensive. The cost of 10 mg of 93% purity ecdysterone from such a well-known global company as Sigma-Aldritch (USA) [32] was 40 thousand rubles as of May 15, 2022, and 52 thousand rubles as of February 8, 2023, or 4-5 billion rubles/kg, so in practice surrogates are offered. The analysis showed that instead of the 100-500 mg of ecdysterone indicated on the label, the drugs actually contain on average 700 times less, 0.09-4.2 mg per capsule (or 0.38% purity), the deviation among 9 drugs was 0.017-0.75%. This absolutely does not correspond to the declared 20E concentration of 95-99% purity [30]. In July 2021, new data was published: out of 16 studied sports supplements purchased in the USA, Canada, Great Britain, Russia, and China, only 5 contained ecdysterone, and in minute quantities (from 0.00005 to 0.15%). Ecdysterone was not detected in 11 other preparations [31]. Ecdysterone is not synthesized in the body of mammals and cannot be synthesized artificially on an commercial scale (by chemical, microbiological methods or in cell and tissue culture), therefore it is obtained exclusively from plants, the number of which is very limited [9, 33].

The value of a particular potential source of 20E is determined by its uniqueness, which consists of such indicators as the concentration of ecdysterone in biomass, availability, biological activity, intended purpose, and economic feasibility. Obviously, those species that are characterized by a high content of target substances, high productivity, the absence of toxic impurities, resistance, the ability to be introduced and long-term grown in agrocenosis are of industrial interest [33].

Plant resources for industrial production of ecdysterone are subjected to several key requirements of international experts [9]. Plants must accumulate at least 0.5% 20E, have a simple ecdysteroid profile where at least 95-97% is the target highly active component ecdysterone soluble in water and alcohol. Ideally, the raw material should be free of minor and weakly active components. Purification should not require expensive chromatographic methods. The species should not be rare or protected, have little dependence on climate and be immune to pests and diseases, that is, easily introduced and successfully cultivated in an agrocenosis. The costs of cultivation, harvesting and processing of raw materials should be minimal, and the initial processing of the crop should occur close to the place of cultivation. That is, these should be perennial plants in an agricultural population, for which the annually alienated above-ground phytomass serves as the raw material.

Ecdysterone is a low-toxic substance, does not accumulate and quickly disappears from the body after ingestion. The LD_{50} for ecdysterone is 6.4 g/kg intravenously and 9.0 g/kg orally. The half-life of 20E in the body is short and depends on the dose, route of administration, intensity of absorption into the blood, and the type of experimental animals. For example, for sheep, the half-life of ecdysterone is 0.2 hours when administered intravenously, 0.4 h when administered orally, and 2.0 h when administered intramuscularly. Excretion from the body occurs through the liver and bile into the intestines and urine. In rats with a high metabolic rate, when administered intravenously, the half-life was 8 minutes. In humans, the peak content of ecdysterone in blood plasma at single dosages of 350-1400 mg occurs after 2-4 hours after which its amount begins to sharply decrease, and after 1 day only traces of 20E remain [9, 34].

In 2020, the safety of pharmaceutically purified ecdysterone (≥ 97%20-hydroxyecdysone) obtained from Leuzea safflower was studied in rodents and domestic dogs. High dosages were used (up to 1000 mg/kg daily) for 180 days for rats and 270 days for dogs. The drug, when administered orally, demonstrated a good safety profile with no observed side effects. In vitro and in vivo genotoxicity studies were negative at doses of 1.0-1.5 g/kg in rats and dogs for 28 days. The Safety Pharmacology battery of tests (animal behaviour, CNS, respiratory function, hERG test and cardiac telemetry) revealed no abnormalities [10]. Then in 2021, after completing clinical trials, the drug was registered in the United States as a pharmaceutical under the commercial name BIO101 from the American-French company Biophytis (identifier NCT03452488) to enhance muscle growth and inhibit proteolysis (against accelerated protein breakdown, muscle weakness, sarcopenia elderly people, respiratory failure of the pectoral muscles) [9, 35].

However, when using crude extracts from plants that accumulate ecdysterone, it must be taken into account that only some of them have proven safety, for example, *Rhaponticum carthamoides* (Willd.) Iljin and *Serratula coronata* L. Most other species are toxic to varying degrees due to accumulation in their organs of other chemicals. According to the special literature, highly poisonous plants that synthesize ecdysterone include species of hellebore (*Helleborus purpurascens* Waldst. & Kit., *H. caucasicus* A.Braun, *H. niger* L.), crow's eye (*Paris quadrifolia* L., *P. polyphylla* Sm., *P. incomplete* M.Bieb.), members of the genus *Vitex* (*V. canescens* Kurz, *V. scabra* Wall. ex Schauer, *V. cymose* Bert. ex Spreng.), yew (*Taxus baccata* Thunb, *T. cuspidate* Siebold & Zucc), blue cocculus *Diploclisia glaucescens* (Blume) Diels, Daurian moonseed *Menispermum dauricum* DC., morning glory *Ipomoea petaloidea* Choisy and purple bindweed *I. hederacea* Jacq. Less toxic pecies are *Pteridium aquilinum* (L.) Kuhn, *Polypodium vulgare* L., *P. lepidopteris* (Langsd. & Fisch.) Mart., species of Silene L., Brazilian ginseng *Pfaffia paniculata* (Mart.) Kuntze, *P. glomerata* (Spreng.) Pedersen, *P. iresinoides* Spreng., *Cyanotis arachnoidea* C.B. Clarke, *C. vaga* (Lour.) Schult. & Schult. f. Relatively toxic are the species of strawflower (*Achyranthes bidentat*a Blume, *A. aspera* L.), representatives of forest mushrooms *Paxillus atrotomentosa* (Batsch), *Tapinella panuoides* (Batsch) E.-J. Gilbert and Chinese polypore *Polyporus umbellatus* (Pers.) Fr. [36, 37].

Plants that are currently considered in Europe as the best sources of ecdysterone and deserve attention for the large-scale production of substances in sufficient quantities and at a reasonable price are species from the genera *Achyranthes* (strawflower from the family *Amaranthaceae*), cyanotis from the family *Commelinaceae*, *Pfaffia* (the family *Amaranthaceae*), *Leuzea/Stemmacatha/Rhaponticum* (the family *Asteracea*), *Serratula* (the family *Asteraceae*) [9]. According to data presented in a number of analytical reviews [6, 8, 10], among these groups of plants, the above-ground and underground parts of Leuzea safflower (*R. carthamoides*) are primarily suitable for obtaining ecdysterone-containing formulations of pharmaceutical quality.

Other representatives of the world flora with adaptogenic properties and relatively high ecdysterone content, e.g., *Cyanotis arachnoide*a C.B. Clarke, *Cyanotis vaga* (Lour.) Schult. & Schult. f., *Achyranthes aspera* L., *Cyathula capitata* Moq., *Pfaffia paniculata* (Mart.) Kuntze, *Pfaffia glomerata* (Spreng.) Pedersen, arenot officially approved and cannot be sold as food or feed additives due to the content of prohibited substances, toxicity and genotoxicity [36]. When comparing the effectiveness of biologically active compounds from commercially available adaptogen plants *Panax ginseng* C.A.Mey, *Lepidium meyeni*i Walp., *Rhaponticum carthamoides* (Willd.) Iljin, and *Eleutherococcus senticosus* (Rupr. & Maxim.) Maxim., the *R. carthamoides* had the greatest potential for practical use [6].

Several other members of the genus *Rhaponticum*, historically originating from the subalpine zone, for example, *Rhaponticum uniflorum* DC. found in Siberia, China and Mongolia, *Rhaponticum scariosum* (Lam.) in the Alps in Europe also synthesize ecdysterone and its analogues [2, 38, 39]. However, such promising species are only being studied on the example of single specimens and do not belong to field-cultivated crops [40, 41].

Both the underground parts of Leuzea with rhizomes and the aboveground parts, namely the leaves of vegetative (rosette) shoots, are allowed to be used in pharmaceuticals and food additives for humans [42], as well as phytobiotics for animals [35]. The regulated active ingredient in both leaves and roots is ecdysterone, 0.1%, or 1000 mg/kg dry matter [3, 4]. According to the results of comparative tests of extracts from roots and leaves (extract 1:10) at the Bekhtereva Institute of Human Brain RAS (St. Petersburg), leaves of rosette shoots of Leuzea had a multiple advantage over underground organs in terms of complex activity, 66 points vs. 16 [43]. A joint work of British and Austrian scientists reported similar results [44].

Ecdysterone, after biosynthesis in roots or leaves, is redistributed and concentrated in young and developing organs and tissues (e.g., growing leaves, apical parts, buds and seeds). The leaves of vegetative shoots of *R. carthamoides*, compared to the roots, are usually much richer in ecdysterone (the content is usually from 0.25 to 0.5-0.7%) [6, 36], and, due to annual growth, this is a renewable plant raw material. Perennial roots that have completed the growing season perform primarily an anchoring function in the soil, so the low content of ecdysterone in Leuzea roots harvested in the fall is understandable. According to publications from different countries (USSR, Czech Republic, Uzbekistan, France, Austria), the real yield of ecdysterone from 30-50-65 kg of dry roots with rhizomes of Leuzea safflower was 0.013% [45], 0.0153% [46];, 0.03% [44], 0.036% [47], 0.05% [48], 0.075% [49], and 0.101% [50].

It is important to emphasize that during storage and processing of plant materials contaminated with microorganisms, in particular the underground organs of *R. carthamoides*, ecdysterone can quickly be destroyed [9, 51]. For example, ecdysterone was not detected in a powdery substance from the roots with rhizomes of *R. carthamoides* purchased from a pharmacy chain in three large Russian cities and studied in a scientific laboratory [52]. This corresponds to the information published earlier by the laboratory of analytical chemistry (Lomonosov Moscow State University) on the trace content (0.040 mg/tablet) of ecdysterone in Levzea-P tablets (rhizome powder with Leuzea roots, Parapharm LLC, Russia) [53].

In addition, if underground parts are used, the plantation ceases to exist, the medicinal raw materials prepared in this way are of low quality, they quickly lose ecdysterone during storage, and the technological process itself is economically unprofitable for the manufacturer due to high costs. Therefore, it is more promising to collect the annually growing aboveground phytomass of *R. carthamoides* with a high content of ecdysterone,

Indeed, ethnographic primary sources for the Altai-Sayan mountain region indicate that local hunters and leaders of famous tribes (Tsetsen Khan) used deer grass in the form of powder from the leaves of Leuzea, but not from the roots with rhizomes [54]. Practical information about the peculiarities of the use of *R. carthamoides* by ethnic groups from little-known and unstudied regions of Central Asia, primarily from Northwestern Mongolia, was recorded by the Russian traveler and ethnographer G.N. Potanin in 1876-1870 during expeditions of the Imperial Russian Geographical Society.

The statement that marals (mountain deer) dig out the roots of *R. carthamoides* from under the snow and feed on them during the rutting period is not

true. During special research, scientists from the Siberian Branch RAAS found that deer eat cold-resistant rosette leaves that remain green under the snow that falls early in the mountains [55]. All other representatives of the animal world in high mountain pastures (horses, cows, sheep, and deer) also eat Leuzea leaves, but not the roots, and sometimes tear off the inflorescences.

Unfortunately, generally accepted technologies and the study of *R. carthamoides* in Russia and abroad do not cover such aspect as the quality of the resulting medicinal raw materials. In previously published monographs, the authors did not study the biosynthesis and accumulation of ecdysterone and its analogues (phytoecdysteroids) in plant products, much less in agricultural populations [55-57]. Another problem is that, despite almost a century-long history of cultivation (the first crops of *R. carthamoides* in the USSR date back to 1926), it is not possible to ensure long-term economic exploitation of the species. In subalpine meadows, the ontogenesis of *R. carthamoides* lasts from 50-60 to 75-120 or more years [55, 58] while in cultivation it is reduced to 5-6 years, and the duration of economic use usually does not exceed 3-4 years [33].

In this work, for the first time, an alternative technology for obtaining an ecdysterone-containing substance from the leaves of rosette shoots of Leuzea safflower *R. carthamoides* has been implemented. The proposed technology is simple and agriculturally scalable in the European North and satisfies the key requirements set by international experts and specialists for sources of industrial production of ecdysterone. In particular, the species can be successfully cultivated for a long time (up to 30 years or more) without reseeding in agrocenosis in cold and cool climates with high humidity, is resistant to diseases and pests, and serves as a source of annually renewable medicinal raw materials. Plant raw materials accumulate a large amount of ecdysterone (0.4-0.6%), the raw material has a simple ecdysteroid profile that is more than 97% ecdysterone, the substance meets all requirements of regulatory authorities.

Our goal was to analyze a 32-year experience in cultivating Leuzea safflower at an commercially exploited plantation located in the European northeast of Russia (Arkhangelsk Province), to identify the potential for longevity and productivity of the agropopulation of *Rhaponticum carthamoides* by age period, to study the patterns of ecdysterone accumulation in annually alienated aboveground phytomass, and to assess the quality of the resulting plant raw materials with regard to the content of standardized substances.

Materials and methods. The studies were carried out in the southeast of the Arkhangelsk Province, in the middle taiga subzone (Kotlas District; 61°20′N, 47°E) in the agropopulation of *R. carthamoides* (a single 1-hectare area) during 1989 to 2022. The agropopulation of seed origin was laid in 1989. The seeds were obtained from the Botanical Garden of the Komi Scientific Center, the Ural Branch RAS (Syktyvkar). Initially, the seeds originaited from the Altai natural population were primary collected in 1956, then in the same site (IB Komi Scientific Center) 3-4 reproductions were carried out with individual selection and reseeding.

The predecessors in crop rotation were potatoes, annual crops and grain crops. Pre-sowing tillage included plowing to a depth of 22-25 cm, disking and 2-fold cultivation with simultaneous harrowing. Before sowing, the area was rolled with smooth water-filled rollers. Sowing (a four-row mounted vegetable seeder SON-2.8A, Russia), was perdormed in mid-October after the onset of autumn frosts. The seed rate was 2.7 kg/ha with a field germination rate of 58%, 70 cm of row spacing, and 2-3 cm of sowing depth. Mineral fertilizers (NPK_{60-90}) were applied only in the first three years after sowing. Further cultivation was as in organic farming (no mineral and organic fertilizers, chemical protection products,

plant growth regulators, and herbicides were used). The rosette leaves from the aerial parts were once harvested annually during the budding stage. The seeds were harvested during the fruiting stage.

To determine the complex of soil agrochemical parameters of the site, samples were collected and studied using generally accepted methods (Agrokhimtsentr Kirovsky, Kirov).

The study of age states in plant ontogenesis and its periodization in the life cycle, population density (number of plants per unit area), gross production of aboveground and underground organs, seed yield with regard to the actual density of the arocenosis was carried out as outlined in the publication on other *R. carthamoides* agropopulation studies in the same region [59]. Plant age state (virginal, or pregenerative, generative and postgenerative periods of ontogenesis) were assessed based on the dominant group of individuals. Seedlings (p), juvenile (j), immature (im) and adult vegetative plants (v) were distinguished in the virginal period, young (g1), middle-aged (g2) and old generative plants (g3) in the generative period and subsenile age-related state (ss) in the post-generative period. The calendar (absolute) age of populations was counted from the time of seedling emergence. We proceeded from the following criteria: young generative age state corresponds to formation of reproductive shoots, weak fruiting, absence of rhizome dying processes; adult generative state corresponds to relative maximum of reproductive shoots, high intensity of growth and fruiting processes, balance of processes of new formation and death; old generative state means a sharp decrease in the proportion of reproductive shoots, weakened growth, inferiority and frequency of fruiting, the predominance of dying processes on the branches of the rhizome. In the post-generative period, the subsenile age state was distinguished by the absence of generative shoots in most individuals, a sharp decrease in the quality of fruiting and a weakened ability to form renewal buds and rhizome particulation [60].

In phenological observations, shoot regrowth, budding, beginning of flowering, mass flowering, fruiting, shoot death, dormancy were recorded annually:. Growth dynamics were assessed by the height of the most developed shoots of the plant from the soil level to the top of the straightened shoot. The width of the leaf blade was measured at the widest point by straightening the leaf.

The gross productivity of populations was determined as dry aboveground and underground phytomass multiplied by the actual density of the agrocenosis at the studied age. Plant density was assessed by counting in 60-80 m^2 areas at 6-9 points along the diagonal of the field. Seed fruiting was assessed by counting all fruiting inflorescences within the studied community, based on the yield of completed seeds (%) and the weight of 1000 seeds (g).

Samples were collected at the optimal phases of plant development, the aboveground phytomass during budding stage (decades 1 and 2 of June), the rhizomes in the fall after the end of the growing season (October) or in early spring before the growing season (April). In the aboveground phytomass, morphologically heterogeneous organs were distinguished, i.e., shortened vegetative shoots (rosette) and stem generative (reproductive) shoots with inflorescence. In each sample, 275-300 vegetative shoots containing 1100-1500 rosette leaves and up to 30-35 generative shoots were examined. Commercial harvest of plant raw materials was carried out in late May-early June, during the budding perios, which is characterized by the maximum concentration of the active substance (ecdysterone) in the rosette leaves of vegetative shoots (a mixture of fractions of young and adult leaves). Plant material (organs, elements and fractions) was dried at 23-25 to 35-40 °C and 25-40% air humidity in accordance with the rules for the preparation and drying of medicinal raw materials. The residual moisture content of air-dried raw materials, determined by the accelerated drying method at 130 °C, was 10-12%. Samples

from air-dried raw materials for further determination of the content of primary and secondary metabolites were formed by the quartering method. Before analysis, they were stored for 3-5 months in plastic bags at room temperature.

The ecdysterone in dry samples was quantified by reverse-phase high-performance liquid chromatography (RP-HPLC) with computer data processing wih the internal standard [3]. Analyzes were performed in the laboratory of biochemistry and plant biotechnology (for 1989-2000) and the biochemical laboratory of the Botanical Garden (2001-2021) of the Institute of Biology, the Komi Scientific Center of the Ural Branch RAS (IB Federal Research Center, Syktyvkar). We used a liquid microcolumn chromatograph Milikhrom-5 (80×2 mm column, Nucleosil C18 sorbent with a 5 μm particle size) (Medicant LLC, Russia). Eluent was a solution of acetonitrile and ethanol in water acidified with acetic acid, in the mode of gradient elution of components at 100 μl/min with UV detector (λ = 242 nm). The average values of 2 biological and 3 analytical replicates (% of air-dry matter) were recorded. The qualitative composition was determined by the proportion of the most active compound ecdysterone (20E) to the weakly active ecdysone (E), the 20E/E. This parameter should be ≥ 20:1, which corresponds to a ≥ 95% qualitative purity, or the ratio of ecdysterone to other ecdysteroids 95:5 [9, 61].

Chemical analyzes of dry samples for the content of potentially hazardous substances (heavy metals Hg, Cd, As, Zn, Ni, Cu, Cr, chlorine and organophosphorus compounds, radionuclides ^{90}Sr and ^{137}Cs, nitrates and nitrites) were carried out in accordance with accepted research methods (accredited laboratories of the Agrochemical Center Kirov, Kirov) (see website http://www.agrobiology.ru).

Safety of *R. carthamoides* and identified active substances were assessed based on information about poisonous plants and herbal medicines with undesirable side effects, including guidelines on toxic substances synthesized by plants [37, 62, 63], Technical Regulations of the Customs Union "On Food Safety" [42] and reviews by A.C. Brown [64-68] on toxic world flora found in food additives. The names of plant genera and species mentioned in the article are given according to the classification IPNI (International Plant Names Index) [69].

Data was processed by standard methods of variation and correlation statistics using the Statistica module in Microsoft Excel 2016. The general totality (based on the complete accounting) and sample parameters were used. To exclude systematic errors, samples were not taken from the edge areas of the agrocenosis, and the surface layers of the soil samples were not analyzed; data on single specimens that differ sharply from normal ones in appearance were excluded. The random sampling method was applied to the residual material and typical individuals. The mean values and standard deviations (M±SD), coefficients of variation (Cv, %), lim, min-max values were calculated based on the sample size collected from the population (N).

Variability was assessed on a scale expressed by the coefficient of variation for biological studies: ≤ 7% for very low, 7-15% for low, 15-25% for moderate, 26-35% for increased, 36-50% for high, ≥ 50% for very high values [70]. The correlations between the accumulation of ecdysterone and plant phytomass over 32 years of life of the agricultural population were analyzed. The experimental data approximation curves show the corresponding value of their reliability in the form of the determination coefficient, or approximation coefficient (R^2), which assesses the strength and direction of the relationship between the two quantitative variables being studied with a 95% confidence interval (at a $p = 0.05$ significance level).

Results. In the area where the experimental site is located, the terrain is slightly undulating, elevated, the soils are sandy loam, soddy-medium podzolic, formed on two-layer deposits. The upper horizon (0-28 cm) consists of sand particles; from a depth of 70-85 cm, the medium loamy fraction predominated.

According to a set of agrochemical indicators, the soil of the site was classified as highly cultivated mineral (soil samples were studied using generally accepted methods at the Agrochemical Center Kirovsky, Kirov). The arable layer is 3.6% humus and 3.1% organic matter. The acidity of the root layer was optimal (pH_{KCl} exchangeable 6.4-6.5; hydrolytic pH 0.7 mEq), base saturation was high (12.4 mEq, or 93.5%). In terms of nutrients, the supply of phosphorus was high (mobile P_2O_5 was 31.2 mg/100 g of soil), potassium was moderate (mobile K_2O was 9.6 mg/100 g); the Ca content was 6.4 mEq/100 g, Mg was 1.0 mEq/100 g.

The research area belongs to the middle taiga subzone and is part of the European-West Siberian taiga-forest bioclimatic region with moderately cool summers and moderately cool winters, a short frost-free period, significant cloudiness and a lack of sunlight in the ultraviolet range, and excess moisture. The zonal humidification coefficient (the ratio of precipitation to evaporation) is close to 1.5. The duration of daylight at the beginning of the regrowth of *R. carthamoides* (decade 1 of May) is 16-17 hours, during flowering (decades 2-3 of June) 20 hours. The growing season lasted 165-186 days and the frost-free period was 105 days. (with an amplitude of fluctuations over the years of 77-139 days). Average annual temperatures above 15 °C were 911 °C (54-57 days), above 10 °C were 1577°C (107-110 days), above 5 °C were 1936 °C (153 days). The average temperature of the warmest month (July) was +17.4 °C, the coldest month (January) was –14.3 °C. Stable snow cover with an average height of 52-58 cm appeared on November 11-16 and persisted until April 17-19.

The temperature at the depth of the tillering node of perennial grasses remained within the range of –l 1.5... –l 2.2 °C, in certain periods it decreased to –l 3.5... –l 4.0 °C. When the air temperature passed through +5 °C on April 29, the growing season of perennial crops began. However, returns of cold weather with frosts on the soil surface (up to –l 5...–7 °C) and repeated snowfall often inhibited the growth and development of plants until the beginning of the second decade of May. Frosts stopped completely from the second ten days of June and resumed from the beginning of September. The completion of the growing season was observed at the beginning of October, which coincided with the autumn transition of temperature through +5 °C. During the year, 495-538 mm of precipitation fell, including 367-387 mm during the warm period. The reserves of productive moisture in the soil layer of 0-20 cm during the warm period were 37-44 mm, in the layer of 0-50 cm 55-70 mm, which is enough for the most perennial crops. Average ten-day relative humidity in the daytime was 62-74%, the lowest rates were at midday, 54-57%. In some dry periods, humidity could drop to 25-35% or lower.

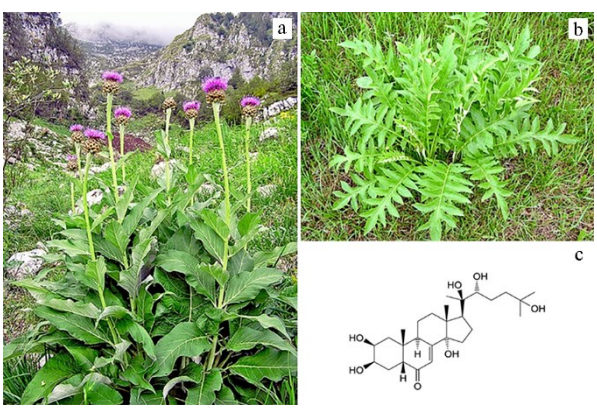


Fig. 1. Representatives of the genus *Rhaponticum* that synthesize ecdysterone: a — *R. scariosum* Lam. with generative shoots (photo by Adriano Bruna, Alps, 2009; https://www.actaplantarum.org/forum/viewtopic.php?t=13006); b — *R. carthamoides* (Willd.) Iliin with rosette leaves (photo by N.P. Timofeev; Arkhangelsk region, Kotlas district, May 2022), c — chemical structure of ecdysterone [71].

Parameters of growth and development of shoots in ontogenesis. Leuzea safflower (Fig. 1) is a perennial winter-hardy and cold-resistant plant; adult individuals form a bush 90-150 cm high

(sometimes 50-250 cm). The species was introduced from the high-mountain zone of the subalpine belt (up to 3000 m above sea level) and since the early 1960s it has been introduced into production in the European North [33].

In terms of life form, *R. carthamoides* is a large herbaceous semi-rosette polycarpic plant with two types of shoots that die annually, the vegetative rosette shoots and generative stem shoots with inflorescences of varying degrees of development. It has two types of reproduction - seed and vegetative (clones). Ontogenesis of *R. carthamoides* in subalpine meadows lasts 50-75 years, where the average relative age of individuals is 25-35 years. The generative period lasts from 6-9 to 30-48 years. Senile individuals are most often absent in natural cenoses [55, 72].

Fig. 2. Plants of R*haponticum carthamoides* (Willd.) Iliin from an agro-population cultivated in the conditions of the European North-East: a — seeds, b — plants of the 1st year of life, c — plants of the generative period in the flowering phase (photo by N.P. Timofeeva; Arkhangelsk Province, Kotlas District, July 2001).

The life activity of the species in the conditions of an agropopulation (Fig. 2) based on the results of 32 years of observations could be divided into two stages: the formation of a coenopopulation, from the 1st to the 5th year of life (Table 1); further sustainable production of above-ground mass with high biosynthesis of ecdysterone (Table 2).

1. Average above-ground parameters of *Rhaponticum carthamoides* (Willd.) Iliin in a European North-East agropopulation during the first 5 years of ontogenesis (Arkhangelsk Province, Kotlas District)

Parameter	Year and plant age								*N*
	1990			1991		1992	1993	1994	
	p	j	im	im	v	v	g1	g1-g2	
Shoot mumber:									
total	1.0	1.0	1.0	1.2	4.2	5.7	17.2	35.6	15-20
vegetative	1.0	1.0	1.0	2.8	3.8	5.1	16.0	31.1	15-20
generative					0.4	0.6	1.2	4.2	15-20
fruit-bearing						0.01	0.16	0.84	186-4233
Height of generative shoot, cm						3-42	90.3	114.0	15-20
Height of vegetative shoot, cm	1.5	17.4	21.1	63.7		58.3	75.0	89.8	15-20
Width of rosette leaves, cm	0.6	3.0	5.0	12.0	14.0	14.5	17.5	22.5	15-20
Weight of the above-ground part:									
total, g	0.01	0.27	0.42	6.2	10.2	16.4	56.8	210.7	12-15
proportion of rosette leaves, %	100	100	100	100	100	93.3	84.0	85.4	12-15
20E content in rosette leaves, %	0.04	0.06	0.11	0.19	0.22	0.25	0.27	0.28	3-4

N o t e. 20E — ecdysterone. Age of plants in ontogenesis is p for seedling, j for juvenile, im for immature, v for virginal, g1, g2 for young and mature generative

In the first 5 years, intensive development of individuals occurred against the background of multiple annual increases in phytomass (see Table 1): seedlings in the 1st year passed through immature and juvenile age states, in the 2nd year they reached the virginal state, which continued into the 3rd year. The first year of life, and in the 4th year the transition to the generative age began, which was fixed in the 5th calendar year. Subsequently (years 6-32), the intensive growth stopped. Individuals entered adult generative (6-8 years), old generative (9-12 years) and subsenile ages (13-32 years), which differed in the ability to produce

seeds, the structure and integrity of rhizomes.

2. Parameters of aboveground organs of *Rhaponticum carthamoides* (Willd.) Iliin in a European North-East agropopulation during sustainable production of above-ground phytomass with high biosynthesis of ecdysterone from the 5th to the 32nd year of life (Arkhangelsk Province, Kotlas District, 1994-1921)

Parameter	*M*±SD	*Cv*, %	max	min	*N*
Shoot mumber:					
total	31.00±12.40	40.1	60.4	17.9	15-20
vegetative	28.30±10.70	37.9	54.6	14.8	15-20
generative	2.71±2.13	78.6	3.8	0.6	15-20
fruit-bearing	0.19±0.31	167.9	1.1	0.001	20188-52
Height of generative shoot, cm	125.0±10.1	8.1	143.1	107.9	15-20
Height of vegetative shoot, cm	87.3±11.3	13.0	119.1	65.2	15-20
Width of rosette leaves, cm	24.5±3.8	15.6	33.0	19.4	15-20
Weight of the above-ground part:					
total, g	223.4±74.4	33.3	354.0	95.1	6-9
proportion of rosette leaves, %	84.4±5.3	6.3	93.9	73.2	6-9
20E content in rosette leaves, %	0.41±0.10	24.4	0.64	0.28	3-4

The senile period in the population as a whole did not occur: the plants continued to grow normally and vegetate into the 31st-33rd years of life. During an anatomical study of the underground parts at the beginning of the 33rd year of life (April 29, 2022), it was found that the processes of death and new formation of individuals are in relative dynamic equilibrium (1:1). The total number of shoots (31.5, $Cv = 28.0\%$) and perennial renewal buds (52.7, $Cv = 42.5\%$) was comparable to the total number of dead shoots (83.7, $Cv = 47.7\%$).

Plants during the growth of aboveground organs and wintering rhizomes were not affected by diseases and pests. There were short periods in the life cycle (seedling phase) when leaves did not have the potential for resistance to leaf-eating phytophages. Birds of the family *Passeridae* were pests of seeds during ripening, *Oxythyrea funesta*, *Potosia cuprea* ssp. *metallica* (*Cetoniinae*), and *Trichius fasciatus* (*Scarabaeidae*: *Trichiinae*) were pests of inflorescences (single lesions in some years) during ovule formation [61].

Rosette leaves of vegetative shoots, the main source for the medicinal raw materials of *R. carthamoides* were large petiolate, in adult plants, more or less deeply pinnately dissected into 15-22 lobes (from no dissections to 27 lobes), light in color, yellow or dark green, formed a rosette with an average diameter of 55-90 cm (from 37 to 112 cm). When young, the surface of the leaves was cobwebby and pubescent, giving them a silvery tint. The size of adult leaves reached 60-90 cm, sometimes up to 120 cm in length and 25-33 cm in width of the leaf blade. The appearance of new leaves, their maturation and death were not confined to certain phases of development; they functioned throughout the entire growing season, changing each other over time, from the moment the snow cover melted until the onset of stable autumn frosts.

Flowering (generative) shoots had a height of 110-140 cm, sometimes up to 180 cm. The stem grew due to the intercalary growth of internodes, on which 28-55 leaves of varying structural complexity were arranged in a spiral. At the top of the hollow, unbranched stem, a single inflorescence formed was a large spherical basket with a diameter of 4-6 cm (range from 3 to 8 cm), with bisexual violet-lilac flowers. The flowering period of individuals in the agricultural population usually fell on June 14-26. At the end of June, less than 1% of the shoots bloomed. The dates of the three earliest deviations for the agricultural population are 06/10/1995, 06/10/2005, 06/09/2015; one late date is 07/07/2017. The appearance of new generative shoots and their flowering were not observed in July-September. In general, the development of *R. carthamoides* to the budding phase takes 15-23 days, flowering lasts 44-56, fruiting 72-77 days. After fruiting in mid-July,

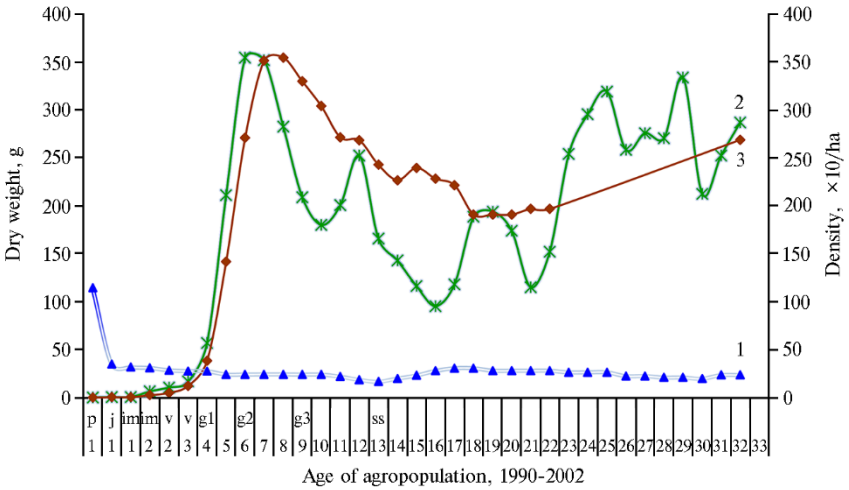
reproductive shoots died off, rosette shoots continued to vegetate until the average daily temperature dropped below 0 °C in the second decade of October, gradually decreasing in number and size. In the above-ground sphere, vegetative (rosette) shoots predominated: their share in the mass of above-ground organs in the first 3 years of life was 100-93.3%, in 4-5 years — 84.0-85.4%, subsequently (5-32 th years) remained virtually unchanged, 84.4% (with a minimum *Cv* = 6.3%). The contribution of generative shoots to the phytomass value turned out to be insignificant throughout the 32-year life cycle: 6.7% in the 3rd year, 6.0% in the 4th year, 4.6% in the 5th year, 5.6% on average for the period from the 5th to the 32nd years of life.

The total number of shoots in individuals in the first 5 years consistently increased (see Table 1): from 1.0 to 4.2 for the 2nd year; 5.7 in the 3rd year; 17.2 in the 4th year; 35.6 in the 5th year of life. The maximum number was recorded during the adult generative period during years 6-9 — 60.4-52.1, the minimum occurred at the beginning of subsenile age, during years 13-15 —17.9-20.6. During the period of stable functioning of the agropopulation with sustainable production of aboveground phytomass from year 5 to year 32 (see Table 2), the average number of shoots was 31.0 (*Cv* = 40.1%), and at the beginning of the 33rd year, it was 31.5.

The number of vegetative shoots increased until the 5th year of life (from 1.0 to 31.1), and in subsequent years the average value was 28.3 pieces (*Cv* = 37.9%). Generative shoots with inflorescences began to appear from the 3rd year, but they died off before reaching the fruiting phase (with height of 3-42 cm). In the 4th year, the plant formed on average 1.2 generative shoots, of which only a small part (0.16 pieces) bloomed and set full-fledged seeds; in the 5th year there were an average of 0.84 fruiting shoots per plant. At the subsenile age, the number of generative shoots again turned out to be insignificant (see Table 2), 0.19 per plant, which was close to the parameters of natural populations [55] and indicated a predominantly vegetative type of reproduction by clones after the disintegration of the mother plant into relatively independent daughter plants.

It is noteworthy that with a stable costs for the generative shoot formation (no more than 15-16% of the phytomass throughout the entire ontogenesis with a minimum Cv = 6.3%), the number of fruiting reproductive shoots in 27 years (from the 5th to 32 years) with average values of 0.19 per plant varied very much, from 0.001 to 1.13 (*Cv* = 167.9%). This was due both to the age characteristics of individual plants in the population and to late spring frosts up to –7... –10 °C from arctic air masses penetrating into the territory, causing irreversible damage and death of inflorescences (the rosette leaves of *R. carthamoides* are cold resistant). A similar influence of climatic factors on the variation of fruiting parameters occurred in natural mountain populations [55].

Phytomass and variability of its accumulation in ontogenesis. The productivity of the population will be determined by multiplying the number of plants per unit area by the dry phytomass of individuals. The density of *R. carthamoides* seedlings in the agrocenosis in the 1st year of life was 114 thousand/ha, from the 3rd-4th to the 10th year 27-24 thousand/ha, from the 13th to 15th 16-20 thousand/ha, then fluctuated over the years from 22-30 to 20-23 thousand/ha (Fig. 3). Over the whole subsenile age (from the 13th to the 32nd years), the average density was 24.5 thousand/ha (with fluctuations from 16.4 to 30.5 thousands, *Cv* = 16.0%). In terms of the norm for winter sowing (with a 4-5-fold reserve per unit area) with qualified care, the need for seeds for sowing was about 3 kg/ha (with an average 1000-seed weight of 15 g and field germination rate of apprx. 60%).



Fir. 3. Dynamics of density (1) and the value of dry phytomass of above-ground (2) and underground (3) organs of *Rhaponticum carthamoides* (Willd.) Iliin in a European North-East agropopulation over plant ontogenesis. Age of plants in ontogenesis: p - seedling, j - juvenile, im - immature, v - virginal, g1, g2, g3 - young, mature and old generative; ss - subsenile (Arkhangelsk Province, Kotlas District, 1990-2021).

Other seeding rates recommended in monographs on the cultivation of *R. carthamoides* and amounting to 10-15 kg/ha [57] and 20-25 kg/ha of first class seeds [56] should be considered overestimated. With high seeding rates in subsequent years, self-thinning and stabilization of the population occurred. Environmental factors causing a decrease in the number of individuals in the cenosis for *R. carthamoides* may be waterlogging, causing rotting of the root system, competitive suppression by fast-growing perennial weeds in the first three years of life, early cutting of aboveground phytomass (from the 2nd year), timing of seed sowing (spring instead of winter), soil moisture deficiency (drought), aging, etc.

The biology of *R. carthamoides* showed gradual germination over 3 years, characteristic of alpine plants: in the spring of the 1st year, in the 2nd-3rd decade of May, 85-88% of the total number of plants appeared, in the 2nd year 10-12%, on the 3rd 2-3% (provided that the soil was not waterlogged and the seeds did not rot). The first 2 months were the most vulnerable period due to the underdevelopment of the primary root system, formed by the lateral branches of the main root with a diameter of 0.03-0.05 mm, which were located in the surface layer of the soil. Mass death of individuals was possible due to suboptimal soil conditions from waterlogging and drying out. The underground sphere developed intensively after the formation of the stem root (with the appearance of adventitious roots from the hypocotyl zone), which followed in time the phase of development of the rosette embryonic shoot in juvenile age. At immature age, the proportion of the root system of plants increased from 19-21 to 43% of the total phytomass, which led to increased resistance to summer drought and ensured the growth of axillary buds of renewal into numerous lateral vegetative shoots.

In the first 3 years of life, the dry aboveground phytomass of juvenile, immature and virginal plants was 0.3, respectively; 6.2 and 16.4 g and was not of interest for alienation (Table 3). From the 4th year, when the transition of the agropopulation to the generative age began, the aboveground mass increased to 56.8 g. From the 5th year of life, a massive transition of the population into the generative state and achievement of average development parameters was observed, 210.7 g of aboveground mass per plant with an average value of 223.4 g

for 5-32 years (Table 4). In the 6th-8th years (mature generative age), the maximum values of the phytomass of individuals were recorded, 354, 352 and 282 g, respectively. In the 9th-12th years of life, the population was at an old generative age, the mass of aboveground organs was 208.7-179 .9 g (see Fig. 3).

3. Productivity of the *Rhaponticum carthamoides* (Willd.) Iliin European North-East agropopulation over the first 5 years of ontogenesis (Arkhangelsk Province, Kotlas District)

Parameter	Year and plant age								
	1990			1991		1992	1993	1994	*N*
	p	j	im	im	v	v	g1	g1-g2	
Stand density, $\times 10^3$/ha	114.3	34.6	31,5	30,8	28,3	27,5	27,3	24,0	40-48
Plant weight, g:									
above-ground part	0.013	0.27	0.42	6.2	10.2	16.4	56.8	210.7	12-15
undergrounf part	0.003	0.07	0.30	2.3	4.7	11.9	38.2	141.3	12-15
Number of developed inflorescences per plant						0.01	0.16	0.84	186-4233
Peoductivity, kg/ha:									
above-ground part	1.5	9.3	13.2	191	289	452	1553	5046	12-15
undergrounf part	0.3	2.4	9.5	71	133	328	1044	3384	12-15
seeds						0.17	8.0	30.3	186-4233

N o t e. Age of plants in ontogenesis: p - seedling, j - juvenile, im - immature, v - virginal, g1, g2.

4. Productivity of the *Rhaponticum carthamoides* (Willd.) Iliin European North-East agropopulation during the period of above-ground phytomass sustainable formation (from the 5th to the 32nd year of life, Arkhangelsk Province, Kotlas District, 1994-1921)

Parameter	*M*±SD	*Cv*, %	max	min	*N*
Stand density, $\times 10^3$/ha	24.1±3.5	14.7	30.5	16.4	40-48
Plant weight, g:					
above-ground part	223.4±74.4	33.3	354.0	95.1	6-9
undergrounf part	246.3±58.6	23.8	354.4	190.6	6-9
Number of developed inflorescences per plant	0.19±0.31	167.9	1.13	0.0005	20188-52
Peoductivity, kg/ha:					
above-ground part	5338.4±1760.6	33.0	8483	2634	6-9
undergrounf part	6019.3±1337.1	22.9	8548	3977	6-9
seeds	1.31±0.65	49.6	1.98	0.49	20188-52

During plant aging, at the beginning of subsenile age (13-17 years), which was accompanied by gradual destruction of the root system of the primary plant in the main root zone and its division into daughter plants, the minimum value of phytomass of aboveground organs was 95.1 g/plant. In subsequent years, an increase in the phytomass of aboveground organs was noted in the second updated cycle of ontogenesis, when daughter individuals, vegetatively arising in the form of a clone, took the place of maternal plants, that is, a rejuvenation of the population occurred. At the same time, the average value of aboveground phytomass for the period from the 5th to the 32nd year of life (223.4 g) turned out to be close to the value for the 5th year of life (210.7 g). The increased variability of the discussed indicator over the years of observation (Cv = 33.3%) (see Fig. 3) is caused by the strong influence of humidity (intense rains) and air temperature (35-38 °C and frosts up to –5... 10 °C) for seasonal development.

The weight of the underground parts (roots with rhizomes in dry form) increased following the aboveground parts (see Tables 3, 4): in the first 3 years it was also insignificant (0.3, 4.7 and 11.9 g/plant), in the 4th year it was 38.2 g, in the 5th year 141.3 g. The maximum value during the generative period with an increase to a maximum from 270.6 to 354.4 g and a subsequent decrease to 303.7 g was noted in the 6th-10th years of life. The average value of the mass of underground organs at subsenile age (13-32 years) was equal to 217.3 g, and in the 33rd year of life (after overwintering on April 29, 2022) 268.5 g (Cv = 28.6 %). The average value

of the mass of roots with rhizomes during the period with relatively stable production of above-ground phytomass (from the 5th to the 32nd year) was 246.3 g (Cv = 23.8%) which is slightly higher than the average value for aboveground parts (223.4 g, Cv = 33.3%) and indicates their importance as a storage organ for the formation of above-ground phytomass in the future period.

Thus, fluctuations in the size of the underground mass over the years were generally smoother, since it is less dependent on seasonal temperatures compared to the aboveground mass and is formed due to the outflow of organic substances from aboveground organs in the process of their gradual death.

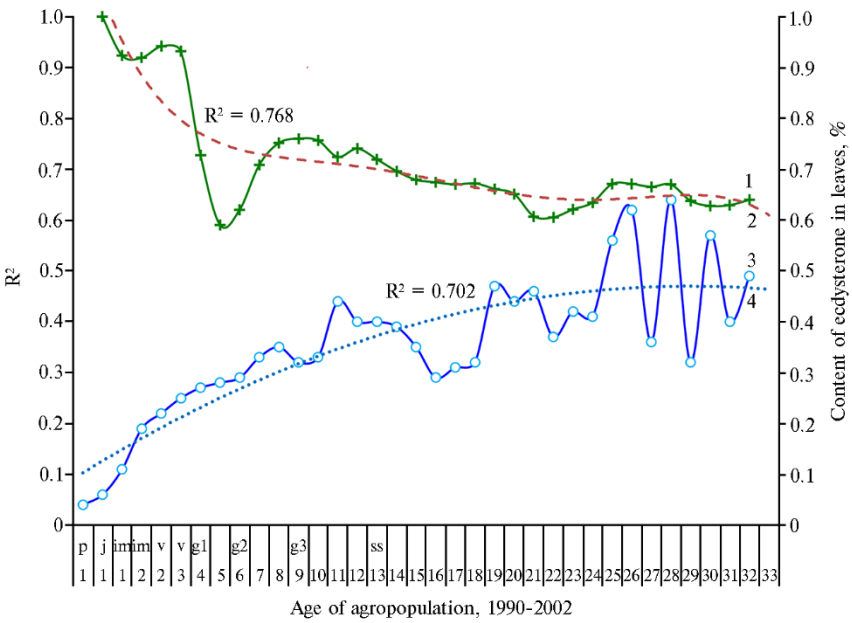


Fig. 4. Correlation between the plant aboveground mass and the content of ecdysterone (1)**, the reliability of the approximation** (2)**, the accumulation of ecdysterone 20E** (3) **and the trend of its accumulation** (4) **in the leaves of vegetative shoots of *Rhaponticum carthamoides* (Willd.) Iliin from a European North-East agropopulation during ontogeny.** Age of plants in ontogenesis: p — seedling, j — juvenile, im — immature, v — virginal, g1, g2, g3 — young, mature and old generative; ss — subsenile (Arkhangelsk Region, Kotlas District, 1990-2021).

Patterns of ecdysterone accumulation in the aboveground parts of plants. The processes of biosynthesis and accumulation of ecdysterone are dependent on growth processes in the aboveground sphere, which, in turn, are determined by the development of the root system of the mother plant and its disintegration into daughter plants. Rosette leaves of vegetative shoots of *R. carthamoides* accounted for 84-94% of the mass of aboveground organs. The ecdysterone content in them was minimal in the 1st year (0.06-0.11%), then increased consistently to 0.19-0.28% from the 2nd to the 5th year of life (see Table 1, Fig. 4). During the generative period (with fruiting flowering shoots) it was 0.29-0.33% (at years 6-10) and decreased from 0.44 to 0.40% during the transition from generative to subsenile age for years 11-13 (see Table 2).

Over the subsequent years of subsenile age (16-32 years), with minimal reproduction (on average 1 fruiting inflorescence per 5 plants), the ecdysterone content in vegetative shoots remained high - 0.41% (variation of 20E over the years from 0.28 to 0.64% when the number of fruiting inflorescences fluctuates from 0.001 to 1.13 pieces). During this period, seed reproduction sharply decreased and synthesized ecdysterone, previously redistributed with the water flow of assimilates from the leaves to the ovules of the inflorescences and seeds, remained in the

rosette leaves. Previously, using the example of 8 populations of *R. carthamoides* and *Serratula coronata* under the age of 15 years, we showed that the dynamics of ecdysteroid content in their vegetative organs is inversely and strongly dependent on seed reproduction [59]. The model of mutual relationship obtained in this work ($R^2 = 0.768$) between the total value of the aboveground mass of R. carthamoides and the content of ecdysterone in rosette shoots with a coefficient of determination of about 80% (which corresponds to $r = 90\%$) can be recognized as explaining the dependence of the biosynthesis and accumulation of ecdysterone on development vegetative shoots.

We have established a set of correlative parameters of plants in ontogenesis, combined with the highest (0.56-0.64%) accumulation of ecdysterone in the vegetative shoots of *R. carthamoides*. The length of rosette leaves is 97-119 cm (maximum), the proportion of rosette leaves in the structure of phytomass is 91-94% (maximum), the number of fruiting inflorescences is 0.016-0.021 pcs/plant (minimum), the total value of above-ground phytomass (together with generative shoots) is 270-320 g (above the average value of 223 g by 20-40%).

Agropopulation productivity. Gross production per unit area of the population (taking into account density) serves as an integral indicator characterizing the ecological optimum and reflects the attitude of the organism to the entire set of factors of the external and internal environment. Under natural conditions, the yield of the aboveground mass of wild thickets of *R. carthamoides* at the Gorno-Altai Agricultural Experimental Station is 2200-4000 kg/ha. The maximum bioproductivity of individual fragments of pure thickets can reach 6500-7000 kg/ha. The productivity of underground organs of *R. carthamoides* in the Altai-Sayan mountain region ranges from 80-1500 kg/ha. The largest areas of subalpine meadows are occupied by cenoses where the average weight of rhizomes is apprx. 57-75 kg/ha, and 12-20% of resource areas have a productivity of 570-640 kg/ha [55]. In relatively small areas in the Kazakhstan Altai, the production of dry roots of *R. carthamoides* is estimated at 1.0-1.1 t/ha [73].

In culture, the rhizomes of Leuzea safflower for use as pharmacopoeial medicinal raw materials begin to be removed from the 3rd year of life. Considering that the most intensive growth of root mass occurs at the end of the growing season, harvesting is carried out in September-October. The average yield of dry roots (excluding actual density) in the Moscow region was 2000-2500 kg/ha]56]. In Finland, the expected yield of roots (in terms of square meters per hectare) after 3 years of cultivation was about 2000 kg/ha, of aboveground parts 1000-2500 kg/ha [57]. In the conditions of the Perm region, the yield of above-ground mass without the use of fertilizers on average over 6 years amounted to 2520 kg/ha [74]. In Siberia, in the fields of the experimental farm of the Central Siberian Botanical Garden, the yield of the aboveground mass of *R. carthamoides* at the age of 4-5 years reached 3600 kg/ha [56]. In older crops, a decrease in yield was noted; for example, in the Leningrad region, in crops 7-8 years old, it did not exceed 700-800 kg/ha. In the Moscow region, on medium loamy soil, at the experimental station of the Russian State Agrarian University — Timiryazev Moscow Agricultural Academy productivity in the 6th year was 3740 kg/ha and by the 9th year it decreased 3.6-fold [59].

In the studied conditions of the European North, the gross production of the agropopulation (see Table 3) was scanty in the first 2 years and in the 3rd year of cultivation and amounted to only 8% and 5%, respectively, with regards to the average values for the 5th-32nd years of life (or 452 kg/ha of aboveground and 328 kg/ha of underground phytomass). In the 4th year, the productivity of the agropopulation approximately corresponded to the literature data, 1553 kg/ha for the aboveground part and 1044 kg/ha for the underground part. In the 5th year,

productivity continued to increase, amounting to 5046 and 3384 kg/ha, respectively, and reached its peak in mature generative age, about 8500 kg/ha in the 6th-7th years for aboveground and in the 7th-8th years for underground organs. At old generative and subsenile ages, phytomass decreased and varied in accordance with the patterns described above (see Fig. 4). In general, from the 5th to the 32nd years of life (over 27 years of economic exploitation), the average estimated productivity of the aboveground part of the agricultural population was about 5338 kg/ha (Cv = 33.0%), of the underground part 6019 kg/ha (Cv = 22.9%) (see Table 4).

There was no seed yield per unit area in the first 3 years. On the 4th and 5th lives it was 8 and 30 kg/ha, respectively, and the highest seed yield occurred in the 6th and 7th years, 108 and 78 kg/ha, decreasing in the 8-10th years to 50, 26 and 5 kg/ha, respectively. After the transition to the subsenile age, when vegetative reproduction dominated, the average annual seed yield for the period from the 13th to the 32nd year was 1.3 kg/ha (from 0.49 to 1.98 kg/ha). The coefficient of seed reproduction of an individual (the ratio of the number of full-fledged seeds per unit area to density) during this period was 3.3.

For comparison, in subalpine meadows of Altai, the average seed yield was 8-30 kg/ha, and in rare thickets it could reach 150-200 kg/ha [55]. In the conditions of the European North, the seed yield from experimental plots was as follows: in Finland up to 200-290 kg/ha [57], in Komi Republic up to 295-410 kg/ha. In the Perm region [75], in the 9-10th year of life, seed yield was estimated at 386-542 kg/ha (38.6-54.2 g/m^2). Moreover, abundant fruiting in such cases was accompanied by an extremely low ecdysterone content in the leaf organs, from 0.05-0.07 to 0.14% [43] and from 0.04 to 0.09% [44].

This confirms our thesis that one of the key factors in the regulation of ecdysterone concentration in leaf organs is the process of competition between seed and vegetative reproduction. Abundant production of seeds leads to the outflow of ecdysterone from vegetative shoots and the early completion of the plant life cycle due to the massive death of generative shoots after fruiting together with renewal buds, which serve as the basis for the formation in future years of new rosette leaves that synthesize ecdysterone.

An approximate estimate of the gross synthesis and accumulation of ecdysterone at the optimal exploitable age (from the 5th to the 32nd years) was about 21.9 kg/ha in the aboveground sphere with an amplitude of 20E content of 2.8-6.4 g/kg (14.9-34.2 kg/ha) annually, or about 600 kg of ecdysterone over 27 years of operation. In the underground sphere of plants, with an approximate 20E content of 0.3-0.5 g/kg (apprx. 2.4 kg/ha, from 1.8 to 3.0 kg/ha) of ecdysterone accumulated, but these plant parts can be alienated only once .

Thus, 90% of the annually synthesized ecdysterone accumulates in the aerial parts of plants from the *R. carthamoides* agropopulation. If we proceed from the potential of the life cycle of an industrially exploited population, taking into account the possibility of repeated removal of phytomass, then ecdysterone is concentrated in aboveground organs by more than 99%.

Qualitative parameters of medicinal raw materials from leaf organs. An important indicator of the quality of *R. carthamoides* medicinal raw materials for the manufacture of drugs is the ratio of highly active ecdysterone (activity in biotests 7.5×10^{-9} M) to weakly active ecdysone (activity 1.1×10^{-6} M), since less active ecdysteroids can completely or partially block the physiological effect more active compounds contained in plant extracts [8, 48]. It is desirable that the purity of ecdysterone be at least 95% (≥ 20:1), better than 97% (≥ 30:1), and ideally, an almost complete absence of minor and weakly active components (≥ 1000:1) [9]. Otherwise, the feedstock must be subjected to a complex multistage chromatographic procedure for purification from inactive impurities [10].

For a dry crude alcoholic extract isolated from 65 kg of dry roots of *R. carthamoides* with rhizomes, the 20E/E ratio was about 60:1 with 0.37% ecdyster- and 0.006%ecdysone [46]. According to data from the Czech Republic [50], the 20E/E ratio for dry roots of *R. carthamoides* was more than 1000:1. For the aerial parts of *R. carthamoides*, this quality indicator is not constant and depends on the intensity of the formation and development of generative shoots. Previously, using 12 different-aged agropopulations of *R. carthamoides* and *S. coronata* in the first 16 years of cultivation, we showed that in *R. carthamoides*, before entering the generative period, the composition of phytoecdysteroids was represented only by highly active ecdysterone. Ecdysone in aboveground reproductive organs was synthesized synchronously with their development: at the beginning of the growing season it was not detected in generative shoots, during flowering its share reached 9.1%, during seed filling and fruiting 17.8-18.7%. During the period of abundant fruiting, the proportion of ecdysone also increased in vegetative shoots, although to a lesser extent, from 1.5 to 4.7% in the budding stage and to 13.3% in the flowering stage [61].

The qualitative ratio of ecdysterone to ecdysone (20E/E) in vegetative shoots of *R. carthamoides* over 32 years of cultivation under agropopulation conditions changed as follows: In immature and virginal plants, it was higher than 1000:1 (1-3 years), in young generative plants plants in the budding stage, it was was apprx. 980:1. In adult generative plants, during the transition to the flowering phase, 20E/E decreased to 20-6:1, and by the beginning of fruiting to 3-4:1. In the subsenile period (13-32 years), when seed reproduction was suppressed, ecdysone was synthesized in trace amounts ($\leq 0.001\%$), the ratio 20E/E ranged over the years from 560-900:1 to 60-80:1 which met the requirement for a relative purity quality of 20E 97%.

Comparison with other industrial sources of ecdysterone showed that *R. carthamoides* has an advantage over them both in the quantitative content and in the quality of the synthesized ecdysterone. In particular, A. Hunyadi et al. [30] examined the European market of nutritional supplements made from *Cyanotis arachnoidea* C.B. Clarke extract and showed that the ratio of ecdysterone to other (minor) ES was deteriorated, apprx. 0.9:1 with a quantitative content of 0.2-2.4%/0.09-2.49%. A closely related species, *Cyanotis longifolia* Benth., grown in a pot culture in France, had a similar qualitative ecdysterone ratio: in aboveground phytomass 1.8:1 (0.095%/0.052%), in roots 0.63:1 (0.385 %/0.607%) [76]. In general, other species of the family *Commelinaceae* from natural habitats are characterized by a relatively low content of ecdysterone in dry phytomass: *Cyanotis hirsuta* Baker — 0.140%, *Cyanotis kewensis* C.B. Clarke —0.0245%, *Cyanotis longifolia* Benth. — 0.008%, *Cyanotis somaliensis* C.B. Clarke — 0.111%, *Cyanotis speciosa* (L.f.) Hassk. — 0.093% [76]. Additionally, commercial Cyanotis extracts in 2021 have been found to contain contaminants of petroleum-derived semi-synthetic ecdysteroids that interact with ecdysterone receptors as competitive agonists and antagonists, which can lead to a variety of negative pharmacological and toxicological consequences when consumed [29].

Among other indicators of the quality of medicinal raw materials, information about the accumulation of toxic compounds of natural or anthropogenic origin in it, including heavy metals, radionuclides, chlorine and organophosphorus compounds, is important. Early color reaction studies in the early 1970s suggesting the presence of alkaloids in *R. carthamoides* were subsequently not confirmed [77]. There is also no information in modern literature about the accumulation of triterpene saponins by this type, other potent, narcotic or toxic substances that are potentially hazardous to human health or that may pose a danger to animals when used as feed and feed additives (bufadienolides, cardiac glycosides, aristolochic

acid, photosensitizing , cumulative or vitamin-breaking substances, etc.) [42, 63, 65-67].

The toxicity of the aerial parts of R. *carthamoides* used as feed additives has been studied previously. In long-term experiments with the inclusion of crushed *R. carthamoides* leaves in diets, no adverse effects were found. Their harmlessness has been proven in doses up to 0.3-0.5 kg of dry matter of the aboveground mass. Rats and birds could feed on the seeds of this species, which contained up to 1.5% ecdysterone [36].

5. Chemical composition of ecdysterone-containing substance from leaves of *Rhaponticum carthamoides* grown in an commercially exploited plantation (Arkhangelsk Region, Kotlas District, 2016-2020) (78)

Parameter	Norm	Actual values
Active ingredients,%:		
ecdysterone (20-hydroxyecdysone, 20E)	≥ 0.1	0.56-0.61
share of ecdysterone from PES	≥ 95.0	95.3-99.6
extractives	≥ 12.0	50.2
raw protein	≥ 16-19	19-27
raw fiber	≤ 23-26	16-19
Heavy metals, mg/kg:		
Hg	0.05	0.009-0.016
Cd	0.3	0.020-0.115
As	0.5	0.05
Ni	3.0	0.59-1.30
Pb	5.0	0.18-0.30
Cu	30.0	7.9
Zn	50.0	28.4
Organochlorine and phosphorus compounds, mg/kg:		
DDT and its metabolites	0.05	< 0.007
hexachlorocyclohexane and its isomers	0.05-0.20	< 0.001
metaphos	0.00-0.50	Not detected
karbofos	2.0-5.0	Not detected
Nitrogen compounds, mg/kg:		
NO^{2-}	10.0	0.3-2.0
NO^{3-}	2000	700-1200
Radionuclides, Bq/kg:		
^{90}Sr	100.0	5.7
^{137}Cs	600.0	4.8

N o t e. PES — phytoecdysteroids, ДДТ — the sum of organochlorine pesticides (dichlorodiphenyltrichloroethane, dichlorodiphenyldichloroethane, dichlorodiphenyldichloroethylene, etc.), hexachlorocyclohexane - the sum of other insecticides (lindane, hexachlorocyclohexane, heptachlor, keltan, aldrin) (data presented in accordance with testing protocols, see http://www,agrobiology).

In the conditions of industrial cultivation, during the sanitary and toxicological assessment of product safety, the priority is the compliance of the content of heavy metals with regulatory requirements. The relevance of the control of heavy metals in high-mountain plants is associated with their genetic predisposition to the accumulation of mercury, cadmium, nickel, lead and copper. When studying the pharmacopoeial characteristics of medicinal raw materials from the leaf organs of *R. carthamoides* [78], we found (Table 5) that the aerial parts of *R. carthamoides* grown and harvested in the studied agropopulation did not accumulate elements of the first and second hazard classes (Hg, Cd, As, Zn; Ni, Cu, Cr) above the background level and corresponded to the maximum permissible concentration for green mass of perennial grasses. There were no chlorine and organophosphorus compounds prohibited by sanitary standards in the phytomass. The content of radionuclides ^{90}Sr and ^{137}Cs was below the MPC (68.8 and 6.2 Bq/kg compared to the permitted 100 and 600 Bq/kg). The amount of nitrites was within the normal range (0.3-2.0 mg/kg) (see Table 5).

The first results of mass application of additives based on leaf material of *R. carthamoides* grown in the considered agropopulation were obtained in the pig-breeding complex of JSC Kotlas Pulp and Paper Mill (Arkhangelsk Province, Russia) with an average monthly population of 1.6 thousand animals, the duration

of the experiment was 12 months [79]. Pregnant sows, weaned piglets and fattening livestock aged 2-4 months were daily fed granulated grass meal from the aerial part of R. *carthamoides* of the 4th year of life at a rate of 20 g/t live weight. The animals' diet was based on food waste from catering establishments with poor phytosanitary composition, which was accompanied by dyspepsia. As a result, the herd became healthier and the mortality of newborn piglets decreased by 2.1-2.7 times, and the anabolic effect was expressed in an increase in the herd's output in live weight by 40.6%.

Further studies were carried out in the breeding pig breeding farm of JSC Zarechye (Kirov Province) under strictly controlled conditions. According to the data obtained, when the substance from *R. carthamoides* was introduced into the diet of weaned piglets, their live weight exceeded that in the control by 15-22%, the intensity of the average daily weight gaine was higher by 24.0-32.8%, the incidence of animal diseases decreased by 1.6-2.5 times, safety was 100% [22]. Comparable results were obtained in experiments using chemically purified ecdysterone (20-hydroxyecdysone 96% purity) isolated from *R. carthamoide*. The anabolic effect in this case was 12-16% while reducing feed consumption by 11-17% per 1 kg of live weight gain [80].

Summarizing the results obtained, it should be noted that the biosynthesis and accumulation of ecdysterone were directly related to vegetative reproduction (with the intensity of growth of rosette shoots over the years and their power), and inversely proportional to the intensity of seed fruiting. The relationship between the total amount of above-ground mass and the content of ecdysterone in rosette shoots over 32 years of cultivation was characterized by the coefficient of determination $R^2 = 0.768$ (or about 80%) and reflected the dependence of the biosynthesis and accumulation of ecdysterone on the development of vegetative shoots. An approximate estimate of the gross synthesis and accumulation of ecdysterone at the optimal exploitation age (from the 5th to the 32nd years) was about 21.9 kg/ha annually in the aboveground sphere, or about 600 kg of ecdysterone over 27 years of exploitation. The underground sphere contained approximately 2.4 kg/ha of ecdysterone. For the factory production of medicines, food additives and phytobiotics, it is preferable to use rosette leaves of vegetative shoots containing high concentrations of ecdysterone (0.4-0.6% with a standard of 0.1%). The qualitative characteristics of medicinal raw materials prepared in the optimal phase of development (beginning of budding) were high and met the requirements for the manufacture of pharmaceuticals with a relative purity of ecdysterone of 97%. The underground parts (roots with rhizomes) did not accumulate significant concentrations of ecdysterone (on average 0.03-0.05%) and could quickly lose active substances due to infection by soil microflora. Seed productivity increased over the years (8-30 kg/ha) and reached a peak in the 6th and 7th years (108 and 78 kg/ha), decreasing in the old generative age (8-10th years) to 50, respectively. 26 and 5 kg/ha. In the subsenile period (from the 13th to the 32nd year of life and beyond), the type of reproduction changed from seed to vegetative and seed production had extremely low values (1.3 kg/ha with a seed reproduction coefficient of 3.3). The average value of the mass of roots with rhizomes from the 5th to the 32nd year of life was 246.3 g/plant, which is slightly higher than the average value of the phytomass of aerial parts, 223.4 g/plant.

From substances based on leaf material of *R. carthamoides*, ecdysterone was well extracted into aqueous and alcoholic solutions and was well preserved in them without preservatives (93-98% within 1 day). The total yield of extractives was 50.2% (with a norm of 12.0%). In biotests, the extract had a stimulating effect at a strong dilution (10^{-9}-10^{-11} M based on ecdysterone) and an inhibitory effect at a lower (100-fold) dilution (10^{-4}-10^{-5} M) [78].

In the future, it is necessary to study the influence of the multiplicity and frequency of cuttings (the amount of alienated aboveground mass) on the formation and ratio of vegetative and generative shoots of Leuzea safflower, as well as the possibility of influencing the process of ecdysterone synthesis through fertilizers, phytohormones and elicitors.

So, as a result of 32 years of research on the agropopulation of *Rhaponticum carthamoides* cultivated in the Arkhangelsk Prorvince from 1989 to 2022 using the technology of annual one-time alienation of aboveground phytomass, it was found that the natural conditions of the European North-East with cool climate, a leaching type of water regime, long daylight hours and a short growing season, are favorable for the industrial cultivation of Leuzea safflower. The duration of ontogenesis of the agropopulation was close to the parameters of natural populations in subalpine meadows and was over 30 years without a transition to the senile age state at the 33rd year of life. Starting from the 3-4th year of life, the density of plants in the agrocenosis reached optimal values of 28-23 thousand/ha. In the conditions of an agropopulation, in the first 4 years there was intensive growth and development of plants; in the 5th year (after the transition to the generative period), seed reproduction of individuals began. The average annual estimated productivity of the aboveground part of the agropopulation during the period of stable production of aboveground phytomass with a high level of ecdysterone biosynthesis (from the 5th to the 32nd years of life) was about 5300 kg/ha, of underground part apprx. 6100 kg/ha. The accumulation of the largest amount of ecdysterone in the vegetative shoots of *R. carthamoides* (0.56-0.64%) was accompanied by the maximum length of rosette leaves (97-119 cm), the maximum proportion of rosette leaves in the structure of phytomass (91-94%), the minimum number of fruiting inflorescences (0.016-0.021 per plant), The total above-ground phytomass together with generative shoots was 270-320 g (above the average value of 223 g by 20-40%). Ecdysterone from leaf organs was well extracted into aqueous and alcoholic solutions without loss of active substances (when the aqueous extract was stored for 1 day, the preservation of ecdysterone was 93-98%). The total yield of extractive substances from the leaves was 50.2%, with the standard being 12.0%. The resulting medicinal raw materials met all regulatory requirements of regulatory authorities regarding the content of radionuclides, heavy metals, herbicide residues, insecticides and other chemical plant protection products. The use of a substance from leaf material of *R. carthamoides* in commercial animal husbandry was accompanied by improvement of the herd health and a decrease in the mortality of young pigs by 2 or more times, an increase in the intensity of average daily growth by 24-33%, and a decrease in feed consumption by 11-17%.

REFERENCES

1. *Rhaponticum carthamoides*. V knige: *Rastitel'nye resursy SSSR. Tsvetkovye rasteniya, ikh khimicheskiy sostav, ispol'zovanie. Semeystvo Asteraceae (Compositae)* /Otvetstvennyy redaktor P.D. Sokolov [In: Plant resources of the USSR. Flowering plants, their chemical composition and use. Family *Asteraceae* (*Compositae*). P.D. Sokolov (ed.)]. St. Petersburg, 1993, iss. 7: 161-163 (in Russ.).
2. Hidalgo O., Garcia-Jacas N., Garnatje T., Susanna A. Phylogeny of *Rhaponticum* (*Asteraceae*, *Cardueae-Centaureinae*) and related genera inferred from nuclear and chloroplast DNA sequence data: taxonomic and biogeographic implications. *Annals of Botany*, 2006, 97(5): 705-714 (doi: 10.1093/aob/mcl029).
3. *Rhapontici carthamoides rhizomata cum radicibus*. V knige: *Gosudarstvennaya farmakopeya Rossiyskoy Federatsii* [In: State Pharmacopoeia of the Russian Federation]. XIV edition. Moscow, 2018, Vol. 4: 6360-6368 (in Russ.).
4. *Rhapontici carthamoides folium* (Leuzea leaf). V knige: *Gosudarstvennaya Farmakopeya Respubliki Belarus'* [In: State Pharmacopoeia of the Republic of Belarus]. 2nd edition. Molodechno, 2016, Vol. 2: 1257-1258 (in Russ.).
5. Shikov A.N., Narkevich I.A., Flisyuk E.V., Luzhanin V.G., Pozharitskaya O.N. Medicinal plants

from the 14th edition of the Russian Pharmacopoeia, recent updates. *Journal of Ethnopharmacology*, 2021, 268: 113685 (doi: 10.1016/j.jep.2020.113685).
6. Todorova V., Ivanov K., Ivanova S. Comparison between the biological active compounds in plants with adaptogenic properties (*Rhaponticum carthamoides*, *Lepidium meyenii*, *Eleutherococcus senticosus* and *Panax ginseng*). *Plants*, 2022, 11(1): 64 (doi: 10.3390/plants11010064).
7. Liu X.X., Chen C.Y., Li L., Guo M.M., He Y.F., Meng H., Dong Y.M., Xiao P.G., Yi F. Bibliometric study of adaptogens in dermatology: pharmacophylogeny, phytochemistry, and pharmacological mechanisms, drug design. *Development and Therapy*, 2023, 17: 341-361 (doi: 10.2147/DDDT.S395256).
8. Głazowska J., Kaminski M.M., Kamiński M. Chromatographic separation, determination and identification of ecdysteroids: focus on maral root *(Rhaponticum carthamoides, Leuzea carthamoides). Journal of Separation Science*, 2018, 41(23): 4304-4314 (doi: 10.1002/jssc.201800506).
9. Dinan L., Dioh W., Veillet S., Lafont R. 20-Hydroxyecdysone, from plant extracts to clinical use: therapeutic potential for the treatment of neuromuscular, cardio-metabolic and respiratory diseases. *Biomedicines*, 2021, 9(5): 492 (doi: 10.3390/biomedicines9050492).
10. Lafont R., Dilda P., Dioh W., Dupont P., Signore S.D., Veillet S. *20-hydroxyecdysone extract of pharmaceutical quality, use and preparation thereof. Patent France FR 3065644A1. Publ. 2020-02-21.*
11. Lafont R., Serova M., Didry-Barca B., Raynal S., Guibout L., Dinan L., Veillet S., Latil M., Dioh W., Dilda P.J. 20-Hydroxyecdysone activates the protective arm of the RAAS via the MAS receptor. *Journal of Molecular Endocrinology*, 2021, 68(2): 77-87 (doi: 10.1530/JME-21-0033).
12. Latil M., Camelo S., Veillet S., Lafont R., Dilda P.J. Developing new drugs that activate the protective arm of the renin-angiotensin system as a potential treatment for respiratory failure in COVID-19 patients — review. *Drug Discovery Today*, 2021, 26(5): 1311-1318 (doi: 10.1016/j.drudis.2021.02.010).
13. Sláma K. Vitamin D1 versus ecdysteroids: growth effects on cell regeneration and malignant growth in insects are similar to those in humans. *European Journal of Entomology*, 2019, 116: 16-32 (doi: 10.14411/eje.2019.003).
14. Slama K. Approaching a time we can prevent pernicious malignant tumors? Mini review. *EC Pharmacology and Toxicology*, 2020, 8(3): 01-09.
15. Shuvalov O., Fedorova O., Tananykina E., Gnennaya Y., Daks A., Petukhov A., Barlev N.A. An arthropod hormone, ecdysterone, inhibits the growth of breast cancer cells via different mechanisms. *Frontiers in Pharmacology*, 2020, 11: 561537 (doi: 10.3389/fphar.2020.561537).
16. Panossian A.G., Efferth T., Shikov A.N., Pozharitskaya O.N., Kuchta K., Mukherjee P.K., Banerjee S., Heinrich M., Wu W., De-An Guo D.A., Wagner H. Evolution of the adaptogenic concept from traditional use to medical systems: pharmacology of stress- and aging-related diseases. *Medicinal Research Reviews*, 2021, 41(1): 630-703 (doi: 10.1002/med.21743).
17. Isenmann E., Ambrosio G., Joseph J.F., Mazzarino M., Torre X., Zimmer Ph., Kazlauskas R., Goebel C., Botre F., Diel P., Parr M.K. Ecdysteroids as non-conventional anabolic agent: performance enhancement by ecdysterone supplementation in humans. *Archives of Toxicology*, 2019, 93: 1807-1816 (doi: 10.1007/s00204-019-02490-x).
18. Parr M.K., Müller-Schöll A. Pharmacology of doping agents — mechanisms promoting muscle hypertrophy (review). *AIMS Molecular Science*, 2018, 5(2): 131-159 (doi: 10.3934/molsci.2018.2.131).
19. Parr M.K., Ambrosio G., Wuest B., Mazzarino M., Torre X., Sibilia F., Joseph J.F., Diel P., Botrè F. Targeting the administration of ecdysterone in doping control samples. *Forensic Toxicology*, 2020, 38: 172-184 (doi: 10.1007/s11419-019-00504-y).
20. Marciniak A., Nemeczek S., Walczak K., Walczak P., Merkisz K., Grzybowski Ja., Grzywna N., Jaskuła K., Orłowski W. Adaptogens — use, history and future. *Quality in Sport*, 2023, 9(1): 19-28 (doi: 10.12775/QS.2023.09.01.002).
21. *World anti-doping code. International standard. Prohibited List 2023.* Available: https://www.wada-ama.org/sites/default/files/2022-09/2023list_en_final_9_september_2022.pdf. Accessed: 10.05.2022.
22. Ivanovskiy A.A., Timofeev N.P., Ermolina S.A. *Agrarnaya nauka Evro-Severo-Vostoka*, 2019, 20(4): 387-397 (doi: 10.30766/2072-9081.2019.20.4.387-397) (in Russ.).
23. Timofeev N.P. *Agrarnaya nauka Evro-Severo-Vostoka*, 2021, 22(6): 804-825 (doi: 10.30766/2072-9081.2021.22.6.804-825) (in Russ.).
24. Bathori M., Toth N., Hunyadi A., Marki A., Zador E. Phytoecdysteroids and anabolic-androgenic steroids − structure and effects on humans. *Current Medicinal Chemistry*, 2008; 15(1): 75-91 (doi: 10.2174/092986708783330674).
25. Lafont R., Dinan L. Practical uses for ecdysteroids in mammals including humans: and update. *Journal of Insect Science*, 2003, 3(7): 1-30 (doi: 10.1673/031.003.0701).
26. Hornok, S., Csorba, A., Kováts, D., Csörgő T., Hunyadi A. Ecdysteroids are present in the blood of wild passerine birds. *Scientific Reports*, 2019, 9: 17002 (doi: 10.1038/s41598-019-53090-9).
27. *Ecdysterone market, by type (0.95, 0.98, other), by application (pharma & healthcare, cosmetic & skin care, other), by region (North America, Europe, Asia Pacific, Middle East & Africa, and South America) — Market Size & Forecasting To 2028. Report Code: CH2066906. Quince Market Insights. Hadapsar, Pune, India, 2021.* Available: https://www.quincemarketinsights.com/industry-analysis/ecdysterone-marke. Accessed: 10.05.2022.

28. Ambrosio G., Wirth D., Joseph J.F., Mazzarino M., Torre X., Botre F., Parr M.K. How reliable is dietary supplement labelling? Experiences from the analysis of ecdysterone supplements. *Journal of Pharmaceutical and Biomedical Analysis*, 2020, 177: 112877 (doi: 10.1016/j.jpba.2019.112877).
29. Tóth G., Herke I., Gáti T., Vágvölgyi M., Berkecz R., Parfenova L.V., Ueno M., Yokoi T., Nakagawa Y., Hunyadi A.A Commercial extract of *Cyanotis arachnoidea* roots as a source of unusual ecdysteroid derivatives with insect hormone receptor binding activity. *Journal of Natural Products*, 2021, 84(7): 1870-1881 (doi: 10.1021/acs.jnatprod.0c01274).
30. Hunyadi A., Herke I., Lengyel K., Báthori M., Kele Z., Simon A., Tóth G., Szendrei K. Ecdysteroid-containing food supplements from *Cyanotis arachnoidea* on the European market: evidence for spinach product counterfeiting. *Scientific Reports*, 2016, 6: 37322 (doi: 10.1038/srep37322).
31. Kraiem S., Al-Jaber M.Y., Al-Mohammed H., Al-Menhali A.S., Al-Thani N.J., Helaleh M., Samsam W., Touil S., Beotra A., Georgakopoulas C., Bouabdallah S., Mohamed-Ali V., Al Maadheed M. Analytical strategy for the detection of ecdysterone and its metabolites in vivo in uPA(+/+)-SCID mice with humanised liver, human urine samples and estimation of prevalence of its use in anti-doping samples. *Drug Testing and Analysis*, 2021, 13(7): 1341-1353 (doi: 10.1002/dta.3032).
32. *20-Hydroxyecdysone. Product No H5142 (≥93 %, HPLC, powder). 2022.* Available: https://www.sigmaaldrich.com/RU/en/product/sigma/h5142. Accessed: 10.05.2022.
33. Timofeev N.P. Achievements and problems in investigation of biology in medicinal herbs of *Rhaponticum carthamoides* (Willd.) Iljin and *Serratula coronata* L. (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2007, 3: 3-17 (doi: 10.15389/agrobiology.2007.3.3rus) (in Russ.).
34. Dinan L., Balducci C., Guibout L., Foucault A.-S., Bakrim A., Kumpun S., Girault J.-P., Tourette C., Dioh W., Dilda P.J., Veillet S., Lafont R. Ecdysteroid metabolism in mammals: The fate of ingested 20-hydroxyecdysone in mice and rats. *The Journal of Steroid Biochemistry and Molecular Biology*, 2021, 212: 105896 (doi: 10.1016/j.jsbmb.2021.105896).
35. *A double-blind, placebo controlled, randomized interventional clinical trial (SARA-INT) (SARA-INT).* ClinicalTrials.gov Identifier: NCT0345248. Available: https://clinicaltrials.gov/ct2/show/NCT03452488. Accessed: 10.05.2022.
36. Timofeev N.P. *International Agricultural Journal*, 2021, 64(6): 46-112 (doi: 10.24412/2588-0209-2021-10384) (in Russ.).
37. Compendium of botanicals reported to contain naturally occuring substances of possible concern for human health when used in food and food supplements. *EFSA Journal*, 2012, 10(5): 2663 (doi: 10.2903/j.efsa.2012.2663).
38. Das N., Mishra S.K., Bishayee A., Ali E.S., Bishayee A. The phytochemical, biological, and medicinal attributes of phytoecdysteroids: an updated review. *Acta Pharmaceutica Sinica B*, 2021, 11(7): 1740-1766 (doi: 10.1016/j.apsb.2020.10.012).
39. Zhang X.-P., Zhang J., Dong M., Zhang M.-L., Huo C.-H., Shi Q.-W., Gu Y.-C. Chemical constituents of plants from the genus *Rhaponticum*. *Chemistry & Biodiversity*, 2010, 7(3): 594-609 (doi: 10.1002/cbdv.200800275).
40. Carasso V., Mucciarelli M., Dovana F., Müller J.V. Comparative germination ecology of two endemic *Rhaponticum* species (*Asteraceae*) in different climatic zones of the Ligurian and Maritime Alps (Piedmont, Italy). *Plants*, 2020, 9(6): 708 (doi: 10.3390/plants9060708).
41. Timofeev N.P., Punegov V.V., Bindasova T.N. V sbornike: *Khimiya i tekhnologiya rastitel'nykh veshchestv* [In: Chemistry and technology of plant substances]. Syktyvkar, 2022: 197-197 (in Russ.).
42. *Tekhnicheskiy reglament Tamozhennogo soyuza. TR TS № 021/2011 «O bezopasnosti pishchevoy produktsii»*. Reshenie KTS ot 09.12.2011 № 880, v red. ot 14.07.2021 [Technical regulations of the Customs Union. TR CU No. 021/2011 "On food safety". Decision of the CCC dated 12/09/2011 No. 880, amended 07/14/2021]. Astana, Kazakhstan (in Russ.).
43. Barnaulov O.D. *Traditsionnaya meditsina*, 2015, 3: 52-56 (in Russ.).
44. Peschel W., Kump A., Prieto J.M. Effects of 20-hydroxyecdysone, *Leuzea carthamoides* extracts, dexamethasone and their combinations on the NF-κB activation in HeLa cells. *Journal of Pharmacy and Pharmacology,* 2011, 63(11): 1483-1495 (doi: 10.1111/j.2042-7158.2011.01349.x).
45. Girault J.-P., Lafont R., Varga E., Hajdu Zs., Herke I., Szendrei K. Ecdysteroids from *Luzea carthamoides*. *Phytochemistry*, 1988, 27(3): 737-741 (doi: 10.1016/0031-9422(88)84085-8).
46. Bili A.Sh., Meyer M., Sheval'e K., Lorenson L., Fol'er N., Roller M., Birtik S., Fansa-Berton P.E.R, Fal'ko L.D. *Kompozitsii i sposoby dlya uluchshennogo myshechnogo metabolizma. Patent RU 2730853 C2. NATUREKS SA. Frantsiya. Zayavl. 03.02.2016. Opubl. 26.08.2020. Byul. № 24* [Substances and methods to improve muscle metabolism. Patent RU 2730853 C2. NATUREX SA. France. Appl. 02/03/2016. Published 08/26/2020. Bull. No. 24] (in Russ.).
47. Píš J., Buděšínský M., Vokáč K., Laudová V., Harmatha J. Ecdysteroids from the roots of *Leuzea carthamoides*. *Phytochemistry*, 1994, 37(3): 707-711 (doi: 10.1016/S0031-9422(00)90343-1).
48. Mamatkhanov A.U., Shamsutdinov M.-R., Shakirov T.T. *Khimiya prirodnykh soedineniy*, 1980, 5: 528-529 (in Russ.).
49. Baltaev U.A., Abubakirov N.K. *Khimiya prirodnykh soedineniy*, 1987, 5: 681-684 (in Russ.).
50. Vokáč K., Buděšínský M., Harmatha J. Minor ecdysteroid components of *Leuzea carthamoides*. *Collection of Czechoslovak Chemical Communications*, 2002, 67(1): 124-139 (doi: 10.1135/cccc20020124).

51. Timofeev N.P., Lapin A.A., Zelenkov V.N. Quality assessment of *Rhaponticum carthamoides* (Willd.) Iljin as medicinal raw material by the bromic antioxidant capacity estimation. In: *Functional foods for chronic diseases. The modern day cure without the side effects of traditionall treatments.* D.M. Martirosyan (ed.). Richardson (Texas, USA), D&A Incorporated, 2006: 164-172. Accessed: 10.05.2022.
52. *Protokol issledovaniya na soderzhanie ekdisteroidov № 18 ot 31.03.2021 g*. Syktyvkar, IB FITs Komi NTs UrO RAN. Available: https://leuzea.ru/pdf/drug_leuzea-p_from_the_pharmacy.pdf. No date (in Russ.).
53. Sevko D.A. *Kontsentrirovanie i opredelenie fitosteroidov s pomoshch'yu molekulyarno-imprintirovannykh sorbentov i tandemnoy mass-spektrometrii vysokogo razresheniya. Avtoreferat kandidatskoy dissertatsii* [Concentration and determination of phytosteroids using molecularly imprinted sorbents and high resolution tandem mass spectrometry. PhD Thesis]. Moscow, 2016 (in Russ.).
54. Potanin G.N. *Ocherki Severo-zapadnoy Mongolii: rezul'taty puteshestviya, ispolnennye v 1876-1877 g. po poruchen iyu Imp. Rus. geogr. o-va chl. sotr. onogo G.N. Potaninym. Vyp. 4. Materialy etnograficheskie* [Essays on North-Western Mongolia: the results of a journey completed in 1876-1877 by member-employee G.N. Potanin on behalf of Imp. Rus. geogr. society. Issue. 4. Ethnographic materials]. St. Petersburg, 1883: 188-189 (in Russ.).
55. Postnikov B.A. *Maraliy koren' i osnovy vvedeniya ego v kul'turu* [Maral root and the basics of its introduction into culture]. Novosibirsk, 1995 (in Russ.).
56. Terekhin A.A., Vandyshev V.V. *Tekhnologiya vozdelyvaniya lekarstvennykh rasteniy* [Technology of cultivation of medicinal plants]. Moscow, 2008 (in Russ.).
57. Galambozi B., Kirakosyan G.M., Luzhanin V.G., Flisyuk E.V., Makarov V.G., Pozharitskaya O.N., Shikov A.N. *Vyrashchivanie efiromaslichnykh i lekarstvennykh rasteniy v usloviyakh Severa: monografiya* [Growing essential oil plants and medicinal plants in the conditions of the North: monograph]. St. Petersburg, 2018 (in Russ.).
58. Kubentaev S.A., Danilova A.N. *Vestnik Tomskogo gosudarstvennogo universiteta*, 2017, 37: 31-46 (in Russ.).
59. Timofeev N.P. *Rastitel'nye resursy*, 2006, 42(2): 17-36 (in Russ.).
60. Timofeev N.P. Ecological relations of the agropopulations of ecdysteroid-containing plants *Rhaponticum carthamoides* (Willd.) Iljin and *Serratula coronata* L. with the insects-phytophagans. Report 1. *Contemporary Problems of Ecology*, 2009, 2(5):489-500 (doi: 10.1134/S1995425509050166).
61. Timofeev N.P. Composition variability of phytoecdysteroids in agrocenoses and their role in the vulnerability of plants to phytophagans. Report 2. Ecological relations of the agropopulations of ecdysteroid-containing plants *Rhaponticum carthamoides* (Willd.) Iljin and *Serratula coronata* L. with the insects-phytophagans. *Contemporary Problems of Ecology,* 2009, 2(6): 531-541 (doi: 10.1134/S1995425509060071).
62. Baù A., Bottex B. EFSA's compendium of botanicals. *Zenodo*, 2018. (doi: 10.5281/zenodo.1212388).
63. Yang J., Wang, L. Safety pharmacology and toxicity study of herbal medicines. In: *Traditional herbal medicine research methods: identification, analysis, bioassay, and pharmaceutical and clinical sciences.* W.J.H. Liu (ed.). Wiley, 2011 (doi: 10.1002/9780470921340.ch7).
64. Brown A.C. An overview of herb and dietary supplement efficacy, safety, and government regulation in the United States with suggested improvements. Part 1 of 5 series. *Food and Chemical Toxicology*, 2017, 107(Part A): 449-471 (doi: 10.1016/j.fct.2016.11.001).
65. Brown A.C. Liver toxicity related to herb and dietary supplements: online table of medical case reports. Part 2 of 5 series. *Food and Chemical Toxicology*, 2017, 107(Part A): 472-501 (doi: 10.1016/j.fct.2016.07.001).
66. Brown A.C. Kidney toxicity related to herb and dietary supplements: online table of medical case reports. Part 3 of 5 series. *Food and Chemical Toxicology*, 2017, 107(Part A): 502-519 (doi: 10.1016/j.fct.2016.07.024).
67. Brown A.C. Heart toxicity related to herb and dietary supplements: online table of case reports. Part 4 of 5. *Journal of Dietary Supplements*, 2018, 15(4): 516-555 (doi: 10.1080/19390211.2017.1356418).
68. Brown A.C. Cancer related to herbs and dietary supplements: online table of case reports. Part 5 of 5. *Journal of Dietary Supplements,* 2018, 15(4): 556-581 (doi: 10.1080/19390211.2017.1355865).
69. *IPNI (International Plant Names Index)*. Available: https://www.ipni.org/. Accessed: 10.05.2022.
70. Mamaev S.A. V kn.: *Individual'naya i ekologo-geograficheskaya izmenchivost' rasteniy. Trudy Instituta ekologii rasteniy i zhivotnykh UNTs AN SSSR. Tom 94* [In: Individual and ecogeographical variability of plants. Proceedings of the Institute of Plant and Animal Ecology, UNC, USSR Academy of Sciences. Vol. 94]. Sverdlovsk, 1975: 3-14 (in Russ.).
71. *Ecdybase: The ecdysone handbook*. R. Lafont, J. Harmatha, F. Marion-Poll, L. Dinan, I.D. Wilson (eds.). Available: https://ecdybase.org/. Accessed: 10.05.2022.
72. Polozhiy A.V., Nekratova N.A. V knige: *Biologicheskie osobennosti rasteniy Sibiri, nuzhdayushchikhsya v okhrane* [In: Biological features of Siberian plants in need of protection]. Novosibirsk, 1986: 198-226 (in Russ.).
73. Myrzangalieva A.B., Samarkhanov T.N. *Vestnik Evraziyskogo natsional'nogo universiteta imeni L.N. Gumileva. Seriya Biologicheskie nauki*, 2018, 124(3): 55-64 (in Russ.).
74. Matolinets D.A., Voloshin V.A. *Sibirskiy vestnik sel'skokhozyaystvennoy nauki*, 2017, 47(6): 66-72

(doi: 10.26898/0370-8799-2017-6-9) (in Russ.).
75. Maysak G.P., Matolinets D.A. *Kormoproizvodstvo*, 2021, 2: 32-35 (in Russ.).
76. Crouzet S., Maria A., Dinan L., Lafont R., Girault J.-P. Ecdysteroids from *Cyanotis longifolia* Benth. (*Commelinaceae*). *Archives of Insect Biochemistry and Physiology*, 2009, 72(4): 194-209 (doi: 10.1002/arch.20329).
77. Kokoska L., Janovska D. Chemistry and pharmacology of *Rhaponticum carthamoides:* a review. *Phytochemistry*, 2009, 70(7): 842-855 (doi: 10.1016/j.phytochem.2009.04.008).
78. Timofeev N.P. *Agrarnaya nauka Evro-Severo-Vostoka,* 2022, 23(4): 480-495 (doi: 10.30766/2072-9081.2022.23.4.480-495) (in Russ.).
79. Timofeev N.P. *Materialy III Mezhdunarodnoy konferentsii po selektsii, tekhnologii vozdelyvaniya i pererabotki netraditsionnykh rasteniy* [Proceedings of III International Conference on breeding, cultivation technology and processing of non-traditional plantsных растений]. Simferopol', 1994: 166-167 (in Russ.).
80. Kratky F., Opletal L., Hejhalek J., Kucharova S. Effect of 20-hydroxyecdysone on the protein synthesis of pigs. *Zivocisna Vyroba,* 1997, 42: 445-451.

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 142-157
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Plant protection

UDC 633.353:632.7:631.522/.524:581.19:581.573.4

doi: 10.15389/agrobiology.2023.1.142eng
doi: 10.15389/agrobiology.2023.1.142rus

SENSITIVITY OF FABA BEAN (*Vicia faba* L.) CULTIVARS TO *Aphis fabae* Scopoli INFESTATION AND PLANT PARAMETERS RESPONSIBLE FOR LOW SUSCEPTIBILITY TO THE PEST

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Final revision received March 24, 2022
Accepted September 09, 2022

Abstract

The most economically significant pest of V*icia faba* L. beans is the bean aphid *Aphis fabae* Scopoli (*Hemiptera*, *Homoptera*: *Aphididae*). The use of varieties resistant to various aphid species can increase the production of this crop, reduce environmental pollution and the cost of monitoring the condition of crops. It is known about the relationship between the degree of damage by aphids and the morphological features of the plant, however, information on chemical changes during damage by aphids and the role of chemical factors in sensitivity to A. fabae is ambiguous. In the present work, for 12 varieties of *V. faba* from the collection of the Institute of Forage Crops (Pleven), it was shown for the first time that the *A. fabae* infestation led to a decrease in plant height, crude protein, phosphorus, and chlorophyll a + b while the amount of cyanogenic glycosides increased significantly. Therefore, the aim of the present study was to assess the sensitivity of faba bean cultivars to *Aphis fabae* and to define the morphological and chemical parameters responsible for low aphid susceptibility. The field study was carried out at the Institute of Forage Crops (Pleven, 2016-2018) in the experiment laid out in Randomized Block Design (RBD). The infestation was assessed by recording the number of aphids per plant at the stages of budding, flowering and bean formation ($n = 20$, $N = 3$), resistance or susceptibility of plants was classified using a 0-12-point scale. The chemical composition (the content of crude protein, phosphorus, chlorophyll a, chlorophyll b, cyanogenic glycosides) was determined by standard methods (Weende system analysis). It was found that aphids reached the highest abundance at the stage of pod formation. Cultivars Fb 3270 and BGE 029055 were defined as very low susceptible to aphids, while BGE 002106, BGE 032012 and BGE 041470 were medium susceptible. Aphid infestation significantly affected the morphological and chemical traits of cultivars and led to a reduction of the plant height, crude protein, phosphorus and chlorophyll a + b content, while cyanogenic glycosides significantly increased in response to aphid injury. The extent of the decrease in chemical parameters and plant height depends on the aphid abundance, being significantly higher in very high susceptible and high susceptible cultivars. On the contrary, cyanogenic glycosides increase with an increase in the aphid abundance. Thereof, the levels of crude protein, phosphorus and cyanogenic glycosides can serve as key factors indicative of the aphid preference. Cultivars Fb 3270 and BGE 029055 with higher phosphorus and cyanogenic glycosides, lower crude protein, and shorter plants had significantly lower aphid infestations. Therefore, these varieties are significantly less susceptible to *A. fabae* than other varieties and can be involved in breeding programs to improve plant resistance to *A. fabae*.

Keywords: *Aphis fabae*, feed preferences, faba bean cultivars, susceptibility, morphological traits, chemical traits

Faba bean (*Vicia faba* L.) is an important grain legume, protein-rich and widely used for human and animal consumption. In addition, faba bean has also a valuable agronomic function considering its high capacity of N_2 fixation.

The most important common bean pest worldwide is the black bean aphid, *Aphis fabae* Scopoli (*Hemiptera*, *Homoptera*: *Aphididae*), which causes considerable damage to plants and yield loss reaches 37% [1]. Aphids frequently grow and

develop rapidly, allowing aphid populations to fastly exceed economical threshold levels. Numerous colonies of *A. fabae* is very damaging to *V. fabae* because of the direct negative impact on the plant growth and the quantity and quality of the yield [2]. Injury caused by many aphid species is well documented to change the rate of photosynthesis, plant growth and physiological and chemical processes [3-5]. For example, H.K. Shannag [6] found the negative effects of aphid feeding by reduction of crude protein levels of damaged leaves in 14-day-infested plants before signs appeared. On the other hand, P. Mawaand and A.B. Tambe [7] studied qualitative losses caused by aphids in lucerne and found a drastic reduction in chlorophyll, dry matter, crude protein and fibre, phosphorous, potassium, ash and calcium.

The control of aphids currently depends primarily on chemical insecticides. However, insecticides have a negative effect on useful insects and the environment. Thus, there is a need for effective, environmentally friendly alternatives to suppress crop pests. Identification of cultivars resistant to different aphid species has become an important subject of research [8-10]. The use of these cultivars may increase faba bean production affected by aphids and reduce environmental pollution and control costs.

Many authors reported that the application of resistant cultivars is a substantive and indubitable method to control aphids [11, 12]. B. Béji et al. [2] studied faba bean resistance to *A. fabae* and found that the best parameters describing resistance were pod weight and grain number. F. Meradsi and M. Laamari [13] evaluated the resistance response of *V. faba* to black bean aphid by the relationship between the resistance level and plant morphological characteristics. It was reported [8, 14} that there is the relative impact of cultivars' resistance to black bean aphids, based on antibiosis and antixenosis. However, there was less information on the chemical changes that occur after plant damage by aphids and on the role of various chemical factors in the susceptibility of different species to *A. fabae* colonization [15-17].

An integrated approach seems to be the most informative to reveal traits associated with the resistance to aphids. However, there was less information about the possible morphological and chemical parameters in *V. faba* cultivars responsible for resistance to black bean aphid and changes occurring after damage.

Here, for the first time, it was shown that *V. faba* plant height, the content of crude protein, phosphorus and chlorophylls a + b decreased while the amount of cyanogenic glycosides increased significantly in response to damage by aphids *A. fabae*. Among 12 varieties of *V. faba* from the collection of the Institute of Forage Crops (Pleven), the varieties have been identified that are promising in breeding for resistance of faba beans to *A. fabae*. The amount of protein, phosphorus, and cyanogenic glycosides in *V. faba* plants has been proposed as key indicators of plant attractiveness and aphid preference for such plants.

Therefore, the aim of the present study was to assess the sensitivity of faba bean cultivars to *Aphis fabae* and define the parameters responsible for low aphid susceptibility.

Materials and methods. The field study was carried out at the Institute of Forage Crops (Pleven) during the period 2016-2018. Twelve cultivars of faba bean (*Vicia faba* L.), originating in Portugal (Fb 1896, Fb 1903, Fb 1929, Fb 2481, Fb 2486, Fb 3270) and Spain (BGE 002106, BGE 029055, BGE 032012, BGE041470, BGE 043776, BGE 046721) were used. The experiment was laid out in Randomized Block Design (RBD) with three replications and a 4 m^2 experimental plots. The cultivars were planted with a sowing rate of 30 seeds/m^2 and kept devoid of insecticide application throughout the experimentation to assess the susceptibility or resistance response to *Aphis fabae*. Aphid infestation occurred

naturally. Keeping plants without aphid infestation was carried out by triple treatment with alternating insecticides with active substances alfa-cypermethrin 150 g/l and deltamethrin 25g/l (20 ml/ha or 0.02 ml/m^2 for both preparations during budding, flowering and pod formation). The reaction of different cultivars to *A. fabae* was assessed by recording the aphid number per plant at 50% budding, 50% flowering and 50% pod formation stages of the faba bean. Therefore, twenty plants were selected randomly from each replication of the cultivar. The average aphid number was calculated based on three-time counts in each stage within 2-3 days. The height of the plants was measured in parallel.. Plant response was assessed on a point scale [18], where 0 is resistance (0 aphids per plant), 1 is slight sensitivity (20 aphids per plant), 2 is very low sensitivity (> 20-100 aphids per plant), 3 is low sensitivity (> 101-200 aphids per plant), 4 is medium sensitivity (> 201-350 aphids per plant), 5 is high sensitivity (> 351-500 aphids per plant), 6 is very high sensitivity (> 501 aphids per plant).

In order to determine the chemical changes of the aboveground mass of cultivars in the aphid infestation, 15 plant samples taken of each cultivar ($n = 5$, $N = 3$) were fixed for 15 min at 100 °C and dried to a constant weight at a 60 °C in a thermostat. The chemical composition was determined by standard methods of the Weende system [19] and includes crude protein (CP) by Kjeldahl method (CP = total N × 6.25), crude fibre (CF), phosphorus colourimetrically by hydroquinone method, calcium complexometrically [20]. In addition, in fresh plant samples, plastid pigments content (chlorophyll a, chlorophyll b, carotenoids, and total) (mg/100 g dry matter)) was determined according to M.I. Zelenskii and G.A. Mogileva [21] as well as the cyanogenic glycosides contents - according to A.I. Ermakov et al. [22]. The content of phosphorus, chlorophyll a, chlorophyll b was measured spectrophotometrically (a Spekol 11 spectrophotometer, Carl-Zeiss, Germany), the content of crude protein was measured using a KELDAL apparatus (model UDK-127, VELP Scientifica Srl, Italy). Chemical compounds in infested and uninfested cultivars were determined in the bedding of the pod formation.

The data were subjected to one-way ANOVA, the means (M) with standard radiation (±SD) were calculated, the means were compared by Tukey's test ($p \leq 0.05$). The Correlation Analysis was performed using Microsoft Office Excel 2007. Multiple Regression Analysis of Statgraphics Plus (1995) for Windows Ver. 2.1 Software program was used.

Results. Aphids appeared in the formation of the first buds, and at the plant development during the budding stage, their number increased proportionally. Despite the low density in that stage, aphids preferred certain cultivars. BGE 046721 had significantly the highest aphid number, followed by BGE 043776 and Fb 1903 ($F_{11.5} = 12.224$; $p < 0.001$) (Table 1). The differences in the infestation in the other cultivars were mostly inconsiderable, but Fb 3270, Fb 1896, BGE 002106 and BGE 032012 were less preferred. The last ones stood out by a small aphid number, varying in the narrow range of 14.6-16.6 number of winged and wingless individuals/plant. The average population density of the species during budding was low (50 aphids per plant). According to the susceptibility grades, there was a stronger aphid preference for BGE 046721 and BGE 043776 as early as the early stage.

At the beginning of the flowering stage, there was a considerable increase in the number, which reached an average of 197.2 aphid number in the 50% flowering. The number was three times higher than in the earlier stage of the plant development. Fb 3270 had the lowest value, followed by BGE 029055 and the difference was significant ($F_{11.5} = 21.922$; $p < 0.001$). A lower number was also found in BGE 002106 and Fb 2486 with minimal differences between them, followed by Fb 1903 and BGE 032012. The differences between the last three cultivars were insignificant.

1. *Aphis fabae* Scopoli number (number of winged and wingless individuals per plant) **in faba bean (*Vicia faba* L.) cultivars of different origine by plant development stages** ($n = 20$, $N = 3$, $M \pm SD$, Randomized Block Design, a 4 m^2 experimental plot, the Institute of Forage Crops, Pleven, Bulgaria, 2016-2018)

Cultivars	Budding stage		Flowering stage		Pod formation		Susceptability	
							estimates	group
Fb 1896	14.8±1.93	a[1]/a[2]	125.3±27.75	f/b	432.1±17.07	e/c	$F_{2.5} = 47.840$; $p < 0.019$	High
Fb 1903	49.0±3.46	d/a	99.2±22.40	de/b	367.3±19.81	d/c	$F_{2.5} = 18.706$; $p < 0.033$	High
Fb 1929	33.8±4.83	c/a	148.9±23.70	g/b	524.6±15.89	f/c	$F_{2.5} = 9.798$; $p < 0.028$	Very high
Fb 2481	26.2±2.64	abc/a	114.4±24.50	ef/b	352.7±16.57	d/c	$F_{2.5} = 9.117$; $p < 0.048$	High
Fb 2486	20.4±3.40	ab/a	89.5±25.70	cd/b	823.0±20.64	g/c	$F_{2.5} = 24.531$; $p < 0.040$	Very high
Fb 3270	14.6±2.16	a/a	34.9±17.54	a/b	52.6±15.12	a/c	$F_{2.5} = 6.578$; $p < 0.012$	Very Low
BGE 002106	16.1±1.59	a/a	70.2±16.63	bc/b	268.6±18.19	c/c	$F_{2.5} = 12.152$; $p < 0.035$	Medium
BGE 029055	25.0±3.79	abc/a	59.5±17.13	b/b	99.8±19.91	b/c	$F_{2.5} = 14.649$; $p < 0.044$	Very Low
BGE 032012	16.6±2.52	a/a	93.0±23.90	de/b	348.4±24.13	d/c	$F_{2.5} = 39.732$; $p < 0.006$	Medium
BGE041470	31.0±3.01	bc/a	135.5±18.41	fg/b	298.6±20.55	c/c	$F_{2.5} = 24.635$; $p < 0.005$	Medium
BGE 043776	ax	e/a	514.6±23.30	h/b	2029.9±25.09	h/c	$F_{2.5} = 33.671$; $p < 0.029$	Very high
BGE 046721	235.0±8.26	f/a	881.1±29.98	i/b	3773.9±22.23	i/c	$F_{2.5} = 87.942$; $p < 0.031$	Very high
Average	50.0		197.2		781.0			

N o t e. In columns, values before the slash ([1]) and marked with the same letters have no statistically significant differences at $p < 0.05$. In a row, values after the slash ([2]) and marked with the same letters have no statistically significant differences at $p < 0.05$.

2. Height of plants colonized and not colonized by *Aphis fabae* Scopoli in faba bean (*Vicia faba* L.) cultivars of different origine by plant development stages ($n = 20$, $N = 3$, $M \pm SD$, Randomized Block Design, a 4 m^2 experimental plot, the Institute of Forage Crops, Pleven, Bulgaria, 2016-2018), 2016-2018)

Cultivars	Budding stage				Flowering stage				Pod formation			
	colonized		not colonized		colonized		not colonized		colonized		not colonized	
1	23.4±2.79	ab[1]/a[2]	31.1±2.88	abcd/b	41.7±9.04	ab/a	67.7±4.85	bcd/b	69.8±7.45	b/a	91.5±6.94	ef/b
2	31.2±4.18	cde/a	37.2±4.66	ef/b	73.2±10.45	f/a	86.2±10.17	g/b	79.3±10.16	cd/a	101.1±9.75	g/b
3	20.0±6.19	a/a	26.7±5.12	a/b	50.9±6.64	c/a	64.2±4.49	abc/b	58.4±6.45	a/a	77.9±6.15	ab/b
4	27.1±9.01	bc/a	33.7±4.69	cde/b	69.8±6.62	ef/a	77.9±6.15	ef/b	73.5±4.19	bc/a	83.8±9.42	bcd/b
5	19.6±8.44	a/a	28.1±10.73	ab/b	47.9±10.24	bc/a	60.8±9.69	ab/b	56.8±3.00	a/a	80.4±5.39	abc/b
6	32.8±6.99	de/a	39.2±6.74	f/a	65.8±6.33	de/a	72.1±6.06	de/a	84.9±3.11	de/a	86.4±5.60	cde/a
7	24.5±7.50	ab/a	29.7±7.73	abc/a	41.4±10.71	**a/a**	58.9±7.62	a/b	60.0±8.55	a/a	74.5±11.18	a/b
8	33.7±5.76	e/a	37.7±3.97	ef/a	74.0±8.55	fg/a	75.5±10.78	e/a	84.0±5.45	de/a	87.9±4.72	de/a
9	27.7±2.75	bcd/a	31.1±6.50	abcd/b	62.4±3.41	d/a	70.9±8.72	cde/b	75.3±3.20	bc/a	85.3±8.43	cde/b
10	28.9±7.48	bcde/a	32.5±4.08	bcde/a	75.3±3.20	fg/a	85.3±8.43	fg/b	77.3±7.76	c/a	87.8±6.95	de/b
11	28.7±5.81	bcde/a	32.8±6.49	bcde/b	66.4±5.30	de/a	77.1±4.79	e/b	70.0±3.82	b/a	95.8±5.20	fg/b
12	31.8±6.29	cde/a	36.0±4.50	def/b	80.4±5.41	g/a	95.4±11.11	h/b	86.4±5.96	e/a	123.8±7.87	h/b
Average	27.5		33.0		62.4		74.3		73.0		89.7	

Note. 1 — Fb 1896, 2 — Fb 1903, 3 — Fb 1929, 4 — Fb 2481, 5 — Fb 2486, 6 — Fb 3270, 7 — BGE 002106, 8 — BGE 029055, 9 — BGE 032012, 10 — BGE 041470, 11 — BGE 043776, 12 — BGE 046721. In columns, values before the slash ([1]) and marked with the same letters have no statistically significant differences at $p < 0.05$. In a row, values after the slash ([2]) and marked with the same letters have no statistically significant differences at $p < 0.05$.

The unifying trait between all these variants was that the aphid number did not exceed the value of 100. At that stage, they were classified as very low susceptible. Conversely, the most preferred cultivar significantly with the highest *A. fabae* number was BGE 046721, followed by BGE 043776 and the trend of the budding stage was fully preserved. It should be noted that the aphid infestation in BGE 046721 and BGE 043776 exceeded 350 winged and wingless individuals/plant and plants were defined as highly susceptible as early as the flowering stage. Other cultivars were defined as low susceptible.

The most indicative of the black bean aphid preference was the pod formation stage, where the number reached maximum values in the studied cultivars and exceeded on average three times and fifteen times the infestation during the flowering and budding stages, respectively. Bursts of asexual reproduction and live births on faba bean allowed large populations to build up quickly on plants which resulted in an average of 781.0 winged and wingless individuals/plant. Stable position and with the lowest density stood out Fb 3270, followed by BGE 029055 ($F_{11.5} = 44.900$; $p < 0.007$). According to susceptibility grades, the aphid number did not exceed 100 and cultivars were defined as very low susceptible, ie. sustainable. Less preferred and numbers not exceeding 350 aphids were found in BGE 002106 and BGE041470 with negligible differences between them, followed by BGE 032012. That defined them as medium susceptible. Numerous colonies and abundance of *A. fabae* were observed in BGE 046721, which had significantly the highest aphid density, followed by BGE 043776. Cultivars were the most preferred and aphid number was many times higher than the value of 500, which categorically defined them as very high susceptible. Despite significant differences between them and Fb 2486 and Fb 1929, the last ones also belonged to the group of high susceptible cultivars. Other cultivars were high susceptible.

The number of *A. fabae* showed a significant difference between the three stages of growth and plant development. The comparative analysis about the aphid number unequivocally showed that cultivars were the most strongly attacked during the pod formation, followed by the flowering and budding stages infestation.

A.J. Biddle and N.D. Cattlin [23] reported a similar result. According to the authors, colonies of aphids developed rapidly on the upper parts of *Vicia* beans during flowering and pod formation. At first, individual stems were infested, later, aphids spread to surrounding plants, developing into localized patches of the infested plants. *Aphis fabae* population increased rapidly at the flowering stage and reached the highest density on the developing pods. M.R.Amin et al. [24] studied the population dynamic, infestation, and harmful effects of aphids on several bean plant species and found that aphid abundance was on the leaves, flowers and pods in the pod formation stage. In addition, he reported that species with the shortest duration of the growth stages had the highest infestation [24]. M.S.A. Mamun et al. [25] found that usually aphid infestation consistently increase from the early development stage and reached the highest values at the pod formation. Then followed a trend to decrease.

On the other hand, S.A. Dwivedi et al. [26] explored mustard varieties' resistance against aphids and found that the highest aphid infestation index on the based of aphid number was at the full pod formation followed by the flowering stage. In opposite to the present study, M. Esmaeili-Vardanjania et al. [11] found that the maximum number of *A. fabae* in bean cultivars was observed at a two-leaf stage in comparison to the flowering stage and the differences between the various stages of growth were significant. According to the authors during increasing plant age, resistance to black bean aphid was increased and the aphid population in

all cultivars at the flowering stage was declined.

The height of the aphid-infested plants in the budding stage varied in a relatively narrow range and occupied similar values. Only Fb 1929 and Fb 2486 were up to 20 cm high (Table 2) and the differences compared to the other cultivars were significantly to be lower ($F_{11.9} = 5.666$; $p < 0.017$) (except for Fb 1896 and BGE 002106). Significantly higher value was observed for BGE 029055 compared to Fb 1896, Fb 1929, Fb 2481, BGE 002106 and BGE 032012. The trend was similar in the treated plants, as the height in Fb 3270 was significantly higher, followed by BGE 029055, Fb 1903 and BGE 046721 (differences between the last three were minimal) ($F_{11.9} = 5.341$; $p < 0.011$). A comparative analysis between infected and non-infected plants showed that *A. fabae* had a primarily depressant effect on the growth, reducing the values significantly at eight cultivars despite the low aphid number in the budding stage (according to the Table 2 numbers: 1 — $F_{1.9} = 5.341$; $p < 0.011$; 2 — $F_{1.9} = 4.161$; $p < 0.027$; 3 — $F_{1.9} = 5.301$; $p < 0.036$; 4 — $F_{1.9} = 5.750$; $p < 0.046$; 5 — $F_{1.9} = 7.070$; $p < 0.031$; 9 — $F_{1.9}=3.269$; $p < 0.021$; 11 — $F_{1.9}=3.775$; $p < 0.035$; 12 — $F_{1.9} = 4.135$; $p < 0.044$). Only Fb 3270, BGE 002106, BGE 029055 and BGE041470 did not show statistically reduced values under the aphid activity (6 — $F_{1.9} = 6.453$; $p < 0.064$; 7 — $F_{1.9} = 7.158$; $p < 0.017$; 8 — $F_{1.9} = 4.646$; $p < 0.284$; 10 — $F_{1.9} = 5.600$; $p < 0.087$).

The plant height is a genetic trait and the ratio between the cultivars remained relatively constant. In the flowering stage, treated BGE 046721 plants had a significantly higher value, followed by Fb 1903 with a minor difference to BGE041470 ($F_{11.9} = 7.628$; $p < 0.005$). The trend was similar for untreated variants as BGE 046721 had significantly the highest height except for BGE 029055 and BGE041470 ($F_{11.9} = 6.728$; $p <0.004$).

At that stage, as a result of the higher aphid number and intensive nutritional activity, there were more pronounced differences in height and a significant decrease in ten of the studied cultivars (1 — $F_{1.9} = 6.819$; $p < 0.008$; 2 — $F_{1.9} = 9.691$; $p < 0.015$; 3 — $F_{1.9} = 5.326$; $p < 0.022$; 4 — $F_{1.9} = 6.010$; $p < 0.048$; 5 — $F_{1.9} = 9.371$; $p < 0.027$; 7 — $F_{1.9} = 8.738$; $p < 0.003$; 9 — $F_{1.9} = 6.222$; $p < 0.009$; 10 — $F_{1.9} = 5.992$; $p < 0.008$; 11 — $F_{1.9} = 4.744$; $p < 0.014$; 12 — $F_{1.9} = 10.660$; $p < 0.005$). The plant growth was decreased by an average of 21.7%. The low number of black bean aphids in Fb 3270 and BGE 029055 led to an insignificant reduction in the height of 9.6 and 2.0%, respectively ($F_{1.9} = 6.827$; $p < 0.019$; $F_{1.9} = 9.142$; $p < 0.005$).

In the pod formation, the plants reached their maximum height and the trend for the highest plants of BGE 046721 in the treated variants was definitely confirmed, followed by Fb 1903 and BGE 043776 with negligible differences between them ($F_{11.9} = 6.7065$; $p < 0.020$). The height trait was strongly influenced in aphid-infected plants as only the genetically highest cultivar (BGE 046721) retained a leading position but the second and third sites were occupied by resistant Fb 3270 and BGE 029055. The differences between the last three cultivars were minimal ($F_{11.9} = 6.345$; $p < 0.001$). Slightly preferred Fb 3270 and BGE 029055 by aphids during the reproductive stage developed under favourable conditions, ensuring normal growth and metabolic processes, which resulted in higher plants than others. On the based of the *A. fabae* abundance in the pod formation stage, the reduction in growth was the most pronounced as the height decreased by an average of 23.8%.

Particularly indicative change was the considerable reduction of height in Fb 1896, Fb 1929, BGE 043776, Fb 2486 and BGE 046721, reaching 31.1, 33.4,

36.9, 41.5 and 43.3%, respectively. The differences from the treated cultivars were significant (according to the table numbers 1 — $F_{1.9} = 8.339$; $p < 0.043$; 3 — $F_{1.9} = 8.059$; $p < 0.013$; 5 — $F_{1.9} = 3.827$; $p < 0.006$; 11 — $F_{1.9} = 3.934$; $p < 0.009$; 12 — $F_{1.9} = 6.562$; $p < 0.015$). Differences for other cultivars were also significant but the height decreased to a relatively lower degree (2 — $F_{1.9} = 10.361$; $p < 0.045$; 4 — $F_{1.9} = 6.854$; $p < 0.024$; 7 — $F_{1.9} = 9.355$; $p < 0.042$; 9 — $F_{1.9} = 5.992$; $p < 0.008$; 10 — $F_{1.9} = 6.924$; $p < 0.017$). Only the Fb 3270 and BGE 029055 heights were not affected by black bean aphid as the decrease was insignificant, by 1.8 and 4.6% ($F_{1.9} = 4.256$; $p < 0.028$; $F_{1.9} = 4.795$; $p < 0.036$, respectively).

Given the relative preservation of the positions of the uninfested cultivars in the measurement of height, the correlation between the aphid number and the height of the control plants was calculated. It was found that *A. fabae* prefered higher plants as in the budding stage the height had a weak positive effect on the number ($r = +0.244$, $p \leq 0.05$), while in the flowering and pod-forming stages a significant middle and strong positive correlation was found, $r = +0.606$ and $r = +0.803$; $p \leq 0.05$, respectively.

Black bean aphid had a highly significant depressant effect on plant growth during the three stages of development, preferring higher cultivars. An exception was found for very low susceptible plants at Fb 3270 and BGE 02905.

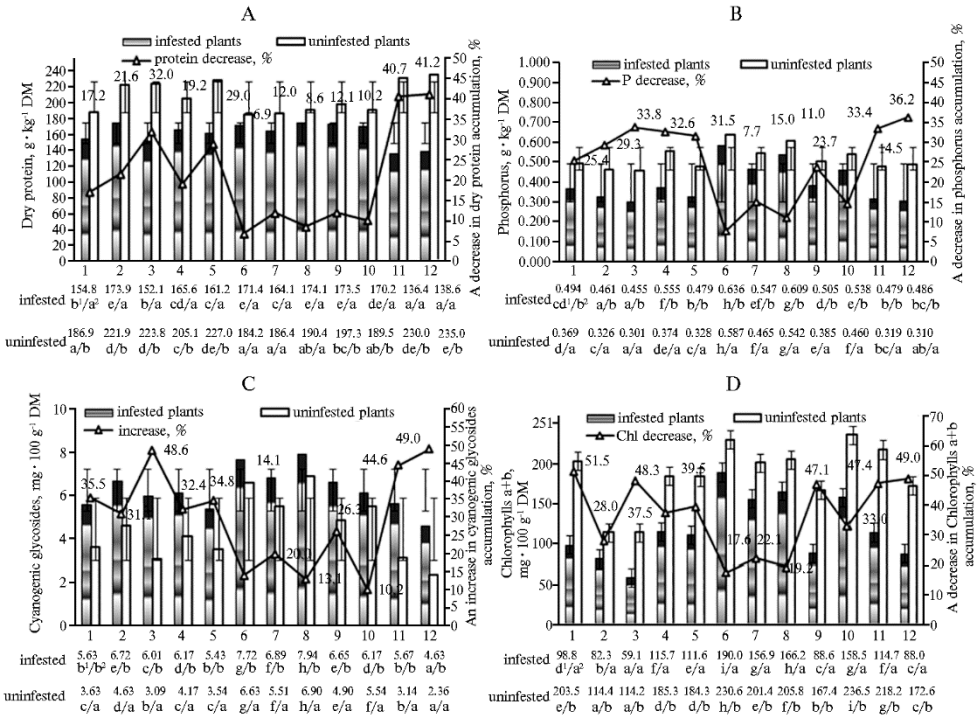
Similar results for a high reduction in plant height under aphid abundance were reported by other authors. According to M.R. Amin et al. [24], aphid infestation and damage had a negative effect not only on the height but also on the leave, flower, and pod number. Furthermore, A.S. Aldawood and A. Soffan [27] supplemented that a cultivar of *V. faba* with the lowest total numbers of aphids had the lowest plant height, while strongly infected cultivars had a higher height. I.A. Khan et al. [28] reported that aphid injury reduced the plant height as height losses were significantly higher (38.78%) in infested cultivars and lower in less infested ones (25.32%). Also, aphid damage delayed plant development and the correlation among aphids and plant height per cent loss was highly significant ($r = 0.75$) [28].

In contrast, F. Meradsi and M. Laamari [13] found that morphological characters as plant height did not affect *A. fabae* infestation, but resistant cultivars had a longer leaflet than highly susceptible cultivars. On the other hand, S. Lebbal [29] mentioned that bean resistant and highly susceptible cultivars had the same morphological characteristics.

The composition of available food in the host plant plays possibly the most important role in determining the relative resistance to aphids. Nitrogen is necessary for many physiological processes of the plant and usually is considered the most important for aphid survival. As *A. fabae* is ingesting only soluble nitrogen sources from plant phloem, its effect on the crude protein resulted in considerable protein reduction up to 41.2% (Fig., A) with significant differences compared to uninfested plants (according to the figure numbers 1 — $F_{1.2} = 26.610$; $p < 0.008$; 2 — $F_{1.2} = 24.259$; $p < 0.001$; 3 — $F_{1.2} = 25.852$; $p < 0.012$; 4 — $F_{1.2} = 21.181$; $p < 0.001$; 5 — $F_{1.2} = 20.257$; $p < 0.022$; 9 — $F_{1.2} = 14.361$; $p < 0.022$; 10 — $F_{1.2} = 7.697$; $p < 0.038$; 11 — $F_{1.2} = 15.178$; $p < 0.012$; 12 — $F_{1.2} = 14.033$; $p < 0.008$). The decrease was usually proportional to the infestation levels of faba bean cultivars and crude protein and aphid numbers showed a medium significant positive correlation ($r = +0.696$, $p \leq 0.05$).

A minimal change was found only in very low susceptible cultivars Fb 3270, BGE 002106 and BGE 029055 where the protein content decreased in the low range from 6.9 to 12.0% (6 — $F_{1.2} = 20.252$; $p < 0.016$; 7 — $F_{1.2} = 25.254$;

$p < 0.001$; 8 — $F_{1.2} = 18.321$; $p < 0.052$).



Changes in the chemical composition of the colonized and not colonized plants in faba bean (*Vicia faba* L.) cultivars affected by *Aphis fabae* Scopoli: 1 — Fb 1896, 2 — Fb 1903, 3 — Fb 1929, 4 — Fb 2481, 5 — Fb 2486, 6 — Fb 3270, 7 — BGE 002106, 8 — BGE 029055, 9 — BGE 032012, 10 — BGE041470, 11 — BGE 043776, 12 — BGE 046721 ($n = 5$, $N = 3$, $M \pm SD$, Randomized Block Design, a 4 m^2 experimental plot, pod formation stage; the Institute of Forage Crops, Pleven, Bulgaria, 2016-2018). In a row, values before the slash ([1]) and marked with the same letters have no statistically significant differences at $p < 0.05$. In columns, values after the slash ([2]) and marked with the same letters have no statistically significant differences at $p < 0.05$.

The sensitive varieties Fb 1903, Fb 1929, Fb 2486, BGE 043776 and BGE 046721 had statistically higher protein than the others ($F_{11,2} = 8{,}643$; $p < 0{,}031$). As a result of the colonization and active nutritional activity of aphids, protein levels significantly decreased and losses were high varying from 21.6 to 41.2% ($F_{11.2} = 5.104$; $p < 0.046$). Our results showed that black bean aphid preferred to settle and colonize on protein-rich plants, while lower protein levels were associated with weakly preference and considerably fewer aphids.

The protein preference of aphids observed in that study was consistent with those reported in several previous experiments. A.M. Mohamed and F.A.A. Siman [30] studied different cultivars/varieties of broad bean for their resistance against *Aphis craccivora* and suggested that high susceptible/heavy infestation of the plant was possibly based on its higher nitrogen and protein content in plant leaves and stems. C.J. Chaudhari et al. [31] reported that resistant lucerne varieties against *Therioaphis maculata* (Buckton) had a lower total chlorophyll, crude protein, sugar and magnesium contents. The authors found also a highly significant positive correlation between the aphid population and chemical component levels in plants. G. Comadira et al. [32] studied the complex relationship between plant N and aphid infestation and found that in N-deficient barley leaves, the progenitor aphids failed to survive until maturity despite the observed large increase in free amino acids.

The present data revealed the key role of plant protein on the quantity and colonization choice of aphids on faba bean plants.

When determining the content of crude fiber, calcium, carotenoids and the total amount of pigments, no differences were found between plants infested and not infested with aphids. The content of these chemical components in plants infested and not infested with aphids was similar in value and did not affect the food preferences of the pest.

Phosphorus (P) is important for the formation of nucleic acids and phospholipids and is needed for the energy metabolism of photosynthesis [33]. In a comparative analysis concerning the P content in uninfested plants (see Fig., B) was found that Fb 3270 had significantly the highest content followed by BGE 029055 ($F_{11.2} = 0.015$; $p < 0.009$) and losses by aphids were low (7.7 and 11.0% respectively). The tendency for statistically higher P levels in the same plants after infection was reserved ($F_{11.2} = 0.013$; $p < 0.018$). In contrast, highly aphid preferred Fb 1929, Fb 2486, BGE 043776 and BGE 046721 had a significantly lower P content in both treated and untreated plants and losses were high varying from 31.5 to 36.2%. Despite the significant P reduction in all infested cultivars (1 — $F_{1.2} = 0.033$; $p < 0.005$; 2 — $F_{1.2} = 0.019$; $p < 0.012$; 3 — $F_{1.2} = 0.043$; $p < 0.001$; 4 — $F_{1.2} = 0.031$; $p < 0.020$; 5 — $F_{1.2} = 0.016$; $p < 0.001$; 6 — $F_{1.2} = 0.034$; $p < 0.007$; 7 — $F_{1.2} = 0.037$; $p < 0.011$; 8 — $F_{1.2} = 0.019$; $p < 0.017$; 9 — $F_{1.2} = 0.026$; $p < 0.026$; 10 — $F_{1.2} = 0.022$; $p < 0.001$; 11 — $F_{1.2} = 0.016$; $p < 0.031$; 12 — $F_{1.2} = 0.022$; $p < 0.015$), the high P content determined a markedly lower *A. fabae* number and low losses. It was found a negative significant correlation between the aphid abundance and the P content in plants ($r = -0.518$; $p \leq 0.05$).

There have been different hypotheses regarding the effects of concentrations of N and P in leaves on the preference of insect pests. For example, R.L. Vannette and M.D. Hunter [34] reported that the greater concentrations of N and P in leaves affected the attractiveness of plants to sap-sucking pests. On the other hand, H.A. Azouz et al. [35] studied how plant mineral status affected the aphid population under field conditions. Authors reported that the susceptible eggplant cultivars had lower potassium, sodium, calcium and phosphorus content and the phytochemical constituents were negatively correlated with the *A. gossypii* (Glover) amount as well as with the level of infestation. S. Facknath and B. Lalljee [36] explained that phosphorus decreases the host suitability for various insect pests by changing secondary metabolites such as phenolics and terpenes and accumulation of phenolics which acts as a barrier having deterring (antifeedant) or directly toxic(insecticidal) effects.

Our results showed that *A. fabae* amount was considerably lower in cultivars with greater concentrations of P but lower content of crude protein in leaves which determined them as important indicators for the aphid preference.

Chemical traits such as cyanogenic glycosides determined the quality of food offered by the host plant and varied from one cultivar to another, with different effects on aphid population levels. Low aphid affected BGE 029055, followed by Fb 3270 had the highest concentration of cyanogenic glycosides in both uninfected ($F_{11.2} = 0.187$; $p < 0.024$) and infected ($F_{11.2} = 0.149$; $p < 0.008$) plants (see Fig., C). The trend was reversed for very high and high susceptible cultivars. Unlike the previous chemical components, where their content decreased after aphid damage, the cyanogenic potential significantly increased in response to injury (1 — $F_{1.2} = 0.443$; $p < 0.005$; 2 — $F_{1.2} = 0.336$; $p < 0.017$; 3 — $F_{1.2} = 0.428$; $p < 0.001$; 4 — $F_{1.2} = 0.424$; $p < 0.035$; 5 — $F_{1.2} = 0.215$; $p < 0.001$; 6 — $F_{1.2} = 0.320$; $p < 0.001$; 7 — $F_{1.2} = 0.308$; $p < 0.027$; 8 — $F_{1.2} = 0.456$; $p < 0.039$; 9 — $F_{1.2} = 0.259$; $p < 0.011$; 10 — $F_{1.2} = 0.272$; $p < 0.001$; 11 — $F_{1.2} = 0.215$; $p < 0.005$; 12 — $F_{1.2} = 0.177$; $p < 0.033$). The considerable higher aphid pressure resulted in a strongly expressed increase of concentrations from 31.1 to 49.0% in high and very high susceptible cultivars as opposed to the slightly preferred ones. Additionally, a

strong significant negative correlation between the cyanogenic glycosides content and aphid number was observed ($r = -0.729$; $p \leq 0.05$).

The results showed that cyanogenic glycosides may play a central protective role against *A. fabae* preventing the colonization and abundance of the species. Bigger differences in the levels of that compound determined not only the different preferences of aphids to the cultivars but also the induction of different cyanogenic content after injury. Probably plants catalyzed their defence mechanisms with increasing aphid pressure, as the induction grade of cyanogenic glycosides was in response to the attack degree.

There was indisputable evidence for the role of cyanogenic glycosides as insect pest deterrents. According to R.M. Gleadow and B.L. Møller [37], these compounds play important role in plant defence producing bitter taste and toxic hydrogen cyanide which repel pests. On the other hand, some authors reported that insect pests damage were responsible for catalyzing the synthesis of cyanogenic glycosides as a defence mechanism [38, 39], while others found that insects were able to detoxify them and either used it as a carbon source or sequester hydrogen cyanide as a defence against predators [40].

In the present study, cultivars with significantly higher cyanogenic glycosides were efficiently directly defended from aphids and responded with a slight increase in compounds, while the preferred plants had a strong increase in cyanogenic glycosides but were not defended by *A. fabae* attack.

Chlorophyll content was used as an indicator of the level of photosynthesis and in turn, the level of carbohydrate produced per leaf. The protein content promoted aphid growth and development while carbohydrates contributed to their energy requirements [41].

Among the uninfected plants with statistically the highest content of chlorophyll a + b was BGE041470, followed by Fb 3270 and BGE 043776 ($F_{11.2} = 2.310$; $p < 0.018$), while the trend in infected cultivars was different and the leading position was occupied by Fb 3270, followed by BGE 029055 ($F_{11.2} = 1,861$; $p < 0.024$) (see Fig., D). No correlation was found between the aphid number and the chlorophyll content, which indicated that the chemical component was not related to the preferences of the species. However, as a result of the attack and nutritional activity of *A. fabae*, a significant reduction in chlorophyll a + b content was found in all cultivars (according to the figure numbers 1 — $F_{1.2} = 2.894$; $p < 0.047$; 2 — $F_{1.2} = 5.204$; $p < 0.001$ 3 — $F_{1.2} = 6.181$; $p < 0.031$; 4 — $F_{1.2} = 3.208$; $p < 0.033$; 5 — $F_{1.2} = 4.391$; $p < 0.001$; 6 — $F_{1.2} = 4.176$; $p < 0.017$; 7 — $F_{1.2} = 4.280$; $p < 0.015$; 8 — $F_{1.2} = 3.130$; $p < 0.001$; 9 — $F_{1.2} = 6.284$; $p < 0.036$; 10 — $F_{1.2} = 5.248$; $p < 0.040$; 11 — $F_{1.2} = 6.177$; $p < 0.023$; 12 — $F_{1.2} = 5.886$; $p < 0.042$). The reduction corresponded to the infestation grade and it was most pronounced in very high and high susceptible cultivars.

Similar results were reported in previous studies. T.I. Huang et al. [42] and M.S. Anjali et al. [43] reported that aphid infestation caused chlorophyll losses and the grade of decreased pigment contents was depended on the aphid density and plant growth stage. In addition, M.R. Amin et al. [24] reported that aphid abundance considerable affected the chemical traits of several bean species and led not only to a high reduction of chlorophyll but also the moisture content in the leaves. Different from our results were reported by D.C. Munthali and A.B. Tshegofatso [41], who studied aphid abundance affected by chlorophyll content in *Brassica oleracea* cultivars. They found that plants with high chlorophyll concentration had a significantly lower aphid infestation. Studies to find out the degree of association of the aphid population with biochemical characters such as chlorophylls are controversial and need further research.

The results of the regression analysis showed that the linear component in

the regression of aphid numbers according to the chemical traits was significant.

Parameters of linear regression:

Dispersion	df	SS	MS	*F*-Ratio	p-value
Model	5	2.73226E7	5.46452E6	15.03	0.00001
Residual	30	1.09078E7	363594.0		
Total (Corr.)	35				

Regression coefficients:

Factors	Coefficient	Standard error	*t*-Stat	p-value
Intercept	–8653.04	2907.290	–2.976	0.005
Height	–26.133	30.273	–0.863	0.009
Protein	33.958	9.175	3.701	0.001
Phosphorus	8471.670	4172.730	2.030	0.051
Cyanogenic glycosides	–506.335	162.323	–3.119	0.004
Chlorophylls a+b	7.65142	3.804	2.011	–0.053

Based on the complex trait study was obtained regression equation indicated the impact of each individual trait on the variation of chemical content:

$Y = -8653.04 - 26.1328X_1 + 33.9578X_2 + 8471.67X_3 - 506.335X_4 + 7.65142X_5$,

where Y is *Aphis fabae* number; X_1 is hieght; X_2 is protein; X_3 is Phosphorus; X_4 is cyanogenic glycosides; X_5 is Chlorophylls a + b.

Results showed that on black bean aphid infestation, the highest negative significant influence had cyanogenic glycosides ($\beta = -506.3$; $p = 0.004$) followed by height ($\beta = -26.1$; $p = 0.009$). Protein content had a positive significant influence ($\beta = 34.0$; $p = 0.001$), while other traits had no significant influence on aphid attack.

Thus, *Aphis fabae* appeared in the formation of the first buds, and at the plant development during the budding and flowering stage, their number increased proportionally and reached a maximum in the pod formation. Stable position and with the lowest density stood out the cultivar Fb 3270, followed by BGE 029055 and according to susceptibility grades, they were defined as very low susceptible, i.e., sustainable to aphid infestation. Medium susceptible to aphids were BGE 002106, BGE 032012 and BGE041470. *Aphis fabae* infestation significant affected the morphological and chemical traits of *Vicia faba* cultivars and led to a reduction of the plant height (by 23.8%, $p \le 0.05$), crude protein (28.2%, $p \le 0.05$), phosphorus (by 31.0%, $p \le 0.05$) and chlorophylls a + b content (28.0%, $p \le 0.05$), while cyanogenic glycosides significantly increased (by 28.6%, $p \le 0.05$) in response to aphid injury. The grade of the decreased content of chemical traits and height was depended on the aphid number and losses were significantly higher in very high and high susceptible plants (BGE 046721, BGE 043776, Fb 2486, Fb 2481, Fb 1896, Fb 1903), while cyanogenic glycosides reciprocally increase with the aphid growth population. Protein and cyanogenic glycosides can be used as key indicators for aphid preference. Phosphorus may also be an important parameter for faba beans influencing *A. fabae* preferences given the negative significant correlation between aphid abundance and plant phosphorus content ($r = -0.518$; $p \le 0.05$). Cultivars Fb 3270 and BGE 029055 with high phosphorus (0.636 and 0.609 g/kg DM, $p \le 0.05$) and cyanogenic glycosides (7.72 and 7.94 mg/100 g DM, $p \le 0.05$) and low plant height and crude protein content (184.2 and 190.4 g/kg DM, $p \le 0.05$) had a significantly lower aphid infestation. Therefore, those cultivars having significantly less susceptibility to black bean aphid than other cultivars can be included in future breeding programmes to improve resistance to *A. fabae*.

REFERENCES

1. Munyasa A.J. *Evaluation of drought tolerance mechanisms in Mesoamerican dry bean genotypes*. University of Nairobi, Nairobi, Kenya, 2013.
2. Béji B., Bouhachem-Boukhris S., Bouktila D., Mezghani-Khémakhem M., Rezgui S., Kharrat M., Makni M., Makni H. Identification of sources of resistance to the black bean aphid, *Aphis*

fabae Scopoli, in Faba bean, *Vicia faba* L., accessions. *Journal of Crop Protection*, 2015, 4(2): 217-224.
3. Cahon T., Caillon R., Pincebourde S. Do Aphids alter leaf surface temperature patterns during early infestation? *Insects*, 2018, 9(1): 34 (doi: 10.3390/insects9010034).
4. Du J.-L., Wu D.-G., Li J.-Q., Zhan Q-W., Huang S.-C., Huang B.-H., Wang X. Effects of aphid disoperation on photosynthetic performance and agronomic traits of different sorghum varieties. *Pakistan Journal of Botany*, 2021, 53(6): 2275-2285 (doi: 10.30848/PJB2021-6(5)).
5. Fuentes S., Tongson E., Unnithan R.R., Gonzalez Viejo C. Early detection of aphid infestation and insect-plant interaction assessment in wheat using a low-cost electronic nose (E-nose), near-infrared spectroscopy and machine learning modeling. *Sensors*, 2021, 21(17): 5948 (doi: 10.3390/s21175948).
6. Shannag H.K. Effect of black bean aphid, *Aphis fabae*, on transpiration, stomatal conductance and crude protein content of faba bean. *Annals of Applied Biology*, 2007, 151(2): 183-188 (doi: 10.1111/j.1744-7348.2007.00161.x).
7. Mawar R., Tambe A.B. Estimation of quantitative and qualitative losses due to major pests of lucerne. *Forage Research*, 2018, 44(2): 105-110.
8. Holt J., Wratten S.D. Components of resistance to *Aphis fabae* in faba bean cultivars. *Entomologia Experimentalis et Applicata*, 1986, 40(1): 35-40 (doi: 10.1111/j.1570-7458.1986.tb02152.x).
9. Birch N. Field evaluation of resistance to black bean aphid, *Aphis fabae*, in close relatives of the Faba bean, *Vicia faba*. *Annals of Applied Biology*, 1985, 106(3): 561-556 (doi: 10.1111/j.1744-7348.1985.tb03147.x).
10. Bond D.A., Lowe H.J.B. Tests for resistance to *Aphis fabae* in field beans (*Vicia faba*). *Annals of Applied Biology*, 1975, 81(1): 21-32 (doi: 10.1111/j.1744-7348.1975.tb00491.x).
11. Esmaeili-Vardanjani M., Askarianzadeh A., Saeidi Z., Hasanshahi G., Karimi J., Nourbakhsh S. A study on common bean cultivars to identify sources of resistance against the black bean aphid, *Aphis fabae* Scopoli (*Hemiptera*: *Aphididae*). *Archives of Phytopathology and Plant Protection*, 2013, 46(13): 1598-1608 (doi: 10.1080/03235408.2013.772351).
12. Sillero J.C., Avila C.M., Rubiales D. Screening faba bean (*Vicia faba*) for resistance to aphids (*Aphis fabae*). In: *International Conference «Advances in grain legume breeding, cultivations and uses for a more competitive value-chain»*, Novi Sad (Serbia), 2017, 27-28 September 2017. Novi Sad, 2017: 46.
13. Meradsi F., Laamari M. Genetic resource of the resistance of *Vicia faba* L. against the black bean aphid, *Aphis fabae* Scopoli. *Journal of Agricultural Studies*, *2016,* 4(2): 107-114 (doi: 10.5296/jas.v4i2.9333).
14. Golizadeh A., Abedi Z., Borzoui E., Golikhajeh N., Jafary M. Susceptibility of five sugar beet cultivars to the black bean aphid, *Aphis fabae* Scopoli (*Hemiptera*: *Aphididae*). *Neotrop. Entomol.*, 2016, 45(4): 427-432 (doi: 10.1007/s13744-016-0383-0).
15. Ismail M., Zanolli P., Muratori F., Hance T. Aphids facing their parasitoids: a first look at how chemical signals may make higher densities of the pea aphid *Acyrthosiphon pisum* less attractive to the parasitoid *Aphidius ervi*. *Insects*, 2021, 12(10): 878 (doi: 10.3390/insects12100878).
16. Babikova Z., Gilbert L., Randall K.C., Bruce T.J., Pickett J.A., Johnson D. Increasing phosphorus supply is not the mechanism by which arbuscular mycorrhiza increase attractiveness of bean (*Vicia faba*) to aphids. *Journal of Experimental Botany*, 2014, 65(18): 5231-5241 (doi: 10.1093/jxb/eru283).
17. Frago E., Mala M., Weldegergis B.T., Yang C., McLean A., Godfray H.C.J., Gols R., Dicke M. Symbionts protect aphids from parasitic wasps by attenuating herbivore-induced plant volatiles. *Nature Communications*, 2017, 8(1): 1860 (doi: 10.1038/s41467-017-01935-0).
18. Teotia T.P.S., Lal O.P. Differential response of different varieties and strains of oleiferous *Brassicae* to the aphid, *Lipaphis erysimi* (Kalt.). *Labdev Journal of Science and Technology*, 1970, 8B: 219-226.
19. *AOAC. Official methods of analysis, 18-th ed.* Association of Analytical Chemists, Gaithersburg, Maryland, 2001, USA.
20. Sandev S**.** *Chemical* methods *for analysis of forages*. Zemizdat, Sofia, 1979 (Bg).
21. Zelenskiy M.I., Mogileva G.A. *Sravnitel'naya otsenka fotosinteticheskoy sposobnosti sel'skokhozyaystvennykh rasteniy po fotokhimicheskoy aktivnosti khloroplastov* [Comparative assessment of the photosynthetic ability of agricultural plants according to the photochemical activity of chloroplasts]. Leningrad, 1980 (in Russ.).
22. Ermakov A.I., Arasimovich V.V., Yarosh N.P. *Metody biokhimicheskogo issledovaniya rasteniy. 2-e izd.* /Pod redaktsiey A.I. Ermakova [*Methods of biochemical research of plants. 2nd edition*. A.I. Ermakov]. Leningrad, 1987 (in Russ.).
23. Biddle A.J., Cattlin N.D. *Pests, diseases and disorders of peas and beans: a color handbook*. Manson Publishing Ltd, London, 2007.
24. Amin M.R., Sharmin M.A., Miah U., Akanda A.M., Suh S.J., Kwon Y.J., Kwon O. Aphid population abundance and pestiferous effect on various bean plant species. *Entomological Research*, 2020, 50(6): 257-266 (doi: 10.1111/1748-5967.12432).
25. Mamun M.S.A., Ali M.H., Ferdous M.M., Rahman M.A., Hossain M.A. Assessment of several

mustard varieties resistance to mustard aphid, *Lipaphis erysimi* (Kalt.). *J. Soil Nature*, 2010, 4(1): 34-38.
26. Dwivedi S.A., Singh R.S., Gharde S.K., Raut A.M., Tomer A. The screening of mustard varieties resistance against mustard aphid *Lipaphis erysimi* Kalt. *Plant Archives*, 2019, 19: 1167-1172.
27. Aldawood A.S., Soffan A. Biology and demographic growth parameters of cowpea aphid (*Aphis craccivora*) on faba bean (*Vicia faba*) cultivars. *Journal of Insect Science*, 2014, 14(1): 120 (doi: 10.1093/jis/14.1.120).
28. Khan I.A., Ahmad M., Hussain S., Akbar R., Saeed M., Farid A., Ali Shah R., Fayaz W., Ud Din M.M., Shah B., Naeem M. A study on correlation between aphid density and loss in yield components of 12 *Brassica* genotypes under screen house conditions. *Journal of Entomology and Zoology Studies*, 2015, 3(6): 29-33.
29. Lebbal S. *Contribution to the study of natural resistance of the broad bean, Vicia faba L. against the cowpea aphid, Aphis crassivora (Homoptera: Aphididae). Master degree thesis in Plant protection*. The University of Batna. Algeria, 2010 (in French).
30. Mohamed A.M., Siman F.A.A. Susceptibility of some broad bean varieties to natural infestation with *Aphis craccivora* Koch and *Liriomyza trofolii* Burgess at Upper Egypt. *Assiut Journal of Agricultural Sciences (Egypt)*, 2003, 32(1): 167-173.
31. Chaudhari C.J., Patel C.C., Kher H.R., Parmar H.P. Resistance to aphid, *Therioaphis maculata* (Buckton) in lucerne. *Indian Journal of Entomology (India)*, 2013, 75(1): 68-71.
32. Comadira G., Rasool B., Karpinska B., Morris J., Verrall S.R., Hedley P.E., Foyer C.H., Hancock R.D. Nitrogen deficiency in barley (*Hordeum vulgare*) seedlings induces molecular and metabolic adjustments that trigger aphid resistance. *Journal of Experimental Botany*, 2015, 66(12): 639-3655 (doi: 10.1093/jxb/erv276).
33. Wang C., Baoliang T., Zhenzhen Y., Jianqing D. Effect of different combinations of phosphorus and nitrogen fertilization on arbuscular mycorrhizal fungi and aphids in wheat. *Insects*, 2020, 11(6): 365 (doi: 10.3390/insects11060365).
34. Vannette R.L., Hunter M.D. Mycorrhizal fungi as mediators of defense against insect pests in agricultural systems. *Agricultural and Forest Entomology,* 2009, 11: 351-358 (doi: 10.1111/j.1461-9563.2009.00445.x).
35. Azouz H.A., Yassin E.M.A., El-Sanady M.A., Abou-Zaid A.M. Field and laboratory studies on three eggplant cultivars to evaluate their relative susceptibility to some piercing sucking pests with relation of leaf constituents. *Journal of Plant Protection and Pathology*, 2014, 5(11): 995-1005.
36. Facknath S., Lalljee B. Effect of soil-applied complex fertilizer on an insect—host plant relationship: *Liriomyza trifolii* on *Solanum tuberosum*. *Entomologia Experimentalis et Applicata*, 2005, 15(1): 67-77 (doi: 10.1111/j.1570-7458.2005.00288.x).
37. Gleadow R.M., Møller B.L. Cyanogenic glycosides: synthesis, physiology, and phenotypic plasticity. *Annu. Rev. Plant Biol.*, 2014, 65: 155-185 (doi: 10.1146/annurev-arplant-050213-040027).
38. Irmisch S., Clavijo McCormick A., Günther J., Schmidt A., Boeckler G.A., Gershenzon J., Unsicker S.B., Köllner T.G. Herbivore-induced poplar cytochrome P450 enzymes of the CYP71 family convert aldoximes to nitriles which repel a generalist caterpillar. Plant J., 2014, 80(6): 1095-1107 (doi: 10.1111/tpj.12711).
39. Chunming B., Yifei L., Xiaochun L. The application of secondary metabolites in the study of sorghum insect resistance. *IOP Conf. Series: Earth and Environmental Science*, 2018, 128: 012169 (doi: 10.1088/1755-1315/128/1/012169).
40. Jeschke V., Gershenzon J., Vassao D.G. A mode of action of glucosinolate-derived isothiocyanates: Detoxification depletes glutathione and cysteine levels with ramifications on protein metabolism in *Spodoptera littoralis*. *Insect Biochem. Mol. Biol.*, 2016, 71: 37-48 (doi: 10.1016/j.ibmb.2016.02.002).
41. Munthali D.C., Tshegofatso A.B. Factors affecting abundance and damage caused by cabbage aphid, *Brevicoryne brassicae* on four brassica leafy vegetables: *Brassica oleracea* var. *Acephala*, *B. chinense*, *B. napus* and *B. carinata*. *The Open Entomology Journal*, 2014, 28: 1-9.
42. Huang T.I., Reed D.A., Perring T.M., Palumbo J.C. Feeding damage by *Bagradahilaris* (*Hemiptera*: *Pentatomidae*) and impact on growth and chlorophyll content of Brassicaceous plant species. *Arthropod-Plant Interactions,* 2014, 8: 89-100 (doi: 10.1007/s11829-014-9289-0).
43. Anjali M.S., Sridevi G., Prabhakar M., Laxmi N.J. Changes in chlorophyll content of sorghum infested with corn leaf aphid, *Rhopalosiphum maidis* (FITCH) (*Homoptera*: *Aphididae*). *Progressive Research — an International Journal*, 2017, 12(Special-I): 814-817.

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 158-183
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

UDC 633.111.1:632.4:632.937.15:581.1

doi: 10.15389/agrobiology.2023.1.158eng
doi: 10.15389/agrobiology.2023.1.158rus

MULTIFUNCTIONAL BIOPREPARATIONS AND COMPLEXES BASED ON MICROORGANISMS AND CHITOSAN INCREASE DISEASES RESISTANCE, PRODUCTIVITY AND LEAF PHOTOSYNTHETIC PIGMENT CONTENTS IN SPRING SOFT WHEAT (*Triticum aestivum* L.)

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The authors declare no conflict of interests
Acknowledgements:
The work was carried out within the framework of the state task in accordance with the VIR thematic plan under project No. 0481-2022-0001 "Structuring and unlocking the potential of hereditary variability of the world collection of grain and cereal crops of the VIR for the development of an optimized gene bank and rational use in breeding and crop production"
Final revision received April 13, 2022
Accepted 15 June, 2022

Abstract

The application of useful microorganisms and biologically active molecules lies at the basis of the modern concept of agroecosystems phytosanitary optimization. The increase of the protective properties of preparative forms, which include phytopathogen antagonists and chitosan, is due to the ability of chitosan polysaccharide to induce systemic plant disease resistance. In addition, multifunctional compositions with multiple action mechanisms, effective against a wide range of phytopathogens, can positively effect on the functional state of plants, including their photosynthetic activity, quantitative and qualitative changes in the entire pigment system, which often reflect the nature of adaptive reactions under stress. However, studies of changes in the photosynthetic apparatus in relation to the disease resistance and plants productivity under the influence of such compositions are extremely few. It was shown for the first time that the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II significantly increase wheat productivity and disease resistance, while the content of chlorophylls a and b in leaves also turned out to be the highest. The ratio of chlorophylls a + b and carotenoids content, which serves as one of the indicators of plant stress resistance, was maximal when using the Vitaplan, CL + Chitosan II complex. This study aimes to estimate the potential wheat productivity by morphometric indicators of plant development, susceptibility to root rot, brown and yellow rust, powdery mildew, Septoria leaf blotch, and the content of chlorophylls a, b, carotenoids in leaves when using multifunctional biopreparations and complexes combining the useful properties of microorganisms — antagonists of phytopathogens and chitosan as plant disease resistance activator. Seeds of the Leningradka 6 cultivar (k-64900, VIR collection) of soft spring wheat (*Triticum aestivum* L.) were treated before sowing with biopreparations based on *Bacillus subtilis* strains VKM B-2604D and *B. subtilis* VKM B-2605D Vitaplan, SP, Vitaplan, CL and the complexes Vitaplan, CL + Chitosan II, Vitaplan, SC + Chitosan II. In the field during the growing season, plants were sprayed with the same preparations vs. control (without treatment). In general, the used complexes turned out to be more effective than biopreparations by 16.2 %. The multifunctional compositions application significantly reduced wheat plants harm by diseases complex (by 17.9 % at $p < 0.05$). The highest values of potential productivity (0.94±0.02 g/plant) and chlorophyll a (1.32±0.02 mg/g) and b (2.15± 0.04 mg/g) content in the leaves were detected when using the multifunctional complex Vitaplan, CL + Chitosan II, which exceeded the control by 57.1 %, 16.7 % and 4.3 %, the other variants — by 19,7 %, 23,7 %, and 11,0

%. Differences in the content of chlorophyll a and chlorophyll b photosynthetic pigments in wheat flag leaves were revealed when using the multifunctional complex Vitaplan, CL + Chitosan II compared to biopreparations by 16.8 %, 3.7 % and 2.0 %, with Vitaplan, SC + Chitosan II — by 1.1 %, 17.7 %, and 27.0 %, respectively. The strongest correlation was found between the chlorophyll b content in the flag leaves and wheat productivity ($r = 0.69$, $p = 0.03$), the chlorophyll b content in the flag leaves and the grains number per spike ($r = 0.79$, $p = 0.006$), the grains weight per spike and the spike weight ($r = 0.69$, $p = 0.03$; $r = 0.72$, $p = 0.02$). Correlations between a decrease in the yellow rust development and an increase in the chlorophylls a and b content in leaves were found ($r = 0.66$, $p = 0.04$; $r = 0.87$; $p = 0.005$). The highest values of the chlorophyll a to chlorophyll b ratio in the leaves compared to control occurred when using Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II complexes. The ratio of the chlorophylls a and b to the carotenoid pigments, as an indicator of plant resistance to negative external factors, also reached maximum values with Vitaplan, CL + Chitosan II. According to the indicators sum, the most promising for use in wheat cultivation is the multifunctional complex Vitaplan, CL + Chitosan II which has a pronounced growth-stimulating and protective effect on plants upon preventive use.

Keywords: *Triticum aestivum*, soft wheat, multifunctional biological products, chlorophyll a, chlorophyll b, carotenoids, wheat productivity, wheat diseases, brown rust, yellow rust, septoriosis, powdery mildew, root rot

Modern technology for cultivating grain crops requires a set of measures to protect against diseases, in particular chemical treatment of seeds and spraying of crops with fungicides, which is environmentally unsafe and leads to the formation of stable populations of phytopathogens. Therefore, alternative, environmentally friendly means are needed [1].

Bacillus subtilis strains, due to the diversity and high variability of their biochemical properties and the synthesis of a spectrum of bioactive metabolites - cyclic lipopeptides, polypeptides, proteins and non-peptide compounds [2-4], are widely used in the fight against pathogens of agricultural crops. *B. subtilis* strains are known to produce three ribosomal antibiotics (TasA, subtilosin, and sublacin), four nonribosomal antibiotics (bacitracin, bacilisin, plipastatin, and surfactin), the novel phospholipid antibiotic bacilisocin, and the amino sugar antibiotic neotrehalosadiamine (NTD) [2]. Nonribosomal cyclic oligopeptides, such as surfactin, iturin group compounds and fengycin, containing a chain of fatty acids, exhibit high antifugal and antibacterial activity [5]. *B. subtilis* strains produce various hydrolytic enzymes that destroy the cell wall of phytopathogenic fungi [6]. A number of active compounds produced by these microorganisms have elicitor activity and trigger mechanisms of induced resistance [7, 8].

Numerous data have also been published regarding the ability of beneficial microorganisms of the rhizo- and phyllosphere to synthesize metabolites that affect the resistance and growth of plants and have signaling and hormonal functions. Auxins, gibberellins, cytokinins, abscisic (ABA), salicylic, jasmonic acids are natural growth regulators [9-11]. Many strains of bacteria belonging to the genera *Bacillus*, *Azospirillium*, *Pseudomonas* can synthesize auxins, which stimulates the development of the plant root system, as a result, the absorption of water and nutrients is activated. These processes collectively increase disease resistance and allow plants to more quickly pass through those developmental stages when plants are most susceptible to pathogens [12-14].

The most promising for protecting agricultural crops and increasing their productivity are compositions that combine the beneficial properties of microorganisms - antagonists of pathogens and activators of plant disease resistance, such as chitosan and its derivatives [15]. Chitosan and preparations based on it have found practical use as inducers that increase resistance to fungal, bacterial and viral diseases [16-19].

The biological activity of chitosan as an inducer of resistance is determined by its ability to activate protective reactions and induce nonspecific

cellular immunity, one of the lines of defense for the innate immunity of plants [19, 20]. Chitosan and chitin, present in the cell walls of many parasitic microorganisms and fungi, are the molecular patterns of phytopathogenic fungi (pathogen-associated molecular patterns, PAMPs), which are recognized by plant protein receptors (pattern-recognition receptors, PRR) [21] and serve as a signal for activation protective responses (pattern-triggered immunity, PTI), preventing the development of infection [22, 23]. Protective responses induced by chitosan include an increase in cytosolic Ca^{2+} concentration, oxidative burst (formation of reactive oxygen species, ROS) [24-26], hypersensitive response (HR), synthesis of pathogenesis-related proteins (PR), with antimicrobial and lytic activity, induction of defense hormones (abscisic acid, jasmonates, salicylic acid), formation of phytoalexins [26-31]. In addition, defense reactions induced by chitosan in plants are characterized by increased synthesis of lignin [32] and callose [30], which leads to the strengthening of cell walls and the creation of physical barriers to the penetration and movement of pathogens in plants [18]. These reactions, aimed at suppressing the growth of the pathogen, lead to the formation of systemic resistance in plants and protect them from subsequent attack by a wide range of pathogens, and also increase plant resistance to unfavorable abiotic factors.

The mechanisms of the protective action of chitosan and its high efficiency in protecting various types of agricultural plants from the action of a wide range of phytopathogens are discussed in detail in numerous review articles [17, 30]. In addition to protecting against phytopathogens, chitosan accelerates plant growth, increases resistance to stress (frost, drought, excess moisture) and the productivity of grain and vegetable crops [33-36]. Treatment with polysaccharide increases the rate of photosynthesis, increases the number of shoots, leaf size, and plant height in wheat, corn, beans, tomatoes, and rice, which provides increased yield [37-42].

Among the factors determining high grain productivity of wheat, chlorophyll photosynthetic potential of crops, which characterizes the total amount of chlorophyll in leaves or whole plants per unit area of crops during the growing season or a certain period thereof. There is a strong positive relationship between chlorophyll photosynthetic potential during the reproductive period and wheat yield [43]. It has been established [44] that the increase in the yield of the modern winter wheat variety Favoritka compared to the Mironovskaya 808 variety bred in the 1960s is associated with an increase in the content and gross amount of chlorophyll, as well as an extension of the period of functioning of the photosynthetic apparatus of sowing during the reproductive period. The modern variety is characterized by more efficient use of absorbed light energy, which leads to an increase in photosynthetic productivity. The authors conclude that increasing photosynthetic efficiency is a promising strategy for increasing crop productivity (44). Optimizing the operation of the photosynthetic apparatus at different levels of its organization can increase grain productivity by 10-60% [45, 46].

In plants, chlorophylls are found only in pigment-protein complexes (PPCs), since in their free form, being strong photosensitizers, they can cause destruction of thylakoid membranes and chloroplast stroma due to the photodynamic effect. PPCs allow optimizing the operation of the photosynthetic apparatus. There are four main types of PBPCs: two of them are reaction centers of photosystem (PS)I and PSII, the other two are light-harvesting complexes (light-harvesting complexes) of PSI and PSII. In chloroplasts, the antenna complex contains a large number (200-400) of chlorophyll molecules and a relatively small amount of carotenoids non-covalently bound to protein.

Chlorophyll b serves as an auxiliary light-harvesting pigment, capturing

and transmitting light energy to the reaction centers of photosystems. It accounts for approximately 15-25% of the total chlorophyll content. Unlike chlorophyll a, which is part of the core complexes of photosystems, chlorophyll b is found only in the light-harvesting complexes of photosystems (LHC I and LHC II) and in the so-called small antenna of PSII. In LHC I, chlorophyll b makes up apprx. 22% of the total amount of chlorophylls, in LHC II apprx. 43%, in the pigment-protein complex of the small antenna 31-46% [47].

Carotenoids are auxiliary photosynthetic pigments and an essential component of the pigment systems of all photosynthetic organisms. In chloroplasts, carotenoids are found in the PPC and partly in the lipophilic phase of thylakoid membranes. Reaction centers, which are a complex of proteins, pigments and other cofactors and provide the reaction of converting light energy into chemical energy during photosynthesis, include only chlorophyll a and β-carotene, and light-harvesting antennas include chlorophylls a and b, carotenes and xanthophylls. Carotenoids, which are part of the light-harvesting antenna, expand the spectral range of photosynthetically active radiation (PAR). In addition to participating in the absorption of solar radiation energy and its migration from additional pigments to the main ones, carotenoids also perform a protective function (quenching triplet chlorophyll and singlet oxygen), protecting the photosynthetic apparatus from photodestruction.

Previously, the biosynthesis of photosynthetic pigments, in particular chlorophylls, was not considered a factor activating signaling pathways that lead to the initiation or delay of ontogenesis phases. However, recent work has demonstrated the important role of the accessory photosynthetic pigment chlorophyll b in the regulation of plant ontogeny [48]. In addition, data have been published that indicate that the absence of chlorophyll b negatively affects the change in periods of ontogenesis in barley. The chlorina mutants of both species differed from the plants of the parental lines in the later onset of floral transformation. In addition, in 30-40% of barley mutants, the growth and differentiation of the structural elements of the ear stopped [49]. Photosynthetic structures may be involved in the plant's adaptive response to stress [50]. In this case, a change in the content of pigments (chlorophyll a, chlorophyll b, the sum of chlorophylls a + b and carotenoids) is likely. Abiotic stress reduces the efficiency of photosynthesis primarily due to the negative impact on chlorophyll biosynthesis, the functioning of photosystems, electron transport mechanisms, and gas exchange parameters [51, 52].

It should be noted that some researchers either have not identified a direct relationship between the efficiency of photosynthesis and yield, or have established a negative correlation between these indicators in many plants, including grain crops [53].

Nevertheless, photosynthesis is the basis of primary bioproductivity both in natural ecosystems and in the formation of crop yields; therefore, under changing environmental conditions it is necessary to maintain and increase the photosynthetic productivity of plants [54-56].

Indirectly, resistance to abiotic stress can be assessed by quantitative changes in the pigment complex [57-60]. A number of repors reflect the influence of plant damage by pathogens on the composition of the pigment complex. In particular, when woody plants are damaged by parasitic fungi, the content of chlorophylls decreases and the content of carotenoids increases [61]. As a result, structural-functional and physiological-biochemical rearrangements occur, which ultimately leads to a decrease in photosynthetic activity [61]. However, similar studies linking the productivity and resistance of plants to biotic stress (damage by

phytopathogens) with the state of the photosynthetic complex are extremely few.

In the presented work, we showed for the first time that the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II significantly increase the productivity and disease resistance of wheat, while the content of chlorophylls a and b in leaf blades also turned out to be the highest. The ratio of the content of chlorophylls a + b and carotenoids, which serves as one of the indicators of plant stress resistance, was maximum when using the Vitaplan, CL + Chitosan II complex.

The purpose of our study is to assess the potential productivity of wheat based on morphometric indicators of plant development, their susceptibility to the most harmful diseases (root rot, brown and yellow rust, powdery mildew, septoria) and the content of chlorophylls a, b, carotenoids in leaves when using multifunctional biological products and complexes, combining the beneficial properties of microorganisms that are antagonists of phytopathogens and the activator of plant disease resistance, chitosan.

Materials and methods. Experimental studies were carried out in an experimental field (Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources — VIR, St. Petersburg-Pushkin, 2016-2018) on spring soft wheat (*Triticum aestivum* L.) variety Leningradskaya 6 (k-64900, provided by the Department of Wheat Genetic Resources of the VIR). In the field experiment, wheat was sown on plots with an area of 1.0 m^2 in a row with row spacing of 15 cm and a distance in the row of 2 cm. For each sample, the registration plot consisted of 6 rows; 50 grains were placed in each row when sowing. The seeding rate was 300 seeds per 1 m^2. The seed placement depth is 5-6 cm. The field experiment was carried out in three repetitions. All activities were carried out in accordance with generally accepted VIR recommendations and methods.

The experimental design included no treatment (control); treatment with biological product Vitaplan, WP (wettable powder, standard), containing cells of the strains *Bacillus subtilis* VKM B-2604D and *B. subtilis* VKM B-2605D (viable cell titer 1011 CFU/g); Vitaplan, CL, the culture liquid of *B. subtilis* VKM V-2604D and *B. subtilis* VKM V-2605D (1:1) with a titer of viable *B. subtilis* cells of 1010 CFU/ml; multifunctional complex Vitaplan, CL + Chitosan II (the composition of the culture liquid CL in the multifunctional complex Vitaplan, CL + Chitosan II and in the preparation Vitaplan, CL is the same); multifunctional complex Vitaplan, SC (suspension concentrate, viable cell titer 5×10^{10} CFU/ml) + Chitosan II. Chitosan II with a molecular weight of 50 KDa was obtained by the method of oxidative destruction [62] from chitosan with a molecular weight of 300 KDa and a degree of deacetylation of 85% (OOO Bioprogress, Russia). Chitosan was dissolved in succinic acid and, with constant stirring, added to the culture liquid to a final concentration of 0.1% [63].

In field tests, the seeds of wheat variety Leningradka 6 were treated before sowing and vegetative plants were sprayed with the same preparations 4 times during the tillering, booting, heading and flowering phases.

Nineteen parameters were used to analyze the elements of wheat productivity that characterize the morphological characteristics of plants and the structure of the crop in the heading, flowering and ripening phases. During the heading-flowering phases, the following set of indicators was taken into account: productive and general bushiness (pcs.), area of flag and pre-flag leaves (cm^2), plant height (cm), spike length (cm), number of spikelets per ear (pcs.), weight ear (g). In addition, the number and length of roots (main embryonic root, embryonic and coleoptile roots) extending from the epicotyl were determined, and the number

and length of nodal roots were accounted. The masses of the roots and vegetative parts of wheat plants were determined (the mass of the vegetative parts was calculated from the total mass of the stem and leaves without taking into account the mass of roots and ears).

The field germination of samples [64] and the stage of plant ontogeny were determined by the J.C. Zadoks's scale [65]. In the ripening stage (stage of full ripeness), the structure of the wheat harvest was studied with regard to the number of spikelets per ear (pcs.), spike length (cm), weight of the spike with grain (g), the number of grains per spike (pcs.), weight of grains with ear (g), 1000-grain weight (g).

The potential (biological) productivity of a single plant P_p was calculated based on the productive tillering capacity T_p and the weight of grains per ear of one plant W_g (g/plant): $P_p = W_g \cdot T_p$.

Wheat leaf area was calculated by the well-known formula [66], using linear measurement data - length (l, cm) and maximum width of the leaf at the base (d, cm): $S = {}^2/_3\, ld$.

To evaluate field germination, the number of wheat plants in the germination phase was counted for each registration plot, based on the sowing rate of 300 seeds per 1 m^2.

The sample size for each experimental variant with 3-fold repetition of plot placement was 60 plants.

The degree of damage to wheat plants (Pd, %) by helminthosporium root rot, the causative agent is *Cochliobolus sativus* (S. Ito & Kurib.) Drechsler ex Dastur, according to experimental options was assessed in laboratory conditions in the tillering and heading-flowering phases in accordance with the generally accepted methodology [67] according to the formula:

$$P_d = \frac{\sum(ab)100\,\%}{AK},$$

where a is the number of plants with the same signs of damage; b is the corresponding damage score; A is the number of plants in a set (healthy and sick); K is the highest score of the accounting scale (maximum score of damage is 3).

The intensity of damage to wheat by pathogens of leaf diseases was determined using generally accepted accounting scales, as well as additional indicators of pathogenesis [68]. The intensity or degree of development of rust is determined as a percentage according to classical scales [63]: brown rust (*Puccinia recondita* Rob. ex Desm f. sp. *tritici* by Rusakov or Peterson, yellow rust (*Puccinia striiformes* Westend) by Manners, septoria (*Septoria tritici* Roberge in Desmaz.) by James, powdery mildew (*Blumeria graminis* (DC.) Speer f. sp. *tritici* March) by Peterson. In addition, indicators that further characterize the pathogenesis were determined: the number of pustules (total per leaf), the number of stripes with pustules, length stripes with pustules, the area of the pustule and their number in the stripe. Pathogenesis indicators were examined using a stereoscopic microscope MBS-9 (LLC PTP ASMA-Pribor, Russia) and a trinocular microscope Micromed 1 (var. 3 LED) (OOO Observational Devices, Russia).

The size of infectious structures on leaves during pathogenesis (spots, pustules, etc.) was determined using object and ocular micrometers. The values of the pustule area were calculated assuming their elliptical shape: $S = m \cdot \pi ab$ where a and b are the length of the semi-axes of the ellipse (in the lines of the ocular micrometer), m is the microscope scale factor.

The content of chlorophylls a and b in wheat flag leaves was determined spectrophotometrically as described [69] using a SPEKOL-11 spectrophotometer (Carl Zeiss AG, Germany). To determine the content of photosynthetic pigments,

we used samples from the middle part of the leaf blade, from which alcoholic extracts were prepared [69].

The concentration of pigments in the extracts (mg/dm^3) was calculated using the following formulas:

$$C_p = 9.784 \cdot D_{662} - 0.99 \cdot D_{644};$$
$$C_b = 21.426 \cdot D_{644} - 4.650 \cdot D_{662};$$
$$C_a + C_b = 5.134 \cdot D_{662} + 20.436 \cdot D_{644};$$
$$C_{car} = 4.695 \cdot D_{440.5} - 0.268 \cdot (C_a + C_b),$$

where C_p is the concentration of pigments, C_a is the concentration of chlorophyll a, mg/dm^3; C_b is chlorophyll b concentration, mg/dm^3; C_{car} is concentration of carotenoids, mg/dm^3; D_{662} is optical density at $\lambda = 662$ nm, units; D_{644} is optical density at $\lambda = 644$ nm; $D_{440.5}$ is optical density at v 440.5 nm.

The concentration of pigments in flag leaves (mg/100 g) was calculated as $X = 100CVV_2 \cdot (mV_1)^{-1} \cdot 1000$, where C is the pigment concentration, mg/dm^3; V is the initial extract volume, cm^3; V_1 is the volume of the initial extract taken for dilution, cm^3; V_2 is volume of diluted extract, cm^3; m is the mass of the sample, g.

Statistical analysis of the results was carried out in the programs IBM SPSS Statistics 21, Statistica 6.0 (StatSoft, Inc., USA), Microsoft Excel 2016. The calculations used methods of analysis of variance and the Scheffй multiple comparison test, methods of parametric statistics (calculation of means M and their standard errors ±SEM, 95% confidence intervals and Student's t-test), nonparametric method of Spearman's rank correlations, linear and nonlinear regression analysis based on the least squares algorithm.

Results. In the field experiment scheme, we did not use the variant with Chitosan II with a molecular weight of 50 KDa separately, since our earlier field experiments showed that its use caused a significant increase only in the mass of the vegetative part of plants [63]. The complex Vitaplan, CL + Chitosan II caused a significant increase in the largest number of wheat productivity indicators compared to the control (63). In the same variant of the experiment, the maximum significant increase in yield (82.6%) and maximum effectiveness against helminthosporium root rot were recorded (reduction in the development of root rot by 80-100% compared to the control) (63).

1. Grain productivity of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on Bacillus subtilis strains and chitosan ($n = 17$, $N = 3$, M±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018)

Treatments	Productivity, g per plant	Student's *t*-test	Confidence interval at a 5% level of significance	To control, %
Conrol (water)	0.60±0.02		0.56–0.64	
Vitaplan, CL	0.81±0.03	1.17	0.75–0.87	35.3
Vitaplan, CL + Chitozan II	0.94±0.02	2.40	0.90–0.98	57.1
Vitaplan, WP	0.80±0.05	0.90	0.70–0.90	33.7
Vitaplan, SC + Chitozan II	0.93±0.03	1.97	0.88–0.99	55.7

N o t e. For a description of the drugs, see the Materials and methods section.

The results obtained in this work showed a significant positive effect of the studied multifunctional compositions on the phytosanitary state of the agrocenosis of spring soft wheat and its productivity indicators. In 2018, the potential yield of wheat in the Vitaplan, CL + Chitosan II variant exceeded the control by 57.1% (Table 1), and for 2016-2018 on average by 64.1% (Fig. 1, A).

We descrived changes in some morphometric indicators associated with the grain productivity of bread wheat when using biologicals and multifunctional complexes (Table 2-4).

For Vitaplan, CL + Chitosan II, there was a reduction in the period of wheat ripening by ontogenesis phases (by 10.2%), a significant ($p \leq 0.05$) increase in plant height (by 24.3%), and root length (by 11.3%). 0%), root mass (by 50.6%), flag leaf area (by 30.9%), number of spikelets per ear (by 6.1%).

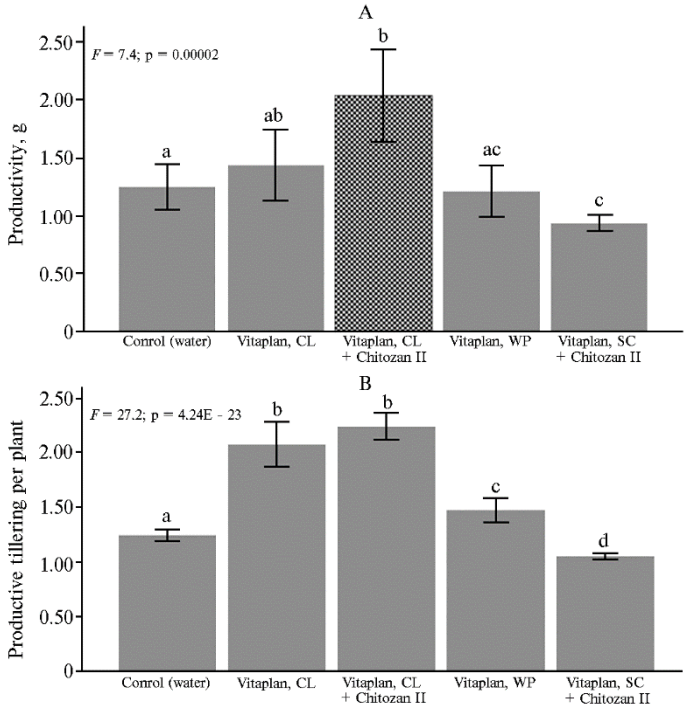

Fig. 1. Potential grain productivity (A) **and productive tillering** (B) **in spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biological products and polyfunctional complexes based on *Bacillus subtilis* strains and chitosan** (n = 51, N = 3, M±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018). For a description of the drugs, see the Materiasl and methods section. The same letters indicate values that do not differ from the control and from each other by the Scheffe's test at $p < 0.05$; F is Fisher's test.

A strong correlation was identified between potential productivity and productive tillering (Spearman non-parametric correlation coefficient r = 0.76; p = 0.029E–9). In our tests, the highest productive tillering was observed when using the multifunctional complex Vitaplan, CL + Chitosan II (see Fig. 1, B).

On average, over the observation period (2016-2018), this figure increased by 80.1% compared to the control.

In 2018, a significant ($p \leq 0.05$) increase in the field germination of wheat was noted for the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II, as well as the biological product Vitaplan, CL (Fig. 2). The maximum increase in field germination was recorded when using the multifunctional complex: Vitaplan, CL + Chitosan II (in 2018 by 20.8%, for the period 2016-2018 by 19.6% compared to the control, $p \leq 0.05$). Perhaps this is due to the most pronounced reduction in the development of helminthosporium rot on wheat compared to other experimental options (in 2018 by 13.7%, in 2016-2018 by 24.8% compared to the control; $p \leq 0, 05$).

The potential productivity of wheat was, to an average extent, positively correlated with root mass (r = 0.46; p = 0.005E-8) (Fig. 3, A) and flag leaf area (r = 0.40; p = 0.006E-11 (see Fig. 3, B) The maximum values of root mass were recorded for the multifunctional complex Vitaplan, CL + Chitosan II.

2. Morphometric parameters of productivity (roots) in spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan (n = 51, N = 3, M±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

Treatments	Parameter	Cource of development		Plant height		Root number		Root length		Nodal root mumber		Nodal root length		Root weight	
		score	to control, %	cm	to control, %	total	to control, %	mm	to control, %	total	to control, %	mm	to control, %	g	to control, %
Conrol	*M*	51.0		48.4		4.9		73.2		9.6		58.0		0.5	
(water)	±SEM	1.5		2.7		0.2		2.6		0.6		2.2		0.0	
Vitaplan, CL	*M*	52.4	2.7	51.4	6.1	5.5	14.1	85.6	17.0	9.4	–2.0	59.3	2.3	0.6	11.5
	±SEM	1.9		3.2		0.3		2.9		0.6		2.0		0.1	
Vitaplan, CL	*M*	56.2	10.2*	60.2	24.3*	5.3	9.5	81.2	11.0*	10.1	5.9	58.6	1.1	0.7	50.6*
+ Chitozan II	±SEM	1.3		3.2		0.3		2.3		0.7		2.4		0.1	
Vitaplan, WP	*M*	52.4	2.8	53.8	11.0	4.9	1.1	74.2	1.3	10.2	6.6	63.7	9.9	0.7	35.6
	±SEM	1.9		3.7		0.3		3.2		0.6		2.7		0.2	
Vitaplan, SC	*M*	48.9	–4.1	37.6	–22.3	4.8	–0.8	67.3	–8.1	8.9	-7.3	48.0	–17.2*	0.3	–32.1*
+ Chitozan II	±SEM	2.0		3.2		0.2		3.5		0.4		1.9		0.0	

N o t e. For a description of the drugs, see the Materiasl and methods section.

* Differences from control are statistically significant according to Student's t-test at $p < 0.05$.

3. Morphometric parameters of productivity (abovt-ground part) in spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan (n = 51, N = 3, M±SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

Treatments	Parameter	Flag leaf area		Pre-flag leaf area		Vegetative part weight		Ear leangth		Spikelet number per ear	
		cm^2	to control, %	cm^2	to control, %	g	to control, %	mm	to control, %	total	to control, %
Conrol (water)	*M*	3.8		4.3		2.2		63.2		13.1	
	±SEM	0.3		0.3		0.2		1.6		0.2	
Vitaplan, CL	*M*	4.8	27.1	4.7	9.1	2.4	8.1	57.7	–8.7	13.2	1.2
	±SEM	0.5		0.4		0.2		1.7		0.3	
Vitaplan, CL + Chitozan II	*M*	5.0	30.9*	4.5	5.1	2.5	14.1	64.6	2.1	13.8*	6.1*
	±SEM	0.3		0.3		0.2		2.4		0.2	
Vitaplan, WP	*M*	5.4	42.6*	4.7	9.8	3.0	33.6*	60.2	–4.9	13.8	6.0
	±SEM	0.6		0.5		0.3		2.3		0.3	
Vitaplan, SC + Chitozan II	*M*	2.3	–40.6*	2.5	–40.8*	2.0	-8.9	62.3	–1.4	11.5	–11.6
	±SEM	0.1		0.2		0.2		2.9		0.2	

N o t e. For a description of the drugs, see the Materiasl and methods section.
* Differences from control are statistically significant according to Student's t-test at $p < 0.05$.

4. Morphometric parameters of productivity (ear structure) in spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan ($n = 60$, $N = 3$, $M \pm$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

Treatments	Parameter	Ear weight		Grain number per ear		Grain weight per ear		1000-grain weight		Ear weigh wigh grain	
		g	to control, %	total	to control, %	g	to control, %	g	to control, %	g	to control, %
Conrol (water)	*M*	0,6		28,9		0,9		30,7		1,1	
	±SEM	0,0		0,7		0,0		0,9		0,0	
Vitaplan, CL	*M*	0,6	–2,6	30,1	4,3	0,9	5,8	31,1	1,3	1,3	12,5
	±SEM	0,0		1,0		0,0		1,1		0,1	
Vitaplan, CL + Chitozan II	*M*	0,6	–5,1	28,3	–1,8	0,8	–6,1	28,6	–6,8	1,1	-3,1
	±SEM	0,0		0,8		0,0		1,0		0,0	
Vitaplan, WP	*M*	0,8	27,3	31,3	8,6	1,0	13,7*	31,0	1,0	1,3	19,5*
	±SEM	0,1		1,0		0,0		1,2		0,0	
Vitaplan, SC + Chitozan II	*M*	0,5	–22,7*	28,4	–1,6	0,9	4,6	33,4	8,8*	1,2	7,9
	±SEM	0,0		0,7		0,0		0,4		0,0	

N o t e. For a description of the drugs, see the Materiasl and methods section.
* Differences from control are statistically significant according to Student's *t*-test at $p < 0.05$.

5. Chlorophylls a and b content in flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan ($n = 17$, $N = 3$, $M \pm$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018)

Treatments	1	2	3		4	5	2	3	6	7	8
Conrol (water)	1.13±0.07		0.99	1.26		2.06±0.20		1.67	2.45		0.55
Vitaplan, CL	1.13±0.10	0.06	0.94	1.33	0.69	2.07±0.12	0.05	1.84	2.30	0.58	0.55
Vitaplan, CL + Chitozan II	1.32±0.02	2.55*	1.27	1.36	16.72	2.15±0.04	0.43	2.07	2.23	4.30	0.61
Vitaplan, WP	1.05±0.20	–0.36	0.67	1.44	–6.62	1.99±0.40	–0.15	1.20	2.78	–3.24	0.53
Vitaplan, SC + Chitozan II	0.96±0.17	–0.90	0.64	1.29	–14.38	1.63±0.27	–1.26	1.10	2.16	–20.70	0.59

N o t e. 1 — chlorophyll a, mg/g; 2 — Student's *t*-test; 3 — confidence interval at a 5% level of significance; 4 — change in chlorophyll a content vs. control, %; 5 — chlorophyll b, mg/g мг/г; 6 — change in chlorophyll b content vs. control, %; 7 — chlorophyll a/chlorophyll b; 8 — change in chlorophyll a/chlorophyll b vs. control, %. For a description of the drugs, see the Materiasl and methods section.

* Differences from control are statistically significant according to Student's *t*-test at $p < 0.05$.

6. Chlorophylls a + b to carotenoids concentration in flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan ($n = 17$, $N = 3$, $M \pm$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018)

Treatments	Chlorophylls a + b to carotenoids	Student's *t*-test	Confidence interval at a 5% level of significance	Change in chlorophyll a + b/carotenoids vs. control, %
Conrol (water)	7.60±2.30		3.09–12.11	
Vitaplan, CL	10.80±4.20	0.67	2.57–19.03	42.09
Vitaplan, CL + Chitozan II	11.80±2.23	1.31	7.42–16.17	55.22
Vitaplan, WP	6.92±1.62	–0.24	3.74–10.10	–8.92
Vitaplan, SC + Chitozan II	8.26±0.87	0.27	6.56–9.97	8.73

N o t e. For a description of the drugs, see the Materiasl and methods section.

7. Brown rust, powdery mildew and septoria damage to flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan ($n = 60$, $N = 3$, $M \pm$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

Treatments	Parameter	Brown rust						Powdery mildew						Septoria	
		damage		number of pustules		pustule area		damage		spot number		spot area			
		%	to control, %	total	to control, %	mm^2	to control, %	%	to control, %	total	to control, %	mm^2	to control, %	%	to control, %
Conrol (water)	*M*	12.5		81.6		0.08934		5.8		7.6		3.6		17.5	
	±SEM	3.8		24.6		0.00880		1.9		2.3		1.0		2.5	
Vitaplan, CL	*M*	7.6	–4.9	133.2	63.2	0.04766	–46.7*	1.8	-4.0	2.8	–63.3	1.3	–63.0	7.5	–10.0
	±SEM	1.8		49.0		0.00524		0.8		1.4		0.4		2.5	
Vitaplan, CL +	*M*	4.8	–7.7	42.2	–48.3	0.07286	–18.5	3.8	–2.0	5.5	–27.9	2.5	–29.2	2.0	–15.5
Chitozan II	±SEM	1.3		22.6		0.01149		1.4		1.7		0.4		1.0	
Vitaplan, WP	*M*	10.2	-2.4	52.2	–36.0	0.10333	15.7	2.9	–2.9	3.1	–59.0	4.3	21.7	0.0	–17.5*
	±SEM	5.0		33.2		0.02187		1.1		1.0		1.3		0.0	
Vitaplan, SC +	*M*	4.1	–8.5*	15.1	–81.5*	0.05016	–43.9*	1.0	–4.8*	1.0	–86.9*	5.5	53.9	0.0	–17.5*
Chitozan II	±SEM	1.2		5.0		0.00688		0.2		0.1				0.0	

N o t e. For a description of the drugs, see the Materiasl and methods section.

* Differences from control are statistically significant according to Student's *t*-test at $p < 0.05$.

8. Powdery mildew and septoria damage to pre-flag leaves and root rot of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan ($n = 60$, $N = 3$, $M \pm$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018)

Treatments	Parameter	Powdery mildew						Septoria		Root rot	
		damage		spot number		spot area					
		%	to control, %	total	to control, %	mm^2	to control, %	%	to control, %	%	to control, %%
Conrol (water)	*M*	22.0		23.1		3.3		31.5		39.7	
	±SEM	5.0		4.7		0.5		8.4		2.9	
Vitaplan, CL	*M*	24.2	2.2	22.3	–3.3	2.9	–11.8	21.9	–9.6	19.7	–20.0*
	±SEM	5.8		3.8		0.4		7.8		5.5	
Vitaplan, CL + Chitozan II	*M*	18.6	–3.4	20.6	–10.7	2.8	–14.4	8.8	–22.8*	14.9	–24.8*
	±SEM	4.3		3.9		0.3		2.7		6.5	
Vitaplan, WP	*M*	30.5	8.5	43.7	88.9	4.8	45.4*	6.3	–25.2*	33.8	–5.9
	±SEM	5.9		13.4		0.5		3.1		6.2	
Vitaplan, SC + Chitozan II	*M*	0.0	–22.0*	0.0	–100.0*	0.0	–100.0*	15.9	–15.6	28.3	–11.3*
	±SEM	0.0		0.0		0.0		8.0		4.7	

N o t e. For a description of the drugs, see the Materiasl and methods section.

* Differences from control are statistically significant according to Student's *t*-test at $p < 0.05$.

9. Yellow rust damage to flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan ($n = 60$, $N = 3$, $M \pm$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018)

Treatments	Parameter	Damage		Strip number		Strip length		Pustules				Pustule area	
								number per strip		sum of pustules			
		%	to control, %	total	to control, %	mm	to control, %	total	to control, %	total	to control, %	mm^2	to control, %
Control (water)	*M*	9.5		2.2		40.9		102.7		221.7		0.02312	
	±SEM	3.3		0.5		7.6		26.4		97.8		0.00391	
Vitaplan, CL	*M*	5.7	–3.8	2.6	18.2	31.9	–22.0	66.0	–35.7	168.8	–23.9	0.02329	0.7
	±SEM	1.4		0.5		2.6		6.0		45.4		0.00183	
Vitaplan, CL + Chitozan II	*M*	2.8	–6.7	1.4	–36.4	28.5	–30.3	58.1	–43.4	106.6	–51.9	0.01327	–42.6*
	±SEM	1.8		0.3		3.4		6.8		28.5		0.00143	
Vitaplan, WP	*wp*	7.7	–1.8	3.3	50.0	18.2	–55.5*	41.9	–59.2*	148.3	–33.1	0.01653	–28.5
	±SEM	4.1		1.1		1.7		6.4		77.6		0.00341	
Vitaplan, SC + Chitozan II	*M*	2.3	–7.2	1.3	–40.9	26.3	–35.7	62.3	–39.3	83.0	–62.6	0.01022	–55.8*
	±SEM	1.3		0.3		8.0		32.8		38.6		0.00086	

N o t e. For a description of the drugs, see the Materiasl and methods section.

* Differences from control are statistically significant according to Student's *t*-test at $p < 0.05$.

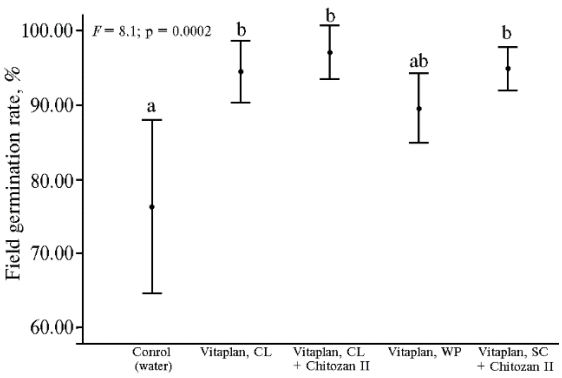

Fig. 2. Field germination of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 treated with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan (n = 1050, N = 3, $M \pm t_{05}$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2016-2018). For a description of the drugs, see the Materiasl and methods section. Identical letters indicate values that do not differ from the control and from each other according to the Scheffe's test at $p < 0.05$; F is Fisher's test.

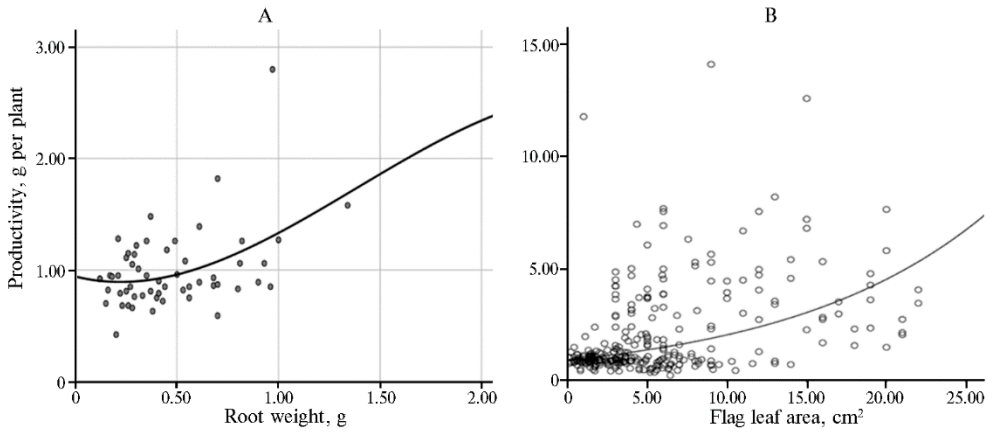

Fig. 3. Regression dependence of potential productivity to root mass (A, W_r) **and flag leaf area** (B, $S_{flag\ leaf}$) **of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 withou trearments with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan (control)** (n = 17, N = 3, $M \pm$SEM; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018). For a description of the drugs, see the Materiasl and methods section. The resulting regression equations:

A — $P_p = 0.94 - 0.45W_r + 1.11W_r^2 - 0.27\ W_r^3$ ($r = 0.46$; p = 0.005E–8);

B — $P_p = 0.807S_{flag\ leaf} - 0.160S_{flag\ leaf}^2 + 0.0085S_{flag\ leaf}^3$ ($r = 0.82$; p = 1.1661E–45).

In 2018, with the Vitaplan complex, CL + Chitosan II, maximum chlorophyll a in flag leaves in the flowering stage occurred, 1.32±0.02 mg/g (16.7% more than in the control, $p \leq 0.05$) (Table 5). The content of chlorophyll b changed slightly, but was 4.3% higher compared to the control (see Table 5). For Vitaplan, CL + Chitosan II, there was the greatest change in the ratio between the content of chlorophylls a, b and carotenoids (by 55.2%) vs. control (Table 6). Some authors believe that the ratio of the content of chlorophylls and carotenoids may be one of the indicators of resistance to external unfavorable factors and reflect the ecological plasticity of plants [70].

Nonparametric correlation analysis revealed that as the content of chlorophyll b (Chl b) in the flag leaves increased, there was an increase in the number of grains per ear (Spearman correlation coefficient $r = 0.79$; $p = 0.006$), in the weight of grains per ear ($r = 0.69$; $p = 0.03$); in ear weight with grains ($r = 0.72$; $p = 0.02$) and in overall potential yield ($r = 0.69$; $p = 0.03$). The dependence of the change in the mass of the ear with grains on the content of chlorophyll b in the flag leaves of wheat can be described by the regression equation: $M_e = 0.26$Chl b + 0.81; $R^2 = 0.45$ (Fig. 4).

The main diseases during the period of phytosanitary monitoring of wheat crops (2016-2018) were helminthosporium root rot caused by the fungus *Bipolaris*

sorokiniana (Sacc.) Shoemaker, brown rust (*Puccinia triticina* Eriks.), yellow rust (*P. striiformes* Westend); powdery mildew (*Blumeria graminis* (DC.) Speer.), septoria caused by *Stagonospora nodorum* (Berk.) Castellani & E.G. Germano and *Zymoseptoria tritici* (Desm.) Quaedvlieg & Crous.

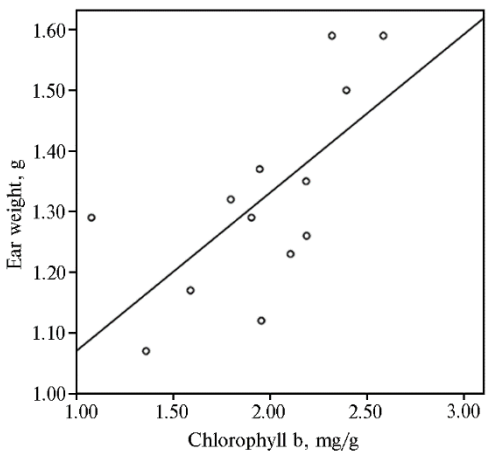

Fig. 4. Dependence of changes in the weight of the ear with grain vs. the content of chlorophyll b in flag leaves of spring soft wheat (*Triticum aestivum* L.) variety Leningradka 6 withou trearments with biologicals and multifunctional complexes based on *Bacillus subtilis* strains and chitosan (control) (n = 13, N = 3; experimental field of the Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg-Pushkin, 2018).

Multifunctional complexes significantly influenced the development of wheat diseases. According to the data obtained (Tables 7, 8), during the observation, the complex Vitaplan, CL and Chitosan II significantly ($p \leq 0.05$) reduced the root rot (by24.8%) and septoria infection on pre-flag leaves (by 22.8 %) compared to the control. The multifunctional complex Vitaplan, SC + Chitosan II significantly ($p \leq 0.05$) reduced the incidence of root rot in wheat (by 11.3%). The development of leaf rust decreased by 8.5%, the number of uredopustules by 81.5%, and the area of uredopustule by 43.9% ($p \leq 0.05$). When affected by powdery mildew, the number of spots on flag leaves decreased by 86.9%. We did not identify any symptoms of powdery mildew development on pre-flag leaves.

In 2018, for multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II, we noted that the intensity of development of yellow rust on wheat (R), the number of pustules (N) and the area of pustules (S) were minimal, R = 2.8±1.7%, N = 106.6±28.5, S = 0.013±0.001 mm^2 and R = 2.3±1.3%, N = 83.0±38.5, S = 0.010±0.0009 mm2, respectively) vs. control, R = 9.5±3.3%, N = 221.7±97.8, S = 0.023±0.004 mm^2 (Table 9). The most pronounced effect was on the area of the pustule, which decreased compared to the control.

Plants are subject to the negative effects of stress factors of various natures throughout the growing season, which leads to a decrease in productivity due to inhibition of growth and photosynthesis. Many researchers note a significant decrease in the photosynthetic activity of plants when attacked by phytopathogenic fungi, which is associated with a decrease in the assimilation surface due to the death of leaf tissue or the growth of mycelium, with the destruction of chloroplasts, a decrease in chlorophyll content, and a violation of the outflow of photosynthetic products due to damage to the phloem [71, 72].

The results of our research showed that the plant treatment with multifunctional complexes significantly reduced the incidence of diseases in wheat, which had a positive effect on the chlorophyll content in the leaves. The content of chlorophylls a and b in wheat leaves increased with a decrease in the degree of yellow rust development as a percentage on the Manners scale (for chlorophyll a r = –0.66; p = 0.04; for chlorophyll b r = –0.87; p = 0.005), a decrease in the number of stripes (r = –0.79; p = 0.02 and r = –0.63; p = 0.04, respectively) and the number of yellow rust pustules (r = –0.73; p = 0.04 and r = –0.97; p = 0.00007). The equations that describe the dependence of the content of chlorophylls in flag leaves on the intensity of yellow rust development are for Chl a R = –37.03 + 52.72Chl a, R^2 = 0.60, for Chl b R = 144.52 – 108.23Chl b + 20.53Chl b^2, R^2 = 0.81.

Thus, as a result of the studies, a statistically significant increase in the content of photosynthetic pigments (chlorophyll a in flag leaves of wheat) and a slight increase in

chlorophyll b were revealed when using biological products. When using the multifunctional complex Vitaplan, CL + Chitosan II, the highest potential productivity and the highest content of chlorophylls a and b in the leaves occurred. A correlation was revealed between an increase in the content of chlorophylls a and b in leaves and a decrease in the intensity of development of yellow rust. Based on the Spearman criterion, the strongest correlations between the chlorophyll b content and the weight of the ear, the weight of grains per ear and the number of grains in the ear are shown.

The observed positive effect of biological products and multifunctional complexes may be associated with the ability of beneficial microorganisms to synthesize complex bioactive complexes, including antibiotics of various chemical classes, enzymes, metabolites with signaling and hormonal functions, phytohormones, which can have a significant effect influence on photosynthetic function, growth and productivity of plants [9-11]. Thus, the stimulating effect of synthetic auxin (indolyl-3-butyric acid, IBA) and cytokinin (6-benzylaminopurine, BAP) on the accumulation of plant biomass, net productivity of photosynthesis, and the functioning of the photosynthetic apparatus of corn has been established [73]. It has been shown that gibberellin enhances the processes of photosynthetic phosphorylation, while the chlorophyll content decreases. Thus, the intensity of chlorophyll use per quantity increases under the influence of gibberellin; in addition, the assimilation number increases [9]. Many bacteria of the genera Bacillus, Azospirillium, and Pseudomonas, as already noted, synthesize auxins that stimulate the development of the root system. Together, these processes increase plant disease resistance [74]. Many strains of bacteria of the genus Bacillus can synthesize gibberellin [75]. Bacteria of the genera *Bacillus*, *Rhizobium*, *Arthtrobacter*, *Azotobacter*, Azospir*i*llium, and *Pseudomonas* are capable of producing cytokinins. When inoculated with cytokinin-producing strains of *B. subtilis*, the content of chlorophyll and cytokinins in plants increased, which subsequently caused an increase in the biomass of the root system and vegetative part of the plant. Strains of *Bacillus*, *Brevibacterium*, *Azospirillum*, *Pseudomonas*, *Lysinibacillus* were found to be able to synthesize abscisic acid and influence its content in plants, which caused optimization of endogenous hormonal balance [76-79].

B. subtilis strains, which form the basis of Vitaplan, produce a variety of antimicrobial metabolites, the lipopeptides and polypeptides [80], which largely determines the fungicidal effect of the biological product against particularly dangerous phytopathogenic fungi. The biological activity of chitosan is determined by its ability to induce biochemical (signaling) pathways leading to the activation of defense reactions and increasing plant resistance to diseases [33-36]. It was reported that chitosan stimulates growth and development, increases the yield of many agricultural plants, e.g., corn [81-83], legumes [84], wheat [85], and rice [86]. It has been convincingly shown that chitosan treatment increases the rate of photosynthesis and chlorophyll content in rice and soybean plants [87, 88], corn [38], cowpea [89], beans [39], tomato [40], and wheat [37]. It is possible that chitosan can be a carbon source for the formation of antioxidants [90]. Although the exact mechanisms of chitosan's influence on photosynthesis have not been established, some studies have shown that in maize, chitosan and its derivatives improved photosynthesis and chlorophyll fluorescence, increasing stomatal activity, transpiration rate, and PSII activity [38]. There are reports that chitosan increases the endogenous level of cytokinins that stimulate chlorophyll synthesis [83). Some researchers associate the increase in yield when using chitosan with the effect of stimulating physiological processes and the subsequent active movement of photoassimilates into the tissues that consume them. This effect was noted in corn [38], beans [39], soybeans [87], cowpea [89], tomato [40], rice [41], and cucumber [91].

Thus, all mechanisms of action of beneficial microorganisms in combination with the biological activity of chitosan, described in the above scientific publications, are capable of optimizing the physiological state of plants by increasing the rate of photosynthesis, stimulating growth and development, which leads to increased stress and disease resistance - vigor and, as a consequence, crop yields.

The results of the studies suggest that the effect of the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II is due to the mechanisms discussed above. It is reflected in a high protection against fungal infection, enhanced growth processes, stimulation of the reproductive properties of plants, increased content of photosynthetic pigments and, ultimately, in increasing the potential productivity of wheat.

In the compositions we have developed, the combination of an inhibitory effect on phytopathogenic microorganisms and a stimulating effect on plants provides higher efficiency and reliable protection compared to biological products. In our opinion, complex mechanisms for increasing disease resistance and ensuring stable plant productivity include adaptive reactions that involve the photosynthetic apparatus and the entire system of photosynthetic pigments. It can be assumed that this mechanism is universal, but the effectiveness of such compositions may depend on the biological characteristics of microorganism strains and the properties of inducers, as well as on the resistance of cultivated plant varieties.

So, it has been shown that the multifunctional complexes Vitaplan, CL + Chitosan II and Vitaplan, SC + Chitosan II optimize the physiological state of wheat plants, significantly increase its productivity and disease resistance (the incidence of plants with a complex of diseases decreased by 17.9%, $p < 0.05$). Plant treatment with the Vitaplan complex, CL + Chitosan II leads to the highest potential wheat productivity (0.94 ± 0.02 g/plant). The Vitaplan complex, SC + Chitosan II provides the least damage to plants by the disease complex. In addition, in these variants the highest content of chlorophylls a and b in the leaves occures. For the Vitaplan, CL + Chitosan II, it was 1.32 ± 0.02 mg/g for chlorophyll a and 2.15 ± 0.04 mg/g for chlorophyll b. For the complex Vitaplan, CL + Chitosan II, the ratio chlorophylls a, b and carotenoid pigments, which serves as one of the indicators of plant stress resistance, was also maximum. The strongest correlation was found between the content of chlorophyll b in flag leaves and wheat productivity ($r = 0.69$, $p = 0.03$), the content of chlorophyll b in flag leaves and the number of grains per ear ($r = 0.79$, $p = 006$), grain weight per ear and ear weight ($r = 0.69$, $p = 0.03$; $r = 0.72$, $p = 0.02$). A decrease in the development of yellow rust correlated цшер an increase in the content of chlorophylls a and b in the leaves ($r = -0.66$, $p = 0.04$; $r = -0.87$, $p = 0.005$). Our study shows that the multifunctional compositions based on the selected strains of bacterial antagonists of plant pathogens and inducers of disease resistance significantly reduces the incidence of a complex of diseases in wheat plants and has a positive effect on the content of photosynthetic pigments (chlorophylls a, b and carotenoids) and plant productivity.

REFERENCES

1. Novikova I.I., Titova Yu.A., Boykova I.V., Zeyruk V.N., Krasnobaeva I.L., Serova T.A. *Vestnik zashchity rasteniy*, 2017, 3(93): 16-23 (in Russ.).
2. Tamehiro N., Okamoto-Hosoya Y., Okamoto S., Ubukata M., Hamada M., Naganawa H., Ochi K. Bacilysocin, a novel phospholipid antibiotic produced by *Bacillus subtilis* 168. *Antimicrobial Agents and Chemotherapy Journal*, 2002, 46(2): 315-320 (doi: 10.1128/aac.46.2.315-320.2002).
3. Tojo S., Tanaka Y., Ochi K. Activation of antibiotic production in *Bacillus* spp. by cumulative drug resistance mutations. *Antimicrobial Agents and Chemotherapy Journal*, 2015, 59(12): 7799-7804 (doi: 10.1128/AAC.01932-15).
4. Hamdache A., Lamarti A., Aleu J., Collado I.G. Non-peptide metabolites from the genus *Bacillus*. *Journal of Natural Products,* 2011, 74(4): 893-899 (doi: 10.1021/np100853e).
5. Quardros C.P., Teixeira Duarte M.C., Pastore G.M. Biological activities of a mixture of biosurfactant from *Bacillus subtilis* and alkaline lipase from *Fusarium oxysporum*. *Brazilian Journal of Microbiology*, 2011, 42: 354-361 (doi: 10.1590/s1517-83822011000100045).
6. Aktuganov G.E., Galimzyanova N.F. Melent'ev A.I., Kuz'mina L.Yu. *Mikrobiologiya*, 2007, 76: 471-479 (in Russ.).
7. Kloepper J.W., Gutierrez-Estrada A., McInroy J.A. Photoperiod regulates elicitation of growth promotion but not induced resistance by plant growth-promoting rhizobacteria. *Canadian Journal of Microbiology*, 2009, 53(2): 159-167 (doi: 10.1139/w06-114).
8. Ohkama-Ohtsu N., Wasaki J. Recent progress in plant nutrition research: cross-talk between nutrients, plant physiology and soil microorganisms. *Plant and Cell Physiology*, 2010, 51(8): 1255-1264 (doi: 10.1093/pcp/pcq095).
9. Dodd I.C., Zinovkina N.Y., Safronova V.I., Belimov A.A. Rhizobacterial mediation of plant hormone status. *Annals of Applied Biology*, 2010, 157: 361-379 (doi: 10.1111/j1744-7348.2010.00439.x).
10. Forchetti G., Masciarelli O., Alemano S., Alvarez D., Abdala G. Endophytic bacteria in sunflower (*Helianthus annuus* L.): isolation, characterization, and production of jasmonates and abscisic acid in culture medium. *Applied Microbiology and Biotechnology*, 2007, 76(5): 1145-1152 (doi: 10.1007/s00253-007-1077-7).
11. Kudoyarova G.R., Melentiev A.I., Martynenko E.V., Timergalina L.N., Arkhipova T.N., Shendel G.V., Kuz'mina L.Y., Dodd I.C., Veselov S.Y. Cytokinin producing bacteria stimulate amino acid deposition by wheat roots. *Plant Physiology and Biochemistry*, 2014, 83: 285-291 (doi: 10.1016/j.plaphy.2014.08.015).

12. Sivasakthi S., Kanchana D., Usharani G., Saranraj P. Production of plant growth promoting substance by *Pseudomonas fluorescens* and *Bacillus subtilis* isolates from paddy rhizosphere soil of Cuddalore district, Tamil Nadu, India. *International Journal of Microbiological Research*, 2013, 4(3): 227-233 (doi: 10.5829/idosi.ijmr.2013.4.3.75171).
13. Kumar P., Dubey R.C., Maheshwari D.K. *Bacillus* strains isolated from rhizosphere showed plant growth promoting and antagonistic activity against phytopathogens. *Microbiological Research*, 2012, 167: 493-499 (doi: 10.1016/j.micres.2012.05.002).
14. Maksimov I.V., Veselova S.V., Nuzhnaya T.V., Sarvarova E.R., Khayrullin R.M. *Fiziologiya rasteniy*, 2015, 62(6): 763-775 (doi: 10.7868/S0015330315060111) (in Russ.).
15. Kolesnikov L.E., Novikova I.I., Surin V.G., Popova E.V., Priyatkin N.S., Kolesnikova Yu.R. Evaluation of the effectiveness of the combined use of chitosan and antagonist microbes in protection of spring soft wheat from diseases using spectrometric analysis. *Applied Biochemistry and Microbiology*, 2018, 54(5): 546-552 (doi: 10.1134/S0003683818050083).
16. El Amerany F., Meddich A., Wahbi S., Porzel A, Taourirte M., Rhazi M., Hause B. Foliar application of chitosan increases tomato growth and influences mycorrhization and expression of endochitinase-encoding genes. *International Journal of Molecular Sciences*, 2020, 21(2): 535 (doi: 10.3390/ijms21020535).
17. Yang H., Zhang Y., Zhou F., Guo J., Tang J., Han Y., Fu C. Preparation, bioactivities and applications in food industry of chitosan-based maillard products: A review. *Molecules*, 2021, 26(1): 166 (doi: 10.3390/molecules26010166).
18. Tyuterev S.L. *Prirodnye i sinteticheskie induktory ustoychivosti rasteniy k boleznyam* [Natural and synthetic inducers of plant disease resistance]. St. Petersburg, 2014 (in Russ.).
19. Badawy M.E.I., Rabea E.I**.** A biopolymer chitosan and its derivatives as promising antimicrobial agents against plant pathogens and their applications in crop protection. *International Journal of Carbohydrate Chemistry*, 2011, 2011: Article ID 460381 (doi: 10.1155/2011/460381).
20. Abdul Malik N.A., Kumar I.S., Nadarajah K. Elicitor and receptor molecules: orchestrators of plant defense and immunity, review. *International Journal of Molecular Sciences*, 2020, 21(3): 963 (doi: 10.3390/ijms21030963).
21. Lopez-Moya F., Suarez-Fernandez M., Lopez-Llorca L.V. Molecular mechanisms of chitosan interactions with fungi and plants. *International Journal of Molecular Sciences,* 2019, 20(2): 332 (doi: 10.3390/ijms20020332).
22. Meenakshi T., Baldev S.S., Role of elisitors in inducing resistanse in plant against pathogen infection: a review. *ISRN Biochemistry*, 2013, 2013: Article ID 762 (doi: 10.1155/2013/762412).
23. Iriti M., Faoro F. Chitosan as a MAMP, searching for a PRR. *Plant Signaling and Behavior*, 2009, 4(1): 66-68 (doi: 10.4161/psb.4.1.7408).
24. Deepmala K., Hemantaranjan A., Bharti S., Nishant Bhanu A. A future perspective in crop protection: chitosan and its oligosaccharides. *Advances in Plants & Agriculture Research*, 2014, 1(1): 23-30 (doi: 10.15406/apar.2014.01.00006).
25. Bigeard J., Colcombet J., Hirt H. Signaling mechanisms in pattern-triggered immunity (PTI). *Molecular Plant*, 2015, 8(4): 521-539 (doi: 10.1016/j.molp.2014.12.022).
26. Silva W.B., Silva G.M.C., Santana D.B., Salvador A.R., Medeiros D.B., Belghith I., Misobutsi G.P. Chitosan delays ripening and ROS production in guava (*Psidium guajava* L.) fruit. *Food Chemistry*, 2018, 242: 232-238 (doi: 10.1016/j.foodchem.2017.09.052).
27. Gai Q.Y., Jiao J., Wang X., Liu J., Wang Z., Fu Y.J. Chitosan promoting formononetin and calycosin accumulation in *Astragalus membranaceus* hairy root cultures via mitogen-activated protein kinase signaling cascades. *Scientific Reports*, 2019, 9: 10367 (doi: 10.1038/s41598-019-46820-6).
28. Li Y., Zhang Q., Ou L., Ji D., Liu T., Lan R., Li X., Jin L. Response to the cold stress signaling of the tea plant (*Camellia sinensis*) elicited by chitosan oligosaccharide. *Agronomy*, 2020, 10: 915 (doi: 10.3390/agronomy10060915).
29. Dubin A., Likhanov A., Klyachenko O., Subin A., Kluvadenko A. Effect of chitosan formulations of different biological origin on tobacco (*Nicotiana tabacum* L.) PR-genes expression. *Journal of Microbiology, Biotechnology and Food Sciences*, 2021, 9(6): 1141-1144 (doi: 10.15414/jmbfs.2020.9.6.1141-1144).
30. Chun S.C., Chandrasekaran M. Chitosan and chitosan nanoparticles induced expression of pathogenesis-related proteins genes enhances biotic stress tolerance in tomato. *International Journal of Biological Macromolecules,* 2019, 125: 948-954 (doi: 10.1016/j.ijbiomac.2018.12.167).
31. Liu J., Zhang X., Kennedy J., Jiang M., Cai Q., Wu X. Chitosan induces resistance to tuber rot in stored potato caused by *Alternaria tenuissima*. *International Journal of Biological Macromolecules*, 2019, 140: 851-857 (doi: 10.1016/j.ijbiomac.2019.08.227).
32. Jiang X., Lin H., Lin M., Chen Y., Wang H., Lin Y., Lin Y. A novel chitosan formulation treatment induces disease resistance of harvested litchi fruit to *Peronophythora litchii* in association with ROS metabolism. *Food Chemistry*, 2018, 266: 299-308 (doi: 10.1016/j.foodchem.2018.06.010).
33. Xing K., Zhu X., Peng X., Qin S. Chitosan antimicrobial and eliciting properties for pest control in agriculture: a review. *Agronomy for Sustainable Development*, 2015, 35(2): 569-588 (doi: 10.1007/s13593-014-0252-3).

34. El Hadrami A., Adam L.R., El Hardrami I., Daayf F. Chitosan in plant protection. *Marine Drugs*, 2010, 8(4): 968-987 (doi: 10.3390/md8040968).
35. Yin H., Du Y., Dong Z. Chitin oligosaccharide and chitosan oligosaccharide: two similar but different plant elicitors. *Frontiers in Plant Science*, 2016, 7: 522 (doi: 10.3389/fpls.2016.00522).
36. Torres-Rodriguez J.A., Reyes-Perez J.J., Castellanos T., Angulo C., Quinones-Aguilar E.E., Hernandez-Montiel L.G. A biopolymer with antimicrobial properties and plant resistance inducer against phytopathogens: chitosan. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca*, 2021, 49(1): 12231 (doi: 10.15835/nbha49112231).
37. Zhang Z., Li K., Liu S., Xing R., Yu H., Chen X., Li P. Size effects of chitooligomers on the growth and photosynthetic characteristics of wheat seedlings. *Carbohydrate Polymers*, 2016, 138: 27-33 (doi: 10.1016/j.carbpol.2015.11.050).
38. dos Reis C.O., Magalhaes P.C., Avila R.G., Almeida L.G., Rabelo M., Carvalho D.T., Cabral D.F., Karam D., de Sousa T.C. Action of N-succinyl and N,O-dicarboxymethyl chitosan derivatives on chlorophyll photosynthesis and fluorescence in drought-sensitive maize. *Journal of Plant Growth Regulation*, 2019, 38: 619-630 (doi: 10.1007/s00344-018-9877-9).
39. Mondal M.M.A., Malek M.A., Puteh A.B., Ismail M.R. Foliar application of chitosan on growth and yield attributes of mungbean (*Vigna radiata* (L.) Wilczek). *Bangladesh Journal of Botany*. 2013, 42(1): 179-183 (doi: 10.3329/bjb.v42i1.15910).
40. Khan H., Basit A., Alam M., Ahmad I., Ullah Iz., Alam N., Ullah In., Khalid M.A., Shair M., ul Ain N. Efficacy of chitosan on performance of tomato (*Lycopersicon esculentum* L.) plant under water stress condition. *Pakistan Journal of Agricultural Research*, 2020, 33(1): 27-41 (doi: 10.17582/journal.pjar/2020/33.1.27.41).
41. Rutairat P., Chonlada D.T. Effect of chitosan on physiology, photosynthesis and biomass of rice (*Oryza sativa* L.) under elevated ozone. *Australian Journal of Crop Science*, 2017, 11(5): 624-630 (doi: 10.21475/ajcs.17.11.05.p578).
42. Zeng D., Luo X. Physiological effects of chitosan coating on wheat growth and activities of protective enzyme with drought tolerance. *Open Journal of Soil Science*, 2012, 2: 282-288 (doi: 10.4236/ojss.2012.23034).
43. Dudenko N.V., Andrianova Yu.E., Maksyutova N.N. *Fiziologiya rasteniy*, 2002, 49(5): 684-687 (in Russ.).
44. Pryadkina G.A. *Sortoizuchenie i okhrana prav na sorta rasteniy*, 2018, 14(1): 97-108 (doi: 10.21498/2518-1017.14.1.2018.126524) (in Russ.).
45. Furbank R.T., Quick W.P., Sirault X.R.R. Improving photosynthesis and yield potential in cereal crops by targeted genetic manipulation: prospects, progress and challenges. *Field Crops Research*, 2015, 182: 19-29 (doi: 10.1016/j.fcr.2015.04.009).
46. Gu J., Yin X., Stomph T.J., Struik P.C. Can exploiting natural genetic variation in leaf photosynthesis contribute to increasing rice productivity? A simulation analysis. *Plant Cell and Environment*, 2014, 37(1): 22-34 (doi: 10.1111/pce.12173).
47. Voitsekhovskaja O.V., Tyutereva E.V. Chlorophyll b in angiosperms: functions in photosynthesis, signaling and ontogenetic regulation. *Journal of Plant Physiology*, 2015, 189: 51-64 (doi: 10.1016/j.jplph.2015.09.013).
48. Tyutereva E.V., Dmitrieva V.A., Voytsekhovskaya O.V. Chlorophyll b as a source of signals steering plant development (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2017, 52(5): 843-855 (doi: 10.15389/agrobiology.2017.5.843eng).
49. Tyutereva E.V., Ivanova A.N., Voytsekhovskaya O.V. *Uspekhi sovremennoy biologii,* 2014, 134: 249-256 (in Russ.).
50. Mamadyusupova M.G., Saboiev I.A., Nigmonov M., Nasyrova F.Yu., Aliev K. The influence of stress factors on the content of photosynthetic pigments and biometric indicators in wheat varieties and their wild relatives. *News of the Academy of Sciences of the Republic Tajikistan*, 2012, 3(180): 35-42.
51. Sharma A., Kumar V., Shahzad B., Ramakrishnan M., Shreeya Bali A., Yadav P., Rehman A., Daman R., Renu P., Gagan Preet B., Sidhu S., Handa N., Khanna K., Kohli S., Yuan H., Zheng Bi., Kapoor D., Bakshi P., Khan E.A., Kumar Thukral A. Photosynthetic response of plants under different abiotic stresses: a review. *Journal of Plant Growth Regulation*, 2020, 39(2): 509-531 (doi: 10.1007/s00344-019-10018-x).
52. Muhammad I., Shalmani A., Ali M., Yang Q.-H., Ahmad H., Li F.B. Mechanisms regulating the dynamics of photosynthesis under abiotic stresses. *Frontiers in Plant Science*, 2021, 11: 615942 (doi: 10.3389/fpls.2020.615942).
53. Abdullaev Kh.A., Giyasidinov B.B., Solieva B.A., Mirakilov Kh.M. *Tezisy Vserossiyskoy nauchnoy konferentsii s mezhdunarodnym uchastiem i Shkoly dlya molodykh uchenykh «Rasteniya v usloviyakh global'nykh i lokal'nykh prirodno-klimaticheskikh i antropogennykh vozdeystviy» (21-26 sentyabrya 2015 g.)* [Abstracts of the All-Russia Conference with international participation and the School for young scientists "Plants under the conditions of global and local climatic and anthropogenic influences" (September 21-26, 2015)]. Petrozavodsk, 2015: 19 (in Russ.).
54. Andrianova Yu.E., Tarchevskiy I.A. *Khlorofill i produktivnost' rasteniy* [Chlorophyll and plant productivity]. Moscow, 2000 (in Russ.).

55. Yurin V.M. *Fiziologiya rasteniy* [Plant physiology]. Minsk, 2010 (in Russ.).
56. Mokronosov A.T., Gavrilenko V.F., Zhigalova T.V. *Fotosintez. Fiziologo-ekologicheskie i biokhimicheskie aspekty* [Photosynthesis. Physiological, ecological and biochemical aspects]. Moscow, 2006 (in Russ.).
57. Amunova O.S., Lisitsyn E.M. *Samarskiy nauchnyy vestnik*, 2019, 8(3): 19-25 (doi: 10.24411/2309-4370-2019-13102) (in Russ.).
58. Sui N., Li M., Meng Q.-W., Tian J., Zhao S. Photosynthetic characteristics of a super high yield cultivar of winter wheat during late grown period. *Agricultural Sciences in China*, 2010, 9(3): 346-354 (doi: 10.1016/S1671-2927(09)60103-6).
59. Morgun V.V., Pryadkina G.A. *Fiziologiya rasteniy i genetika*, 2014, 46(4): 279-301 (in Russ.).
60. Martirosyan L.Yu., Kosobryukhov A.A., Martirosyan V.V., Martirosyan Yu.Ts. The influence of different light sources on photosynthetic performance and productivity of *Cucumis sativus* L. hybrid Tristan F_1 in aeroponic phytotron facilities. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2021, 56(5): 934-947 (doi: 10.15389/agrobiology.2021.5.934eng).
61. Kornilina V.V. *Fundamental'nye issledovaniya,* 2012, 9(3): 568-572 (in Russ.).
62. Muzzarelli R.A.A. *Chitin*. Pergamon Press, Oxford, 1977.
63. Kolesnikov L.E., Popova E.V., Novikova I.I., Priyatkin N.S., Arkhipov M.V., Kolesnikova Yu.R., Potrakhov N.N., Van Duijn B., Gusarenko A.S. Multifunctional biologics which combine microbial anti-fungal strains with chitosan improve soft wheat (*Triticum aestivum* L.) yield and grain quality. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2019, 54(5): 1024-1040 (doi: 10.15389/agrobiology.2019.5.1024eng).
64. Zakharova N.N., Zakharov N.G. *Vestnik Ul'yanovskoy gosudarstvennoy sel'skokhozyaystvennoy akademii,* 2016, 4(36): 17-23 (doi: 10.18286/1816-4501-2016-4-17-23) (in Russ.).
65. Zadoks J.C., Chang T.T., Konzak C.F. A decimal code for the growth stages of cereals. *Weed Research*, 1974, 14: 415-421 (doi: 10.1111/j.1365-3180.1974.tb01084.x)
66. Dmitriev N.N., Khusnidinov Sh.K. *Vestnik KrasGAU*, 2016, 7(118): 88-93 (in Russ.).
67. Popov Yu.V. *Zashchita i karantin rasteniy*, 2011, 8: 45-47 (in Russ.).
68. Novikova I.I., Popova E.V., Kovalenko N.M., Krasnobaeva I.L. *Vestnik zashchity rasteniy*, 2022, 105(3): 122-134 (doi: 10.31993/2308-6459-2022-105-3-15320) (in Russ.).
69. Ermakov A.I., Arasimovich V.V., Yarosh N.P. *Metody biokhimicheskogo issledovaniya rasteniy* [Methods of research in plant biochemistry]. Leningrad, 1987 (in Russ.).
70. Kalinina A.V., Lyashcheva S.V. *Izvestiya Samarskogo nauchnogo tsentra Rossiyskoy akademii nauk*, 2018, 20(2): 286-290 (in Russ.).
71. Khalil R.R., Bassiouny F.M., El-Dougdoug K.A., Abo-Elmaty S., Yousef M.S. A dramatic physiological and anatomical changes of tomato plants infecting with tomato yellow leaf curl germinivirus. *Journal of Agricultural Technology*, 2014, 10(5): 1213-1229.
72. Akinshina N.G., Rashidova D.K., Azizov A.A. Seed encapsulation in chitosan and its derivatives restores levels of chlorophyll and photosynthesis in wilt-affected cotton (*Gossypium* L., 1753) plants. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2016, 51(5): 696-704 (doi: 10.15389/agrobiology.2016.5.696eng).
73. Kalinina E.A. Vliyanie biologicheski aktivnykh soedineniy na rost, fotosintez i produktivnost' kukuruzy (*Zea mays L.*). *Izvestiya TSKh*, 2009, 3: 181-186 (in Russ.).
74. Kilian M., Steiner U., Krebs B., Junge H., Schmiedeknecht G., Hain R. FZB24® *Bacillus subtilis* — mode of action of microbial agent enhancing plant vitality. *Pflanzenschutz-Nachrichten Bayer*, 2000, 1: 72-93.
75. Arkhipova T.N., Prinsen E., Veselov S.U., Martynenko E.V., Melentiev A.I., Kudoyarova G.R. Cytokinin producing bacteria enhance plant growth in drying soil. *Plant and Soil*, 2007, 292: 305-315 (doi: 10.1007/s11104-007-9233-5).
76. Cohen A.C., Travaglia C.N., Bottini R., Piccoli P.N. Paticipation of abscisic acid and gibberellins produced by endophytic *Azospirillum* in the alleviation of drought effects in maize. *Botany*, 2009, 87: 455-462 (doi: 10.1139/B09-023).
77. De Meyer G., Audenaert K., Hufte M. *Pseudomonas aeruginosa* 7NSK2-induced systemic resistance in tobacco depends on in planta salicylic acid accumulation but is not associated with PR1a expression. *European Journal of Plant Pathology*, 1999, 105: 513-517 (doi: 10.1023/A:1008741015912).
78. Belimov A.A., Dodd I.C., Safronova V.I., Dumova V.A., Shaposhnikov A.I., Ladatko A.G., Davies W.J. Abscisic acid metabolizing rhizobacteria decrease ABA concentrations in planta and alter plant growth. *Plant Physiology and Biochemistry*, 2014, 74: 84-91 (doi: 10.1016/j.plaphy.2013.10.032).
79. Porcel R., Zamarreso A.M., Garcia-Mina J.M., Aroca R. Involvement of plant endogenous ABA in *Bacillus megaterium* PGPR activity in tomato plants. *BMC Plant Biology*, 2014, 14: 36 (doi: 10.1186/1471-2229-14-36).
80. Pavlyushin V.A., Novikova I.I., Boykova I.V. Microbiological control in phytosanitary optimization technologies for agroecosystems: research and practice (review). *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2020, 55(3): 421-438 (doi: 10.15389/agrobiology.2020.3.421eng).
81. Lizarraga-Pauli E.G., Torres-Pacheco I., Moreno Martinez E., Miranda-Castro S.P. Chitosan application in maize (*Zea mays*) to sounteract the effects of abiotic stress at seedling level. *African Journal of Biotechnology*, 2011, 10(34): 6439-6446 (doi: 10.5897/ajb10.1448).

82. Mondal M.M.A., Puteh A.B., Dafader N.C., Rafii M.Y., Malek M.A. Foliar application of chitosan improves growth and yield in maize. *Journal of Food Agriculture and Environment*, 2013, 11: 520-523.
83. Martins M., Carvalho M., Carvalho D.T., Barbosa S., Doriguetto A.C., Magalhaes P.C., Ribeiro C. Physicochemical characterization of chitosan and its effects on early growth, cell cycle and root anatomy of transgenic and non-transgenic maize hybrids. *Australian Journql of Crop Science*, 2018, 12: 56 (doi: 10.21475 /ajcs.18.12.01.pne649).
84. Ibrahim E.A., Ramadan W.A. Effect of zinc foliar spray alone and combined with humic acid or/and chitosan on growth, nutrient elements content and yield of dry bean (*Phaseolus vulgaris* L.) plants sown at different dates. *Sci. Hort.*, 2015, 184: 101-105 (doi: 10.1016/j.scien ta.2014.11.010).
85. Wang X., Vigjevic M., Liu F., Jacobsen S., Jiang D., Wollenweber B. Drought priming at vegetative growth stages improves tolerance to drought and heat stresses during grain filling in spring wheat (*Triticum aestivum* L. cv. Vinjett). *Plant Growth Regulation*, 2015, 75: 677-687 (doi: 10.1007/s1072 5-014-9969-x).
86. Pongprayoon W., Roytrakul S., Pichayangkura R., Chadchawan S. The role of hydrogen peroxide in chitosan-induced resistance to osmotic stress in rice (*Oryza sativa* L.). *Plant Growth Regulation*, 2013, 70: 159-173 (doi: 10.1007/s1072 5-013-9789-4).
87. Costales M., Falcon R. Combination of application forms of chitosan in the development of biofertilized soybean. *Cultivos Tropicales*, 2018, 39(3): 71-79.
88. Chamnanmanoontham N., Pongprayoon W., Pichayangkura R., Roytrakul S., Chadchawan S. Chitosan enhances rice seedling growth via gene expression network between nucleus and chloroplast. *Plant Growth Regulation*, 2015, 75: 101-114 (doi: 10.1007/s10725-014-9935-7).
89. Farouk S., Amany A.R. Improving growth and yield of cowpea by foliar application of chitosan under water stress. *Egyptian Journal of Biology*, 2012, 14: 14-26 (doi: 10.4314/ejb.v14i1 .2).
90. Ashaeva O.V., Monin D.S. *Vestnik Nizhegorodskoy gosudarstvennoy sel'skokhozyaystvennoy akademii*, 2012, 1: 111-113 (in Russ.).
91. Shehata S.A., Fawzy Z.F., El-Ramady H.R. Response of cucumber plants to foliar application of chitosan and yeast under greenhouse conditions. *Australian Journal of Basic and Applied Sciences*, 2012, 6(4): 63-71.

AGRICULTURAL BIOLOGY,
2023, V. 58, Iss. 1, pp. 184-199
[SEL'SKOKHOZYAISTVENNAYA BIOLOGIYA]

ISSN 2412-0324 (English ed. Online)
ISSN 0131-6397 (Russian ed. Print)
ISSN 2313-4836 (Russian ed. Online)

Plant microbiology

UDC 633.1:632.3.01/.08:632.913.1:579.64

doi: 10.15389/agrobiology.2023.1.184eng
doi: 10.15389/agrobiology.2023.1.184rus

DETECTION OF BACTERIOSIS PATHOGENS SIGNIFICANT FOR GRAIN EXPORT AND A COMPLEX OF ASSOCIATED MICROORGANISMS IN GRAIN CROPS (ON THE EXAMPLE OF TIMIRYAZEVSKAYA FIELD EXPERIMENTAL STATION

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The authors declare no conflict of interests
Final revision received 03 August, 2022
Accepted 16 November 2022

Abstract

According to official statistics, about 130 million tons of cereals are produced annually in Russia. In the Unified List of quarantine objects of the Eurasian Economic Union is the causative agent of wheat yellow mucous bacteriosis *Rathayibacter tritici*. This species is subject to detection during import and, if the importer requires, during export of wheat. Due to the need for regulation, there is a diagnostic method for *Rathayibacter tritici* in quarantine phytosanitary laboratories. For other pathogens of bacteriosis in grain crops, such as *Rathayibacter rathayi*, *Pseudomonas fuscovaginae*, *Pseudomonas cichorii*, *Pseudomonas fluorescens*, *Pseudomonas syringae, Acidovorax avenae,* E*rwinia rhapontici*, *Xanthomonas translucens*, *Clavibacter tessellarius*, etc., there are no diagnostic methods, due to which no detections have been recorded in the practice of diagnostic phytosanitary laboratories. The listed types are regulated by importing countries that purchase more than half of all grain products intended for export in Russia. Bacterioses pose a serious threat to grain production, and the possible damage they cause to the crop is estimated at 10-40 %. The bacteria can cause disease outbreaks or be latent in plants depending on environmental conditions and almost never cause symptoms on grain. In this regard, it is possible to detect causative agents of bacteriosis only in the laboratory using the method of inoculation on nutrient media, which often takes a week or more. Reliable identification of each type of bacteria is possible only with the use of molecular methods. It is required to develop PCR tests that allow the identification of target bacteria directly in samples without using the cultural method, which will significantly simplify and speed up the procedure for confirming the compliance of the state of Russian grain batches with the requirements of importers. The development of molecular methods for diagnosing causative agents of bacterioses in grain crops is possible only after studying their species composition in plants and grain, while the diversity of living bacteria in vegetative plants is significantly higher than in grain. Information on the species composition of bacteria on grain crops will make it possible, using genomic analysis, to detect species-specific PCR targets and develop diagnostic PCR tests for the rapid identification of bacterial species that are especially dangerous and important for grain export. Previously, a large-scale study of the bacterial composition in grain crops was not carried out, and therefore, there is no list of bacteria that can be found together in one sample. There is also no complete list of all bacteria that can be found in cereals. At the same time, for bioinformatic prediction of a species-specific PCR target, it is necessary to know all the species that can be found in the analyzed sample, from which the target species should be distinguished. The composition of the bacterial microbiota may differ depending on the crop and variety, so the maximum diversity of different crops and varieties will provide more complete information. Humid and moderately warm summer conditions in the Central region are ideal for the development of bacteriosis. In connection with the foregoing, sampling was carried out on the territory of the Timiryazevskaya field experimental station (Moscow), where hybridization, selection and variety testing of several hundred varieties of

grain crops are carried out annually. The work is devoted to the detection and identification of bacteria in samples of grain crops of the Timiryazevskaya field experimental station (Moscow). The objects of the study were bacterial isolates from grain samples in 2020. Bacteria were identified by sequencing the amplicons obtained by PCR with primer pairs PSF/PSR, SyD1/SyD2, and 8UA/519B and comparing the resulting sequences using the BLAST service with sequences posted in GenBank (https://blast.ncbi.nlm. nih.gov). As a result, 55 samples of grain crops were collected, 171 bacterial isolates were isolated and identified, including 34 isolates identified to species. Bacterial diversity is represented by 14 species. Among them, there are phytopathogens *Pantoea ananatis*, *Clavibacter michiganensis*, *Rhodococcus fascians*, *Pseudomonas trivialis*, *Pseudomonas viridiflava* and *Pseudomonas syringae*. The highest frequency of occurrence, 70.9 %, was noted in species belonging to the genus *Pseudomonas*. Representatives of the genera *Frigoribacterium* (36.4 %), *Clavibacter* (16.4 %), *Arthrobacter* (12.7 %) and *Rhodococcus* (10.9%) also have a high frequency of occurrence. The results of the study can be used in the development of fast and reliable methods for diagnosing especially dangerous and important bacterial species for grain export. In addition, during the study, bacteria were isolated that belong to certain genera, but do not belong to any of the known species, which makes them promising for further study to describe new species in the microbiota of grain crops.

Keywords: diagnostics of phytopathogens, grain crops, bacterioses, PCR, sequencing

According to information provided by the Federal State Statistics Service (https://rosstat.gov.ru/), winter and spring grain crops (wheat, rye, barley, triticale, and oats) are grown annually in Russia on an area of more than 41 million hectares, and the gross harvest of products is about 130 million tons. According to the Customs Statistics of Foreign Trade of the Russian Federation (http://stat.customs.ru/), the Russian Federation annually exports more than 39.5 million tons of grain (analysis for the period from 2019 to 2021).

Plant diseases caused by bacterial pathogens significantly limit crop production and cause significant annual losses globally [1-3]. Bacterioses of cereals pose a serious economic threat, since, according to various estimates, they can reduce yields by 10-40% depending on environmental conditions and the stage of plant ontogenesis in which the infection occurred [4, 5]. The problem requires systemic control of the spread of bacterial infections [1, 6]. In accordance with the Decision of the Council of the Eurasian Economic Commission No. 157 dated November 30, 2016 (as amended by the decisions of the Council of the Eurasian Economic Commission No. 31 dated 03.29.2019, No. 74 dated 08.08.2019, No. 54 dated 05.18.2021, No. 98 dated 05.10.2021, and No. 109 dated 07.15.2022), only the causative agent of wheat yellow mucous bacteriosis *Rathayibacter tritici*, is regulated on grain crops, namely on regulated products under HS codes 1001 and 1008600000 (https://www.alta.ru/tamdoc/16sr0157/). The specified species is subject to identification during import and, subject to the requirements of the importer, during export of wheat. Due to the need for regulation, a diagnostic technique for *Rathayibacter tritici* has been developed and used in quarantine phytosanitary laboratories in the Russian Federation. In quarantine phytosanitary laboratories, there are no methods to identify other dangerous plant pathogenic bacteria, e.g., the causative agents of bacteriosis of grain crops *Rathayibacter rathayi*, *Pseudomonas fuscovaginae*, *Pseudomonas cichorii*, *Pseudomonas fluorescens*, *Pseudomonas syringae*, *Acidovorax avenae*, *Erwinia rhapontici*, *Xanthomonas translucens* and *Clavibacter tessellarius*. According to the Federal Service for Veterinary and Phytosanitary Surveillance (https://fsvps.gov.ru/ru) and the European and Mediterranean Plant Protection Organization (https://gd.eppo.int/), the listed species are regulated in grain products by the phytosanitary requirements of a number of countries, including those importing Russian grain. One or more of these bacteria species is regulated in Egypt, Jordan, Turkey, Morocco, Tunisia, Nigeria, Pakistan, Cameroon, Taiwan, Serbia, South Africa, Brazil, Israel, Colombia and Mexico, the countries that, according to External Customs Statistics trade of the Russian Federation, purchase in Russia more than half of all grain products intended for export.

Bacteria that colonize plant vascular tissue [7], cannot be controlled under field conditions. Often, infected plants produce grain which is a source of infection [3, 4, 8]. Plant pathogenic bacteria can survive for a long time in plants and seeds without showing symptoms [9, 10]. Among the causative agents of bacteriosis of grain crops, *Erwinia rhapontici* can cause characteristic symptoms (pink pigmentation) on seeds [11, 12]. For most bacterioses of grain crops, characteristic symptoms are various stripes, streaks and constrictions on the leaves, burns, yellowing, watery spots or necrosis, depending on the stage of the disease. It is noted that the ears, including seeds and glumes, are usually asymptomatic, but can still harbor infection and be a source of infection [13]. The most effective way to prevent the spread of seed-borne diseases is early laboratory diagnosis [14]. Thereof, both fundamental and practical research require reliable, unified and highly sensitive methods for identification of pathogens to differentiate these species despite the diversity of plant microbiota. For example, this is important in studies of plant-pathogen interaction, acquisition of plant resistance, the plant-pathogen system ecology [15, 16], as well as in breeding varieties for disease resistance, biotechnologies of infection-free cell and tissues cultures, phytosanitary monitoring, export and import quarantine control [16-18]

The fastest and most reliable approach to diagnosing plant pathogens is the use of species-specific PCR tests [19]. To predict the PCR target using bioinformatics methods and validate the resulting primers, information about the species composition of the microbiota of the object is required. It is important to have the most complete collections of both target bacterial isolates and possible accompanying microbiota from which the test must differentiate the target regulated species. However, systematic and large-scale screening of grain crops for the presence of bacterial phytopathogens significant for export has not yet been carried out in the Russian Federation.

The climatic conditions of Moscow are characterized by high humidity [20] and can promote the growth of bacteria inside and on the surface of the plant, which increases the likelihood of their detection. In this work, in samples of grain crop varieties collected from testing sites and hybridization plots at the Moscow field experimental station of the Timiryazev Russian State Agrarian University, along with bacteria exhibiting economically useful and neutral properties, we have identified for the first time the plant pathogens *Pantoea ananatis*, *Clavibacter michiganensis*, *Rhodococcus fascians*, *Pseudomonas trivialis*, *Pseudomonas viridiflava* and *Pseudomonas syringae*.

Our goal was to collect and identify bacterial isolates in samples collected at the Timiryazevskaya field experimental station to form a collection of pathogenic and non-pathogenic microbiota of grain crops.

Materials am methods. Samples (one sample for one variety) of wheat, triticale and rye plants were collected on May 13, 2020 at the variety testing sites and hybridization plots (the field experimental station of the Timiryazev Russian State Agrarian University, 2020-2021). The sample of winter crops consisted of 5-15 plant stems cut at the first internode; the sample of spring crops consisted of 15 seedlings. If symptoms were present, both symptomatic plants and healthy vegetative material were selected.

Individual analytical samples were prepared as previously described [21]. Collected plant material stored at 4 °C in the dark was used within 1 week after collection. Plant tissue (5-10 g) crushed using sterilized scissors was added with 20 ml of phosphate-buffered saline (per 1 liter of distilled water, 2.9 g $Na_2HPO_4 \cdot 12H_2O$, 0.2 g $KH_2PO_4 \cdot 2H_2O$, 8 g NaCl and 0.2 g KCl; pH 7.0-7.2) and left on the shaker for 1 hour at 200 rpm. Then the liquid part was passed through filters with a pore size of 3-5 μm and centrifuged for 10 minutes

at 10,000 g and 4 °C. The supernatant was removed, and the pellet was suspended in 1 ml of phosphate-buffered saline.

Bacteria were isolated on CRL medium [21] by plating 20 μl analytical aliquot onto three Petri dishes according to the Drigalski method. After 5-7 days, individual colonies were subcultured onto CRL medium using a sterile bacteriological loop. The entire morphotypical diversity of colonies grown on the plates was collected. Small fragment of individual colony from each pure culture taken with a sterile bacteriological loop was suspended in 200 μl of distilled water.

Suspensions were used for DNA extraction using a commercial Proba-GS kit (ZAO AgroDiagnostica, Russia) in accordance with the manufacturer's instructions.

All DNA samples were tested in duplicate by classical PCR. Amplification (a T100 thermal cycler, Bio-Rad, USA) was performed using oligonucleotides synthesized at ZAO Evrogen (Russia) and ready-made mixtures for PCR 5× Mas^{DD}TaqMIX-2025 (ZAO Dialat, Russia). The first test was performed with primers PSF/PSR (PSF: 5′-AGCCGTAGGGGAACCTGCGG-3′, PSR: 5′-TGACTGCCAAGGCATCCACC-3′) [22]. Several copies of the 610 bp sequence amplified with the indicated primers are located in tRNA in bacteria of the genus *Pseudomonas*. The PCR mixture for one reaction was 16 μl water, 5 μl 5× Mas^{DD}TaqMIX-2025, 1 μl each primer at a concentration of 10 μmol and 2 μl DNA. Amplification program was 95 °C for 10 min; 25 cycles of 95 °C for 20 s, 64 °C for 15 s, 72 °C for 15 s; 72 °C for 2 min. The PCR product was detected after electrophoretic separation in a 1.5% agarose gel using a gel documentation system (Bio-Rad, USA). Representatives of the genus *Pseudomonas* were found among all isolates. DNAs of cultures from which a 610-bp PCR product was obtained were tested with primers SyD1/SyD2 (SyD1: 5′-CAGCGGCGTTGCGTCCATTGC-3′′, SyD2: 5′-TGCCGCCGACGATGTAGACCAGC-3′) [22]. The primers identify *Pseudomonas syringae* and amplify a 1040 bp product. The PCR mixture per reaction was 17.4 μl water, 5 μl 5× MasDDTaqMIX-2025, 0.3 μl each primer at a concentration of 10 pmol and 2 μl DNA. Amplification program was 95 °C for 10 min; then 25 cycles of 95 °°C for 20 s, 64 °C for 15 s, 72 °C for 45 s; 72 °C for 7 minutes. The PCR product was detected in a 1.5% agarose gel horizontal electrophoresis. If a 1040 bp product was present, the amplicon remaining in the tube was purified using the GeneJET PCR Purification Kit (Thermo Fisher Scientific, USA) and used for Sanger sequencing with Big Dye Kit, BigDye®XTerminator™ Purification Kit (Thermo Fisher Scientific, USA) on an AB-3500 genetic analyzer (Applied Biosystems, USA) according to an adapted method [23]. In the absence of 1040 bp amplicons, sequencing of the PCR product was performed with primers PSF/PSR. For DNA samples for which no PCR products were obtained with primers SyD1/SyD2 or PSF/PSR, PCR was performed with primers 8UA/519B (8UA: 5′-AGAGTTTGATCMTGGCTCAG-3′, 519B: 5′-GTATTACCGCGGCKGCTG-3′) for the 16-23S rRNA region [24]. The PCR mixture for one reaction was 14 μl water, 5 μl 5× Mas^{DD}TaqMIX-2025, 2 μl each primer at a concentration of 10 μmol and 2 μl DNA. Amplification protocol was 96 °C for 10 min; 35 cycles of 95 °C for 15 s, 55 °C for 30 s, 72 °C for 30 s; 72 °C for 10 min. The PCR product was detected by a 1.5% agarose gel horizontal electrophoresis. Amplicon residues not used for electrophoresis were subjected to purification and sequencing as described above.

Sequencing results were processed using the BioEdit program (https://bioedit.software.informer.com/). The deciphered nucleotide sequences were compared using the BLAST service with sequences deposited in GenBank (https://blast.ncbi.nlm.nih.gov). The identification result was considered to be

the organism with the maximum similarity (Max score), automatically calculated by the BLAST service based on the calculation of the Query coverage and Percent identity indicators. If several such organisms were found in a taxon, the oldest taxon was considered the result of identification.

For each identified species and genus, the frequency of occurrence (A) was calculated using the formula [25]: $A = B/C \times 100\%$, where B is the number of samples on which a bacterium with a certain species was found, C is the total number of analyzed samples. When calculating the frequency of occurrence of bacterial genera, both isolates identified to species and isolates identified only to genus were accounted.

Results. The period of plant sampling for winter grain crops was during the booting stage, and for spring grain crops during the seedling phase. There were no symptoms of bacterial diseases on the plants during the sampling period of winter grain crops. Chlorosis occurred on spring rye seedlings. A total of 55 samples of grain crops were selected (Table 1).

1. Collected samples of grain crops (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academ, Moscow, 2020)

Crop	Variety
Secale cereale L.	Snezhana, Verasen, Unnamed
× *Triticosecale* Wittm. & A.Camus	Alexander, Victor, Nemchinovsky 56, Valentin 90, Timiryazevskaya 150
Triticum turgidum L.	Donskoy Yantar, Terra
Triticum durum Desf.	Pobeda 70
Triticum dicoccum Schrank	Untitled
Triticum sphaerococcum Percival	Eremeevna
× *Triticosecale* (Wittm. & A. Camus) *sphaerococcum*	Titus
Triticum aestivum L.	Zhiva, Alekseevich, Urup, Morozko, Timiryazevskaya Yubileynaya, Moskovskaya 56, Turquoise, Timiryazevka 150, Count, Vassa, Moskovskaya 39, Doublet, Cavalier, Scarlet Dawn, Nemchinovskaya 24, Legend, Avesta, Inna, Stan, Ascetic, Velena, Vanya, Artel, Nemchinovskaya 85, Videya, Don Lyra, Sineva, Moskovskaya 40, Don 107, Steppe, Governor of the Don, Rostovchanka, Vekha, Nemchinovskaya 57, Augusta, Soberbash, Anka, Gurt, Antonina, Nemchinovskaya 17, Bezostaya 100

N o t e. The sample of winter crops consisted of 5-15 plant stems cut at the first internode; the sample of spring crops consisted of 15 seedlings. One sample was taken from one variety.

Among the collected samples, 14 are rye *Secale cereale* L., triticale × *Triticosecale* Wittm. & A. Camus, × *Triticosecale* (Wittm. & A. Camus) *sphaerococcum*, turgid wheat *Triticum turgidum* L., hard wheat *Triticum durum* Desf. and spherical wheat *Triticum sphaerococcum* Percival, 41 samples are common wheat *Triticum aestivum* L. (see Table 1).

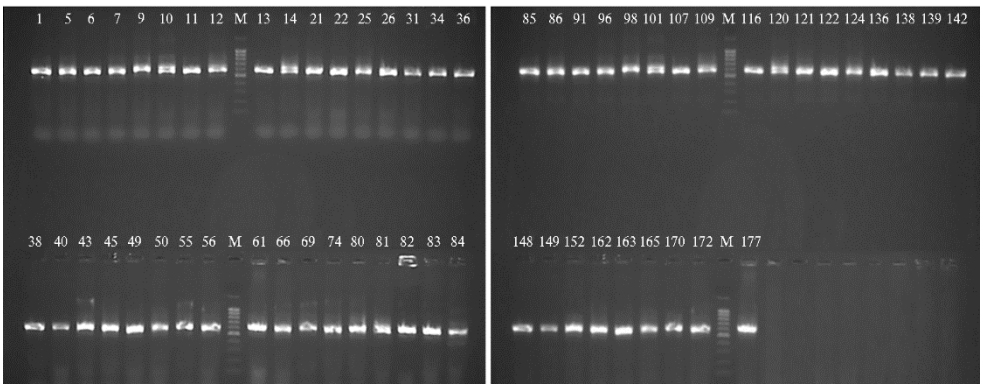

Fig. 1. PCR products with primers PSF/PSR (610 bp) for DNA samples of bacterial isolates from cereal varieties: 1 — Snezhana; 5 — Alive; 6, 7 — Alekseevich; 9-12 — Morozko; 13, 14 — Timiryazevskaya Yubileinaya; 21 — Moskovskaya 56; 22 — Turquoise; 25, 26 — Timiryazevka 150; 31 —

Alexander; 34, 36, 38 — Donskoy amber; 40 — Victor; 43 — *Triticum dicoccum* Schrank (no name); 45 — Nemchinovsky 56; 49 — Eremeevna; 50 — Titus; 55, 56 — Moskovskaya 39; 61 — Doublet; 66-69 — Cavalier; 74 — Scarlet dawn; 80 — Nemchinovskaya 24; 81, 82 — Victory 70; 83, 84 — Legend; 85, 86 — Avesta; 91, 96, 98 — Verasen; 101 — Inna; 107 — Terra; 109 — Timiryazevskaya 150; 116 — Stan; 120-122 — Ascetic; 124 — Velena; 136 — Videa; 138, 139 — Don lyre; 142 — Sineva; 148 — Don 107; 149 — Steppe; 152 — Rostovchanka; 162 — Soberbash; 163, 165 — Anka; 170 — Antonina; 172 — Nemchinovskaya 17; 177 — *Secale cereale* L. (no name); M — DNA length marker 100+ bp DNA ladder (100-1000 bp (ZAO Evrogen, Russia) (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020).

A total of 171 bacterial isolates derived from the collected samples. PCR with primers PSF/PSR revealed a 610 bp amplicon in 60 tested DNA samples of bacterial cultures (Fig. 1).

PCR with primers SyD1/SyD2 generated a 1040 bp amplicon for eight bacterial culture DNA samples tested (Fig. 2).

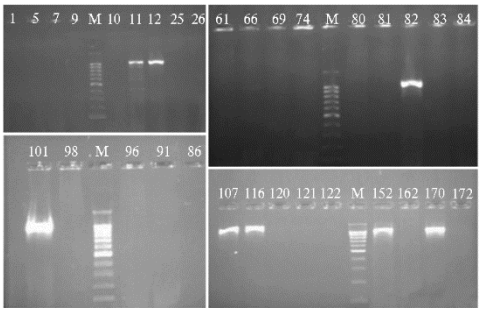

Fig. 2. PCR products with primers SyD1/SyD2 (1040 bp) obtained for DNA samples of bacterial isolates from cereal varieties: 1 — Snezhana; 5 — Zhiva; 7 — Alekseevich; 9-12 — Morozko; 25, 26 — Timiryazevka 150; 61 — Doublet; 66, 69 — Cavalier; 74 — Scarlet dawn; 80 — Nemchinovskaya 24; 81, 82 — Victory 70; 83, 84 — Legend; 86 — Avesta; 91, 96, 98 — Verasen; 101 — Inna; 107 — Terra; 116 — Stan; 120-122 — Ascetic; 152 — Rostovchanka; 162 — Soberbash; 170 — Antonina; 172 — Nemchinovskaya 17; M — DNA length marker 100+ bp DNA ladder (100-1000 bp (Evrogen, Russia) (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020).

For the remaining 103 DNA samples from bacterial cultures, 500 bp amplicons were obtained in PCR with primers 8UA/519B (Fig. 3).

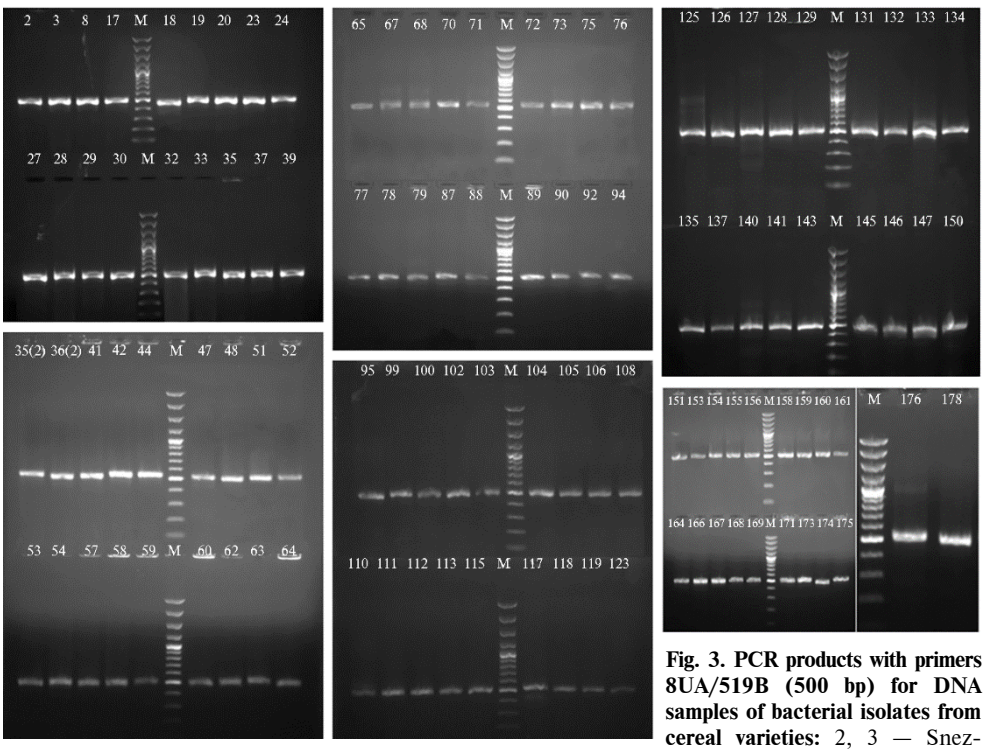

Fig. 3. PCR products with primers 8UA/519B (500 bp) for DNA samples of bacterial isolates from cereal varieties: 2, 3 — Snezhana; 8 — Urup; 17-20 — Timiryazevskaya Yubileinaya; 23-24 — Turquoise; 27 — Timiryazevka 150; 28-30 — Count; 32, 33, 35, 37 — Donskoy amber; 39 — Victor; 35(2), 36(2) — Donskoy amber; 41-42 — Vassa; 44 — Nemchinovsky

56; 47-48 — Eremeevna; 51-52 — Titus; 53-54, 57-58 — Moskovskaya 39; 59-60 — Valentin; 62-65 — Doublet; 67-68 — Cavalier; 70-73, 75-76 — Scarlet dawn; 77-79 — Nemchinovskaya 24; 87-90 — Avesta; 92, 94 — Verasen; 95, 97, 99 — Verasen; 100, 102-103 — Inna; 104-106, 108 — Terra; 110-111 — Timiryazevskaya 150; 112-114, 117 — Stan; 118-119 - Ascetic; 123 — Velena; 125 — Va-nya; 126-129, 131-132 — Artel; 133-134 — Nemchinovskaya 85; 135 — Videa; 137 — Don lyre; 140-141 — Sineva; 143, 145-146 — Moskovskaya 40; 147 — Don 107; 150 — Governor of Don; 151 — Rostovchanka; 153 — Milestone; 154-155 — Nemchinovskaya 57; 156, 158-159 — Augusta; 160-161 — Soberbash; 164 — Anka; 166-169 — Edge; 171 — Nemchinovskaya 17; 173-175 — Bezostaya 100; 176, 178 — spring rye Secale cereale L. (no name); M — molecular weight marker GeneRuler 100 bp Plus (100-1000 bp) (Thermo Fisher Scientific, USA) (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020).

2. Alignment of nucleotide sequences from Sanger sequencing for the collected bacterial isolates (BLAST service, https://blast.ncbi.nlm.nih.gov; Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020)

1	2	3	4	5	6	7
			Secale cereale L.			
Snezhana	1	SF/PSR	*Pseudomonas* sp.	990	100 %	99.09 %
Snezhana	2	8UA/519B	*Rhodococcus* sp.	353	75.0 %	86.73 %
			Rhodococcus fascians	350	89.0 %	83.62 %
Snezhana	3	8UA/519B	*Rhodococcus* sp.	619	91.0 %	91.23 %
Verasen	97	8UA/519B	*Arthrobacter* sp.	787	100 %	97.22 %
Verasen	98	PSF/PSR	*Pseudomonas* sp.	959	100 %	98.53 %
			Pseudomonas graminis	948	100 %	98.17 %
Verasen	99	8UA/519B	*Staphylococcus pasteuri*, *Staphylococcus* sp., *Staphylococcus warneri*	909	100 %	99.80 %
			Triticum aestivum L.			
Zhiva	5	PSF/PSR	*Pseudomonas trivialis*	902	96.0 %	96.26 %
Alexeyevich	6	PSF/PSR	*Pseudomonas* sp.	959	100 %	98.53 %
			Pseudomonas graminis	948	100 %	98.17 %
Alexeyevich	7	PSF/PSR	*Pseudomonas poae*	1075	99.0 %	99.49 %
Urup	8	8UA/519B	*Erwinia papayae*, *Erwinia* sp., *Erwinia billingiae*	551	99.0 %	98.71 %
Morozko	9	PSF/PSR	*Pseudomonas viridiflava*	712	99 %	88.17 %
Morozko	10	PSF/PSR	*Pseudomonas syringae*, *Pseudomonas syringae* pv. *aptata*	575	100 %	99.68 %
Morozko	11	PSF/PSR	*Pseudomonas syringae* pv. *atrofaciens*, *Pseudomonas syringae*	1596	100 %	97.18 %
Morozko	12	PSF/PSR	*Pseudomonas syringae* pv. *atrofaciens*, *Pseudomonas syringae*	1596	100 %	97.18 %

Note. 1 — variety from which the isolate was isolated, 2 — isolate number, 3 — pair of primers (for more details, see the Materials and methods section), 4 — microorganism with maximum similarity, 5 — maximum score, 6 — query coverage (query coverage), 7 — percent identity. The table is presented in full on the http://www,agrobiology.ru.

Purification, sequencing, and processing using the BioEdit program allowed us to obtain nucleotide sequences for each of isolates and align these sequences in the BLAST service (https://blast.ncbi.nlm.nih.gov). Examples of the results obtained are shown in Table 2 (see in full on the website http://www.agrobiology.ru).

Some species of identified bacteria were found only in one sample of grain crops (Table 3). The Gram-positive bacterium *Rathayibacter festucae* isolated from a sample of triticale cv. Timiryazevskaya 150, was originally identified in 2002 from a leaf gall caused by the nematode *Anguina graminis* on red fescue [26]. The genus *Rathayibacter* includes 6 species, among which *Rathayibacter tritici* and *Rathayibacter rathayi* are pathogenic for grain crops and are regulated by phytosanitary requirements of a number of countries (https://fsvps.gov.ru/ru, https://gd.eppo.int/).

The species *Pseudoclavibacter helvolus* identified in the winter rye sample (see Table 3) is a gram-positive bacterium that does not have phytopathogenic properties [27]. The gram-negative bacterium Paucimonas lemoignei, isolated from turgid wheat variety Donskoy Yantar (see Table 3), is not characterized as a phytopathogen [28].

3. Identification of bacteria in samples of grain crops (except for *Triticum aestivum* L.) (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020)

Crop	Variety	Identified as
Secale cereale L.	Snezhana	*Pseudomonas* sp., *Rhodococcus* sp.
	Verasen	*Pseudomonas trivialis*, *Micrococcus* sp., *Staphylococcus* sp., *Pseudomonas* sp., *Arthrobacter* sp.
	Unnamed	Actinomycetales bacterium, *Pseudomonas* sp., *Pseudoclavibacter helvolus*
× *Triticosecale* Wittm. & A.Camus	Alexander	*Pseudomonas* sp.
	Victor	*Frigoribacterium* sp., *Pseudomonas* sp.
	Nemchinovsky 56	*Dyadobacter* sp., *Pseudomonas* sp.
	Valentin 90	*Clavibacter michiganensis*, *Frigoribacterium faeni*
	Timiryazevskaya 150	*Pseudomonas* sp., *Frigoribacterium* sp., *Rathayibacter festucae*
Triticum turgidum L.	Donskoy amber	Uncultured bacterium, *Frigoribacterium faeni*, *Paucimonas lemoignei*, Uncultured Enterobacteriaceae bacterium, Uncultured soil bacterium, *Pantoea ananatis*, *Frigoribacterium* sp., *Salinibacterium* sp., *Pseudomonas* sp.
	Terra	*Arthrobacter* sp., *Rhodococcus* sp., *Clavibacter michiganensis*, *Pseudomonas syringae*, *Frigoribacterium* sp.
Triticum durum Desf.	Pobeda 70	*Pseudomonas viridiflava*, *Pseudomonas syringae*
Triticum dicoccum Schrank	Untitled	*Pseudomonas* sp.
Triticum sphaerococcum Percival	Eremeevna	*Frigoribacterium* sp., *Sanguibacter* sp., *Pseudomonas* sp.
× *Triticosecale* (Wittm. & A. Camus) *sphaerococcum*	Titus	*Pseudomonas* sp., Bacterium, Uncultured bacterium

N o t e. The sample of winter crops consisted of 5-15 plant stems cut at the first internode; the sample of spring crops consisted of 15 seedlings. One sample was taken from one variety.

The species *Pantoea ananatis* identified in a sample of turgid wheat variety Donskoy Yantar (see Table 3) is a gram-negative bacterium that is the causative agent of various plant bacterioses [29] and, according to the Federal Service for Veterinary and Phytosanitary Surveillance (https://fsvps.gov .ru/ru), is regulated by the Colombian quarantine list. *Pantoea ananatis* was reported to promote active metabolism in plants [30].

In single samples of 41 winter soft wheat specimens (Table 4), we found both phytopathogenic and economically useful bacteria.

The gram-positive bacterium *Arthrobacter chlorophenolicus* isolated from wheat variety Sineva (see Table 4) is economically useful and increases the drought resistance of plants [31]. The gram-negative soil bacterium *Pseudomonas chlororaphis* from wheat variety Asket (see Table 4) is used as a bioagent against plant diseases [32].

Bacteria were also isolated the presence of which was noted in several samples of cereal crops (see Tables 3, 4). Species of gram-positive bacteria *Clavibacter michiganensis* and *Rhodococcus fascians* and gram-negative *Pseudomonas trivialis*, *Pseudomonas viridiflava* and *Pseudomonas syringae* (including the pathovar *Pseudomonas syringae* pv. *syringae*) have been identified, for which phytopathogenic properties have been described [4, 33-37]. In addition, the gram-positive bacteria *Frigoribacterium faeni*, usually isolated from soil, plant phyllosphere and other sources, for which economically valuable or pathogenic properties have not been noted, have been isolated and identified [38]. Gram-negative bacteria *Pseudomonas graminis* and *Pseudomonas poae* have also been isolated (see Tables 3, 4) which are usually found in soil or plants and are used to control plant diseases [39, 40].

4. Identification of bacteria in samples of winter soft wheat *Triticum aestivum* L. (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020)

Variety	Identified as
Alive	*Pseudomonas trivialis*
Alexeyevich	*Pseudomonas* sp., *Pseudomonas poae*
Urup	*Erwinia* sp.
Morozko	*Pseudomonas viridiflava*, *Pseudomonas syringae*
Timiryazevskaya Yubileinaya	*Pseudomonas* sp., *Frigoribacterium* sp., Uncultured bacterium, *Clavibacter* sp., *Kineococcus* sp.
Moskovskaya 56	*Pseudomonas* sp.
Turquoise	*Pseudomonas* sp., *Pantoea* sp., Uncultured bacterium
Timiryazevka 150	*Pseudomonas* sp., *Pseudomonas poae*, Uncultured bacterium
Graph	*Curtobacterium* sp., *Arthrobacter* sp., *Streptomyces* sp.
Vassa	*Frigoribacterium* sp.
Moskovskaya 39	*Frigoribacterium* sp., *Clavibacter* sp., *Pseudomonas trivialis*, *Pseudomonas* sp.
Doublet	*Frigoribacterium* sp., Bacterium, *Curtobacterium* sp.
Cavalier	*Pseudomonas* sp., Uncultured bacterium, Bacterium, *Pseudomonas graminis*
Scarlet dawn	*Oerskovia* sp., *Cellulomonas* sp., *Frigoribacterium* sp., *Microbacterium* sp., *Pseudomonas* sp., Bacterium
Nemchinovskaya 24	Microbacteriaceae bacterium, *Frondihabitans* sp., *Curtobacterium* sp., *Pseudomonas* sp.
Legend	*Pseudomonas graminis*, *Pseudomonas poae*
Avesta	*Pseudomonas poae*, *Clavibacter* sp., *Frigoribacterium* sp., *Sphingomonas* sp.
Inna	*Clavibacter michiganensis*, *Pseudomonas syringae,* Uncultured bacterium, *Microbacterium* sp.
Mill	*Rhodococcus fascians*, *Arthrobacter* sp., *Phycicoccus* sp., *Pseudomonas syringae* pv. *syringae*, *Frigoribacterium* sp.
Ascetic	*Frigoribacterium* sp., *Bacillus* sp., *Pseudomonas viridiflava*, *Pseudomonas chlororaphis*, *Pseudomonas* sp.
Velena	*Rhodococcus* sp., *Pseudomonas viridiflava*
Vania	*Plantibacter* sp.
Artel	Uncultured bacterium, Unidentified microorganism, *Frigoribacterium* sp., *Curtobacterium* sp., Rhizosphere soil bacterium, *Sphingomonas* sp.
Nemchinovskaya 85	*Clavibacter* sp., *Frigoribacterium* sp.
Videya	Bacterium, *Pseudomonas* sp.
Don lyre	*Plantibacter* sp., *Pseudomonas* sp., *Pseudomonas syringae* pv. *syringae*
Sineva	*Frigoribacterium* sp., *Arthrobacter chlorophenolicus*, *Pseudomonas* sp.
Moskovskaya 40	*Clavibacter* sp., *Arthrobacter* sp.
Don 107	*Frigoribacterium* sp., *Pseudomonas* sp.
Steppe	*Pseudomonas* sp.
Governor of Don	*Frigoribacterium* sp.
Rostovite	Bacterium, *Pseudomonas* sp.
Milestone	*Erwinia* sp.
Nemchinovskaya 57	*Rhodococcus fascians*, Uncultured bacterium
Augusta	*Bacillus* sp., Uncultured bacterium
Soberbash	*Clavibacter* sp., *Pseudomonas* sp., Bacterium
Anka	*Pseudomonas trivialis*, Bacterium, *Pseudomonas* sp.
Gurt	*Pseudomonas* sp., *Agreria* sp., *Frondihabitans* sp., *Sphingomonas* sp.
Antonina	*Pseudomonas syringae*
Nemchinovskaya 17	*Rhodococcus* sp.
Bezostaya 100	*Athrobacter* sp., *Micrococcus* sp., *Frigoribacterium* sp.

N o t e. The sample of winter crops consisted of 5-15 plant stems cut at the first internode; the sample of spring crops consisted of 15 seedlings. One sample was taken from one variety.

These data revealed that the frequency of occurrence of bacteria of the genera *Pseudomonas*, *Frigoribacterium*, *Clavibacter*, *Arthrobacter* and *Rhodococcus* on grain crops of the Timiryazev field experimental station was more than 10% (Table 5). The diversity of pseudomonads, the most common bacteria in the studied samples with 70.9% frequency of occurrence, is represented by six species (see Table 5). Of these species the *Pseudomonas chlororaphis*, *Pseudomonas graminis* and *Pseudomonas poae*, according to the sprcial literature, have properties beneficial to plants, and three species, the *Pseudomonas syringae*, *Pseudomonas trivialis* and *Pseudomonas viridiflava* are plant pathogens.

Bacteria of the genus *Frigoribacterium*, the second most abundant group (see Table 5), are common members of the plant microbiota, promoting plant growth and adaptation [41]. Common representatives of soil and plant microbiota also include the bacteria *Arthrobacter* sp., which we found in samples of grain

crops with an occurrence frequency of 12.7%.

5. Frequency of occurrence of bacterial species and genera in grain crop samples (Timiryazevskaya field experimental station, Russian State Agrarian University — Timiryazev Moscow Agricultural Academy, Moscow, 2020)

Genus	Frequency, %	Species	Frequency, %
Agreria sp.	1.8	–	
Arthrobacter sp.	12.7	*Arthrobacter chlorophenolicus*	1.8
Bacillus sp.	5.5	–	
Cellulomonas sp.	1.8	–	
Clavibacter sp.	16.4	*Clavibacter michiganensis*	5.5
Curtobacterium sp.	7.3	–	
Dyadobacter sp.	1.8	–	
Erwinia sp.	3.6	–	
Frigoribacterium sp.	36.4	*Frigoribacterium faeni*	3.6
Frondihabitans sp.	3.6	–	
Kineococcus sp.	1.8	–	
Microbacterium sp.	3.6	–	
Micrococcus sp.	3.6	–	
Oerskovia sp.	1.8	–	
Pantoea sp.	3.6	*Pantoea ananatis*	1.8
Paucimonas sp.	1.8	*Paucimonas lemoignei*	1.8
Phycicoccus sp.	1.8	–	
Plantibacter sp.	3.6	–	
Pseudoclavibacter sp.	1.8	*Pseudoclavibacter helvolus*	1.8
Pseudomonas sp.	70.9	*Pseudomonas chlororaphis*	1.8
		Pseudomonas graminis	3.6
		Pseudomonas poae	7.3
		Pseudomonas syringae	12.7
		Pseudomonas trivialis	7.3
		Pseudomonas viridiflava	7.3
Rathayibacter sp.	1.8	*Rathayibacter festucae*	1.8
Rhodococcus sp.	10.9	*Rhodococcus fascians*	3.6
Salinibacterium sp.	1.8	–	
Sanguibacter sp.	1.8	–	
Sphingomonas sp.	5.5	–	
Staphylococcus sp.	1.8	–	
Streptomyces sp.	1.8	–	

N o t e. Dashes mean that species within the genus have not been identified.

We found a prevalence of the pathogenic species *Clavibacter michiganensis* at a frequency of 5.5%. It is most likely that the detected bacteria belong to the subspecies *Clavibacter michiganensis* subsp. *tessellarius* (*Clavibacter tessellarius* sp. nov.) which is the causative agent of bacterial mosaic in wheat, since *Clavibacter michiganensis* subspecies are highly specific to the host plant, and it is the tessellarius subspecies that infects wheat [42].

Among the bacteria *Rhodococcus* sp., the frequency of which was 10.9%, only *Rhodococcus fascians* are reported to cause plant diseases [34].

The frequency of occurrence of other identified bacterial genera and species was less than 10%.

Thus, we studied the microbiota of local grain crops (Timiryazevskaya field experimental station, 2020) and revealed field isolates that can enter a unified collection of grain bacteria in order to create species-specific primers that will be a key part of the developed regulatory documents on the detection and identification of quarantine and export-significant pathogens of bacteriosis of grain crops. The specified regulatory documents which are of very great demand will be used by phytosanitary laboratories for phytosanitary control. Gien the regional characteristics of soil-climatic and agrotechnical conditions and the biodiversity of isolates [43-45], we believe that standard strains from foreign collections of microorganisms (if available) are less suitable for these purposes. Let us note that previously no large-scale study of the bacterial composition in grain crops has been carried out in Russia, and therefore there is no information about the species composition of bacteria that can be found together in one sample. There is also no complete list of bacteria that can be found in grain crops in

Russia.

We conducted a study of the composition of the microbiota on crops of economically important crops using classical microbiology methods to isolate bacteria from samples and molecular genetic methods to identify isolated isolates. The data obtained can add to the knowledge of bacteria living in plants and will be useful for developing a general understanding of the microbiome of target crops in the field.

Bacteria were isolated from seedlings of spring crops (rye) and from green plants of winter crops in the stage of emerging into the tube, which could affect the number of some types of bacteria in plants compared to others and, indirectly, the composition of the resulting bacterial associations [4, 5, 13]. Strains were isolated on Petri dishes based on the diversity of morphotypes, which is quite subjective and in any case does not allow detection of uncultivable microorganisms [45]. However, this does not contradict the tasks that we set for ourselves when carrying out the work. i.e., to isolate cultures for a collection of bacterial phytopathogens and their accompanying microbiota that are phytosanitary important for grain export. We do not extend the obtained data on the composition and frequency of occurrence of bacteria to other grain crops, even in the same agroclimatic zone.

It is important to note that we identified pathogenic, neutral and beneficial species in the microbiota of grain plants. More knowledge about pathogens can improve the phytosanitary assessment of cereal crops, while candidate bacteria can be found among beneficial species to develop new drugs for the biological control of phytopathogens. In addition, we identified bacteria that belong to certain genera, but do not belong to any of the known species, which makes them promising for further study and the possibility of describing new species of the grain crop microbiota.

So, at testing sites and hybridization plots of the Timiryazevskaya field experimental station, we collected 55 samples of grain crops of which 171 bacterial isolates were purified and 37 isolates were identified to species using molecular genetic methods. The identified bacterial diversity is represented by 14 species. Among them, phytopathogens include *Pantoea ananatis*, Cl*a*vibacter *michiganensis*, *Rhodococcus fascians*, *Pseudomonas trivialis*, *Pseudomonas viridiflava* and *Pseudomonas syringae*. *Pantoea ananatis* is listed and watched by the North American Plant Protection Organization. *Rhodococcus fascians* is regulated as a quarantine organism in Argentina, Brazil, Chile, Mexico, etc. *Pseudomonas viridiflava* is a quarantine organism for Mexico and is regulated as a non-quarantine organism in Switzerland and the UK. *Pseudomonas syringae* (and, in particular, *Pseudomonas syringae* pv. *syringae* which we discovered on grain crops), is a quarantine organism for such importers of Russian grain as Taiwan, Mexico, Colombia and Jordan, and is also regulated by the phytosanitary requirements of Egypt and Zimbabwe and Great Britain in grain products as a non-quarantine species (https://fsvps.gov.ru/ru, https://gd.eppo.int/). Bacteria with economically useful properties were also isolated and identified, these are *Arthrobacter chlorophenolicus*, *Pseudomonas chlororaphi*s, *Pseudomonas graminis* and *Pseudomonas poae*. Other identified species, the *Rathayibacter festucae*, *Pseudoclavibacter helvolus*, *Paucimonas lemoignei* and *Frigoribacterium faeni*, according to published data, do not have pronounced harmful or beneficial properties. The highest frequency of occurrence (70.9%) was characteristic of the genus *Pseudomonas* species. Representatives of the genera *Frigoribacterium* (36.4%), *Clavibacter* (16.4%), *Arthrobacter* (12.7%) and *Rhodococcus* (10.9%) also have a high frequency of occurrence. The experimental data on the bacteria species composition we obtained in grain crops can be used to identify the spread

of bacterioses on the territory of the Russian Federation and to bioinformaticsally analyze bacterial genomes in the search for species-specific genetic markers of quarantine objects.

REFERENCES

1. Singh J., Chhabra B., Raza A., Yang S.H., Sandhu K.S. Important wheat diseases in the US and their management in the 21st century. *Frontiers in Plant Science*, 2023, 13: 1010191 (doi: 10.3389/fpls.2022.1010191).
2. Krawczyk K., Wielkopolan B., Obr palska-St plowska A. *Pantoea ananatis*, a new bacterial pathogen affecting wheat plants (*Triticum* L.) in Poland. *Pathogens*, 2020, 9(12): 1079 (doi: 10.3390/pathogens9121079).
3. Sapkota S., Mergoum M., Liu Z. The translucens group of *Xanthomonas translucens*: complicated and important pathogens causing bacterial leaf streak on cereals. *Molecular Plant Pathology*, 2020, 21(3): 291-302 (doi: 10.1111/mpp.12909).
4. Tambong J. Bacterial pathogens of wheat: symptoms, distribution, identification, and taxonomy. In: *Wheat*. IntechOpen, 2022 (doi: 10.5772/intechopen.102855).
5. Butsenko L., Pasichnyk L., Kolomiiets Y., Kalinichenko A., Suszanowicz D., Sporek M., Patyka V. Characteristic of *Pseudomonas syringae* pv. *atrofaciens* isolated from weeds of wheat field. *Applied Sciences*, 2021, 11(1): 286 (doi: 10.3390/app11010286).
6. Sundin G.W., Castiblanco L.F., Yuan X., Zeng Q., Yang C.H. Bacterial disease management: challenges, experience, innovation and future prospects: challenges in bacterial molecular plant pathology. *Molecular Plant Pathology*, 2016, 17(9): 1506-1518 (doi: 10.1111/mpp.12436).
7. Wichmann F., Vorhölter F., Hersemann L., Widmer F., Blom J., Niehaus K., Reinhard S., Conradin C., Kölliker R. The noncanonical type III secretion system of *Xanthomonas translucens* pv. *graminis* is essential for forage grass infection. *Molecular Plant Pathology*, 2013, 14(6): 576-588 (doi: 10.1111/mpp.12030).
8. Xie G.L., Zhang G.Q., Liu H., Lou M.M., Tian W.X., Li B., Zhou X.P., Zhu B., Jin G.L. Genome sequence of the rice-pathogenic bacterium *Acidovorax avenae* subsp. *avenae* RS-1. *Journal of Bacteriology*, 2011, 193(18): 5013-5014. (doi: 10.1128/JB.05594-11).
9. Nandi M., Macdonald J., Liu P., Weselowski B., Yuan Z.C. *Clavibacter michiganensis* ssp. *michiganensis*: bacterial canker of tomato, molecular interactions and disease management. *Molecular Plant Pathology*, 2018, 19(8): 2036-2050 (doi: 10.1111/mpp.12678).
10. Coutinho T.A., Wingfield M.J. *Ralstonia solanacearum* and *R. pseudosolanacearum* on *Eucalyptus*: opportunists or primary pathogens? *Frontiers in Plant Science*, 2017, 8: 761 (doi: 10.3389/fpls.2017.00761).
11. McMullen M.P., Stack R.W., Miller J.D., Bromel M.C., Youngs V.L. *Erwinia rhapontici*, a bacterium causing pink wheat kernels. In: *Proceedings of the North Dakota Academy of Sciences*. Grand Forks, ND, 1984, 38: 78.
12. Wise K.A., Zhao Y.F., Bradley C.A. First report of pink seed of pea caused by *Erwinia rhapontici* in North Dakota. *Plant Disease*, 2008, 92(2): 315 (doi: 10.1094/PDIS-92-2-0315A).
13. Asaad S., Sands D.C., Mohan S.K. Chapter 4: Detection of *Pseudomonas syringae* pv. *syringae* in wheat seeds. In: *Detection of plant-pathogenic bacteria in seed and other planting material, Second Edition*. AIP Publications, 2017: 21-26 (doi: 10.1094/9780890545416.004).
14. Boykin L.M., Sseruwagi P., Alicai T., Ateka E., Mohammed I.U., Stanton J.L., Kayuki C., Mark D., Fute T., Erasto J., Bachwenkizi H., Muga B., Mumo N., Mwangi J., Abidrabo P., Okao-Okuja G., Omuut G., Akol J., Apio H.B., Osingada F., Kehoe M.A., Eccles D., Savill A., Lamb S., Kinene T., Rawle C.B., Muralidhar A., Mayall K., Tairo F., Ndunguru J. Tree lab: portable genomics for early detection of plant viruses and pests in Sub-Saharan Africa. *Genes (Basel)*, 2019, 10(9): 632 (doi: 10.3390/genes10090632).
15. Gorshkov V.Yu. *Bakteriozy rasteniy: molekulyarnye osnovy formirovaniya rastitel'no-mikrobnykh patosistem* [Plant bacterioses: molecular basis for the formation of plant-microbial pathosystems]. Kazan', 2017 (in Russ.).
16. Nair S., Manimekalai R. Phytoplasma diseases of plants: molecular diagnostics and way forward. *World Journal of Microbiology and Biotechnology*, 2021, 37(6): 102 (doi: 10.1007/s11274-021-03061-y).
17. Ignat'eva I.M., Slovareva O.Yu. *Tezisy dokladov 22-y Vserossiyskoy konferentsii molodykh uchenykh «Biotekhnologiya v rastenievodstve, zhivotnovodstve i sel'skokhozyaystvennoy mikrobiologii» (Moskva, 07-09 noyabrya 2022 goda)* [Abstracts of the 22nd All-Russian Conference of young scientists "Biotechnology in crop production, animal husbandry and agricultural microbiology" (Moscow, November 07-09, 2022)]. Moscow, 2022: 140 (in Russ.).
18. Ignatyeva I.M., Karimova E.V., Prikhodko S.I. Diagnostics of the bacterial blight pathogen of bean *Xanthomonas axonopodis* pv. *phaseoli* in plant and seed material of grain legumes using molecular genetics methods. *AIP Conference Proceedings*, 2021, 2388: 030014 (doi: 10.1063/5.0068504).

19. Islam M.R., Hossain M.R., Kim H.T., Jesse D.M.I., Abuyusuf M., Jong H.J., Park J.I., Nou I.S. Development of molecular markers for detection of *Acidovorax citrulli* strains causing bacterial fruit blotch disease in melon. *International Journal of Molecular Sciences*, 2019, 20(11): 2715 (doi: 10.3390/ijms20112715).
20. Korneykova M.V., Soshina A.S., Gavrichkova O.V. *Mikologiya i fitopatologiya*, 2021, 55(4): 256-270 (doi: 10.31857/S0026364821040085) (in Russ.).
21. Slovareva O.Y. Detection and identification of pathogens of bacterial diseases of wheat and barley in the Russian Federation. *Microbiology Independent Research Journal (MIR Journal)*, 2020, 7(1): 13-23 (doi: 10.18527/2500-2236-2020-7-1-13-23).
22. Kazempour M.N, Kheyrgoo M., Pedramfar H., Rahimian H. Isolation and identification of bacterial glum blotch and leaf blight on wheat (*Triticum aestivum* L.) in Iran. *African Journal of Biotechnology*, 2010, 9(20): 2860-2865.
23. Belkin D.L., Bondarenko G.N., Yaremko A.B., Uvarova D.A. *Karantin rasteniy. Nauka i praktika*, 2019, 28(2): 31-34 (in Russ.).
24. Honda-Takinami R., Hata J., Matsuoka K., Hoshi S., Koguchi T., Sato Y., Akaihata H., Kataoka M., Ogawa S., Nishiyama K., Suzutani T., Kojima Y. Association between the presence of bacteria in prostate tissue and histopathology in biopsies from men not complaining of lower urinary tract symptoms. *Fukushima Journal of Medical Science*, 2022, 68(3): 161-167 (doi: 10.5387/fms.2022-34).
25. Minaeva L.P., Samokhvalova L.V., Zavriev S.K., Stakheev A.A. First detection of fungus *Fusarium coffeatum* in the territory of the Russian Federation. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2022, 57(1): 131-140 (doi: 10.15389/agrobiology.2022.1.131eng).
26. Dorofeeva L.V., Evtushenko L.I., Krausova V.I., Karpov A.V., Subbotin S.A., Tiedje J.M. *Rathayibacter caricis* sp. nov. and *Rathayibacter festucae* sp. nov., isolated from the phyllosphere of *Carex* sp. and the leaf gall induced by the nematode *Anguina graminis* on *Festuca rubra* L., respectively. *International Journal of Systematic and Evolutionary Microbiology*, 2002, 52(6): 1917-1923 (doi: 10.1099/00207713-52-6-1917).
27. Raghupathi P.K., Herschend J., Røder H.L., Sørensen S.J., Burmшlle M. Draft genome assembly of two *Pseudoclavibacter helvolus* strains, G8 and W3, isolated from slaughterhouse environments. *Genome Announcements*, 2016, 4(2): e00077-16 (doi: 10.1128/genomeA.00077-16).
28. Jendrossek D. Transfer of [*Pseudomonas*] *lemoignei*, a gram-negative rod with restricted catabolic capacity, to *Paucimonas* gen. nov. with one species, *Paucimonas lemoignei* comb. nov. *International Journal of Systematic and Evolutionary Microbiology*, 2001, 51(3): 905-908 (doi: 10.1099/00207713-51-3-905).
29. Weller-Stuart T., De Maayer P., Coutinho T. *Pantoea ananatis*: genomic insights into a versatile pathogen. *Molecular Plant Pathology*, 2017, 18(9): 1191-1198 (doi: 10.1111/mpp.12517).
30. Lu L., Chang M., Han X., Wang Q., Wang J., Yang H., Guan Q., Dai S. Beneficial effects of endophytic *Pantoea ananatis* with ability to promote rice growth under saline stress. *Journal of Applied Microbiology*, 2021, 131(4): 1919-1931 (doi: 10.1111/jam.15082).
31. Miranda-Ríos J.A., Ramírez-Trujillo J.A., Nova-Franco B., Lozano-Aguirre Beltrán L.F., Iturriaga G., Suárez-Rodríguez R. Draft genome sequence of *Arthrobacter chlorophenolicus* strain Mor30.16, isolated from the bean rhizosphere. *Genome Announcements*, 2015, 3(3): e00360-15 (doi: 10.1128/genomeA.00360-15).
32. Liu F., Yang S., Xu F., Zhang Z., Lu Y., Zhang J., Wang G. Characteristics of biological control and mechanisms of *Pseudomonas chlororaphis* zm-1 against peanut stem rot. *BMC Microbiology*, 2022, 22(1): 9 (doi: 10.1186/s12866-021-02420-x).
33. Hwang I.S., Oh E.J., Song E., Park I.W., Lee Y., Sohn K.H., Choi D., Oh C.S. An apoplastic effector Pat-1Cm of the gram-positive bacterium *Clavibacter michiganensis* acts as both a pathogenicity factor and an immunity elicitor in plants. *Frontiers in Plant Science*, 2022, 13: 888290 (doi: 10.3389/fpls.2022.888290).
34. Park J.M., Koo J., Kang S.W., Jo S.H., Park J.M. Detection of *Rhodococcus fascians*, the causative agent of lily fasciation in South Korea. *Pathogens*, 2021, 10(2): 241 (doi: 10.3390/pathogens10020241).
35. Sawada H., Fujikawa T., Tsuji M., Satou M. *Pseudomonas allii* sp. nov., a pathogen causing soft rot of onion in Japan. *International Journal of Systematic and Evolutionary Microbiology*, 2021, 71(1). (doi: 10.1099/ijsem.0.004582).
36. Lipps S.M., Samac D.A. *Pseudomonas viridiflava*: An internal outsider of the *Pseudomonas syringae* species complex. *Molecular Plant Pathology*, 2022, 23(1): 3-15 (doi: 10.1111/mpp.13133).
37. Xin X.F., Kvitko B., He S.Y. *Pseudomonas syringae*: what it takes to be a pathogen. *Nature Reviews Microbiology*, 2018, 16(5): 316-328. (10.1038/nrmicro.2018.17).
38. Zhuo Y., Jin C.Z., Jin F.J., Li T., Kang D.H., Oh H.M., Lee H.G., Jin L. *Lacisediminihabitans profunda* gen. nov., sp. nov., a member of the family Microbacteriaceae isolated from freshwater sediment. *Antonie Van Leeuwenhoek*, 2020, 113(3): 365-375 (doi: 10.1007/s10482-019-01347-8).
39. Crovadore J., Calmin G., Chablais R., Cochard B., Schulz T., Lefort F. Whole-genome sequence of *Pseudomonas graminis* strain UASWS1507, a potential biological control agent and biofertilizer isolated in Switzerland. *Genome Announcement*, 2016, 4(5): e01096-16 (doi: 10.1128/genomeA.01096-16).

40. Sun H., Kong L., Du H., Chai Z., Gao J., Cao Q. Benefits of *Pseudomonas poae* s61 on *Astragalus mongholicus* growth and bioactive compound accumulation under drought stress. *Journal of Plant Interactions*, 2019, 14(1): 205-212 (doi: 10.1080/17429145.2019.1611958).
41. Shen S.Y., Fulthorpe R. Seasonal variation of bacterial endophytes in urban trees. *Frontiers in Microbiology*, 2015, 6: 427 (doi: 10.3389/fmicb.2015.00427).
42. Li X., Tambong J., Yuan K.X., Chen W., Xu H., Lйvesque C.A., De Boer S.H. Re-classification of *Clavibacter michiganensis* subspecies on the basis of whole-genome and multi-locus sequence analyses. *International Journal of Systematic and Evolutionary Microbiology*, 2018, 68(1): 234-240 (doi: 10.1099/ijsem.0.002492).
43. Sennikov V.A., Larin L.G., Rossinskaya T.M., Belolyubtsev A.I., Korovina L.N. *Izvestiya Timiryazevskoy sel'skokhozyaystvennoy akademii*, 2005, 1: 141-146 (in Russ.).
44. Belolyubtsev A.I., Asaulyak I.F. *Izvestiya Timiryazevskoy sel'skokhozyaystvennoy akademii*, 2013, 4: 66-84 (in Russ.).
45. Dumova V.A., Pershina E.V., Merzlyakova Ya.V., Kruglov Yu.V., Andronov E.E. The main trends in dynamics of soil microbiom during a long-term field experiment as indicated by high throughput sequencing the 16S-rRNA gene libraries. *Sel'skokhozyaistvennaya biologiya* [*Agricultural Biology*], 2013, 5: 85-92 (10.15389/agrobiology.2013.5.85rus) (in Russ.).