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ACYL-HOMOSERINE LACTONES FOR CROP PRODUCTION AND STRESS TOLERANCE OF AGRICULTURAL PLANTS (review)

L.M. BABENKO¹✉, K.O. ROMANENKO¹, O.S. IUNGIN^{2, 3}, I.V. KOSAKIVSKA¹

¹*M.G. Kholodny Institute of Botany of the National Academy of Sciences of Ukraine, 2, Tereshchenkivska Str., Kyiv, 01004 Ukraine, e-mail lilia.babenko@gmail.com (✉ corresponding author), katernaromanenko4@gmail.com, irynakosakivska@gmail.com;*

²*Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine, 150, Akademika Zabolotnogo Str., Kyiv, 03143 Ukraine, e-mail olgaungin@gmail.com;*

³*Kyiv National University of Technologies and Design (KNUTD), 2, Nemyrovycha-Danchenka Str., Kyiv, 01011 Ukraine*

ORCID:

Babenko L.M. orcid.org/0000-0001-5391-9203

Iungin O.S. orcid.org/0000-0001-8876-6075

Romanenko K.O. orcid.org/0000-0003-0456-4412

Kosakivska I.V. orcid.org/0000-0002-2173-8341

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Abstract

Acyl homoserine lactones (AHL) are a class of mediator molecules coordinating cell activity in the gram-negative bacteria population. AHLs synchronize individual genomes due to which bacterial populations function as multicellular organisms. AHLs provide remote signaling between bacteria colonizing the phytosphere that enables the bacterial population to respond to external influences and establish symbiotic or antagonistic relationships with the host plant (A.R. Stacy et al., 2018; A. Shrestha et al., 2020). Autoreception of quantitative parameters of the bacterial population is called "quorum sensing" (QS) (R.G. Abisado et al., 2018). QS systems form autoinducer signaling molecules that easily penetrate from cells into the environment and back into the cell (M.B. Miller et al., 2001; B. Bassler, 2002). QS systems play a key role in the regulation of metabolic and physiological processes in a bacterial cell (M. Frederix et al., 2011; M. Whiteley et al., 2017). Bacterial signaling is perceived by eukaryotes, which form a symbiosis with microbial communities (A. Schenk et al., 2015; L.M. Babenko et al., 2016, 2017). Plant growth and development, nutrients assimilation, and stress resistance are largely determined by the pattern of this interaction (H.P. Bais et al., 2006; R. Orthz-Castro et al., 2009; S. Basu et al., 2017). Within the plant, bacterial signaling is controlled by the quorum quenching (QQ) system (N. Calatrava-Morales et al., 2018), whose mechanism of action is to suppress AHL synthesis by plant metabolites, compete with AHL for binding to receptor proteins, and repression of QS-controlled genes (H. Zhu et al., 2008; R. Sarkar et al., 2015). However, to date, the molecular mechanisms by which plants respond to bacterial signaling are not fully understood. Individual metabolites of AHL signaling have been characterized, but their role in the chemical interaction of partners in most cases requires further study. It has been shown that the QS phenomenon and its participants are involved in the regulation of prokaryotic-eukaryotic interactions, including biofilm formation, phytohormones synthesis, plasmids transfer, virulence factors production, bioluminescence, sporulation, and the formation of nodules (L.M. Babenko et al., 2017). Differences in the structure of molecules ensure that bacteria recognize their own AHL and separate foreign ones. The transfer of AHL from bacteria to a host plant is carried out through membrane vesicles (M. Toyofuku, 2019). In recent years, there has been an active study of genetics, genomics, biochemistry, and signaling diversity of QS molecules. Rhizosphere is the most dynamic site of interaction between the plant and the associated microflora with the participation of AHL. The regulation of rhizosphere functions is the task of particular importance in the development of new biotechnological approaches aimed at increasing the yield and stress resistance of crops. One of the effective technologies for increasing resistance to biotic and abiotic stresses is pre-sowing treatment (priming) of seeds (A. Shrestha et al., 2020). Both direct

(on plants) and indirect (on rhizosphere microflora) effects of AHL priming were established (O.V. Moshynets et al., 2019). AHL induce an increase of growth, of photosynthetic pigments content, as well as cause changes in the ratio of phytohormones in organs and tissues, affect the formation of defense mechanisms, which increases the productivity of crops (A. Schikora, S.T. Schenk, 2016; A. Shrestha et al., 2020). AHL meets the requirements of intensive organic farming, they are considered as promising ecological phytostimulants and phytomodulators capable of safely increasing the quantity and quality of agricultural products.

Keywords: acyl-homoserine lactones (AHL), quorum sensing (QS), quorum quenching (QQ), plant-microbial signaling, AHL-priming, AHL-mimicry, phytostimulants, phytomodulators, stress resistance

In recent decades, the study of the mechanisms of plant resistance to unfavorable environmental factors has become one of the most urgent problems of plant molecular physiology in connection with global climatic changes and anthropogenic load on the biosphere. Extreme temperatures are one of the most common abiotic stressors. Global warming threatens not only agriculture but also biodiversity conservation. The predicted increase in average temperature by 1 °C can lead to a reduction in plant species diversity by one-third [1]. At the same time, the cold and frost resistance of plants should not be ignored. Winter thaws, alternating with frosts, cause serious damage to plants and reduce yields. The earlier onset of a meteorological spring increases the likelihood of damage from spring frosts. Another unfavorable climatic factor, the effect of which is increasing, is a lack of moisture and redistribution of the annual precipitation [2]. An increase in temperature and changes in the precipitation pattern lead to a hydrological regime violation and a decrease in water resources. So, within the Ukraine territory in the last quarter of the twentieth century, there was a steady trend towards a decrease in the annual precipitation [3]. At the same time, the frequency of rainfalls increased, which also negatively affects plant productivity. Acid rain and heavy metal pollution are serious threats. The ecological balance is disturbed due to the irrational use of chemical crop protection products. About 2 million tons of pesticides are used annually in world agricultural production. In 2020, an increase in the global use of pesticides was predicted to reach 3.5 million tons [4], but this did not solve the problem of effectively increasing crop yields.

To meet the growing demand for food, safe agrobiotechnologies are needed, which will increase the agricultural product quantity and quality [5-8]. The use of bacterial inoculates that improve plant growth, as well as bacterial growth regulators for presowing seed priming and foliar treatment of plants is considered a promising biotechnological approach [5, 9, 10]. This “green” technology is becoming more and more popular. In some cases, natural phytostimulants can improve the stress tolerance of crops and increase yields without undesirable effects on the environment [11].

In recent years, attention has been focused on a phenomenon called “quorum sensing” (QS). This is a system of bacterial intercellular communication which depends on the cell population density and coordinates the response to changes in environmental conditions [12, 13]. QS systems form autoinducer signaling molecules that easily diffuse from cells into the environment and back [14, 15]. QS regulation has been established for more than 500 species of bacteria. QS systems play a key role in the regulation of metabolic and physiological processes in a bacterial cell [16, 17]. Bacterial signaling is perceived by eukaryotic organisms that form a symbiosis with microbial communities and provide bacteria and plant interaction [7, 18-21]. Plant growth and development, assimilation of nutrients, stress resistance are largely determined by the nature of such interaction [22-24]. Signaling compounds of the acyl-homoserine lactones (AHLs) class [25] are involved in the QS regulation of gram-negative bacteria [25], which have been shown

as effective plant growth stimulators and phytomodulators of resistance to biotic and abiotic stressors [8, 26].

The purpose of this review was to analyze and summarize the latest literature data on the role of bacterial QS and AHLs in the formation and functioning of plant-microbial signaling, the participation of AHLs in the regulation of plant growth and development, the resistance formation, as well as the prospects for using these compounds to create environmentally friendly preparations that can increase the yield of crops.

The influence of PGPR-group microorganisms on plant growth, development, and resistance. Plant growth and development depend on environmental conditions, primarily the soil in which various living organism forms are concentrated. Edaphic microbiota significantly affects mineral nutrition and soil-forming processes in general. For its part, the root system is actively involved in plant habitat formation. The main ecological niche occupied by rhizospheric bacteria is the zone of root exometabolite active release. A root system is a peculiar form of communication between plants and soil microflora, the main source of physiologically active substances, which during the growing season play the role of a link in the donor-acceptor interaction between plants and microbial communities in the soil [22, 27, 28]. The rhizosphere microbiota affects plant immunity and soil suppression [29]. Due to the high root secretory activity, soil microorganisms are provided with a nutrient substrate and form strong associative bonds both in the rhizoplane and in the rhizosphere. At the same time, root exometabolites can be one of the factors determining soil fungistasis. The reaction of pathogens such as stimulation or suppression of development depends on their composition and concentration [30]. Each plant has a protection system against pathogens; however, the method for detecting and distinguishing beneficial microorganisms from pathogens is not fully understood. It is believed that the plant has receptors responding to microbial molecules [31]. This process involves both various signaling mechanisms (chemoattraction, nodulation) and direct chemicals (organic acids, sugars, flavonoids, volatile organic substances) [32]. The presence of a certain connection becomes a signal for the beginning of root colonization or nodulation. After root colonization, bacteria correct the plant metabolism [33].

Bacteria colonizing the surface of roots and rhizosphere and having properties useful for plants are defined as the PGPR group (plant growth-promoting rhizobacteria) [22]. The PGPR group representatives can overcome the endodermal barrier. They enter the plant mainly through the root cortex, infect the vascular system, and form endophytic populations in roots, stems, leaves, and other organs [22]. The PGPR effect on plant growth and development is direct and indirect. Thus, under the PGPR direct influence, an active synthesis of phytohormones that stimulate growth (auxins, cytokinins, and gibberellins) occurs, as well as inhibition of the synthesis of stress phytohormones (ethylene, salicylic, and abscisic acids), the absorption of nutrients and water is enhanced (using N₂ fixatives, phosphate solubilizers, producers of siderophores). The PGPR indirect effects are in the defense mechanism induction, namely, the activation of the anti-biotic synthesis and cell wall lytic enzymes (chitinases), which exhibit phytostimulating and biopesticidal effects [34]. Due to these properties, PGPRs are used as a component of microbiological fertilizers to increase agricultural crop productivity in an environmentally friendly way [5].

PGPRs of *Rhizobium*, *Klebsiella*, *Clostridium*, *Nostoc*, *Anabaena*, *Bacillus* genera have a phytostimulating effect. They have a positive effect on phosphorus solubilization and nitrogen fixation, stimulate phytohormone synthesis, induce

mechanisms against pathogens, and accelerate reversibility after stress [5, 35]. By inducing solubilization and chelation, as well as redox reactions, PGPRs facilitate the soil micronutrient availability and through the biofortification of nitrogen, iron, zinc, and selenium provide the necessary content of these microelements in plants. PGPRs mitigate the negative effects of high temperatures, drought, salinity, and other abiotic stressors which makes it possible to use them in the creation of resistant microbiological preparations [36].

One of the most important tasks of biotechnology is phytoremediation of contaminated soils, the revival of areas of little use for farming agriculture. PGPRs as growth stimulators and modulators of plant resistance are positioned as promising detoxicants [37, 38].

Microbiological fertilizers are an environmentally friendly alternative to chemical fertilizers and agrochemicals which are detrimental to the environment [5]. Four groups of microbiological preparations that increase soil fertility and provide plant protection are currently presented on the market. These are nitrogen fixators (associative and symbiotic), phosphate-mobilizing bacteria, phytoestimulants, as well as bacterial preparations decomposing plant residues [39]. However, the low quality of microbiological fertilizers causes distrust among farmers and complicates commercialization. To increase production and their widespread introduction, it is necessary to stabilize the final product.

Intercellular communication of microorganisms. Biofilm formation is an ancient and integral component of the life cycle of prokaryotes, as well as a key factor ensuring survival in various ecological niches [20, 40, 41]. A biofilm is a highly organized bacterial formation, a kind of ecological niche with stable conditions of existence, in which complex trophic links are formed [14, 17]. Within biofilms, bacteria coordinate and synchronize the work of individual genomes, which allows the population to function like a multicellular organism [42]. The interaction of individual cells in a bacterial population is necessary for its survival in changing environmental conditions and the establishment of symbiotic or parasitic relationships with multicellular organisms [17, 43]. The coordinated activity of bacterial cells within the biofilm is carried out due to the QS interaction of specialized chemical molecules — communicative mediators, or autoinducers (AI), named because of their ability to stimulate their biosynthesis [12, 44].

Signaling AI molecules serve as gene expression regulators. They freely diffuse through cell membranes creating conditions under which the bacterial cell acquires the ability to respond to any changes in their intracellular concentration and thus determine the population size [45]. Intercellular QS signaling coordinates the bacterial population behavior. With an increase in the bacteria number, the AI content increases, and after reaching the AI threshold concentration, they bind and activate receptor proteins [42]. The ligand-receptor complex initiates the transcription of QS-regulated genes and determines the population phenotype which depends on the bacterial cell density.

Bacterial QS systems (with some approximation) can be considered a prototype of complex regulatory systems (hormonal and immune) of higher organisms that use mediators to coordinate various cell functions and form an adaptive response at the level of tissues, organs, and the body as a whole [46]. Among the mediators of bacterial QS, the most studied lactones are low molecular weight chemical compounds of the L-homoserine lactone acyl derivative class. Signaling systems, which include N-AHLs and their receptors, are most common in most gram-negative bacteria [25, 47]. AHLs contain a homoserine lactone five-membered ring and a variable acyl side chain attached to it by an amide bond (Fig. 1). Homoserine lactones were discovered by Nealson et al. [48] in the study of the

bioluminescent symbiont bacterium *Vibrio fischeri*, living in the photophores of Hawaiian calamari and causing the luminescence of these organs at a cell concentration of 10^{11} .

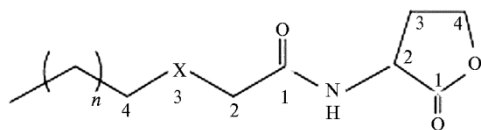


Fig. 1. Molecular structure of acyl-homoserine lactone: X — possible substituents of radicals in the third carbon atom of the side chain (H, OH, O), n — the number of carbon atoms of the acyl side chain.

AHL molecules synthesized by bacteria of different species differ in chain length and the radical presence or absence in the third carbon atom side chain. Differences in the molecular structure ensure bacteria recognizing their own and foreign AHLs [17]. Bacteria synthesize both short-chain AHLs (3-6 carbon atoms

in the acyl group), which freely diffuse through the cell membrane, and long-chain AHLs (10-16 carbon atoms), which can be incorporated into the cell membrane [7]. In addition to participating in the QS system functioning, AHLs can directly affect the eukaryotic organism's cells, in particular, plant cells [49]. AHL biosynthesis is carried out by AHL synthases of the LuxI type. As the bacterial population grows, AHLs accumulate up to a certain threshold value and bind to the corresponding receptor proteins, forming complexes that regulate certain bacterial gene expression [50] (Fig. 2). Recent studies have shown that bacterial signaling molecules are transferred by membrane vesicles. Previously, it was believed that vesicles are formed as a result of the cell membrane blebbing, but later it was found that they also appear after explosive lysis or cell death, which expands the understanding of the intercellular interaction between microorganisms [51].

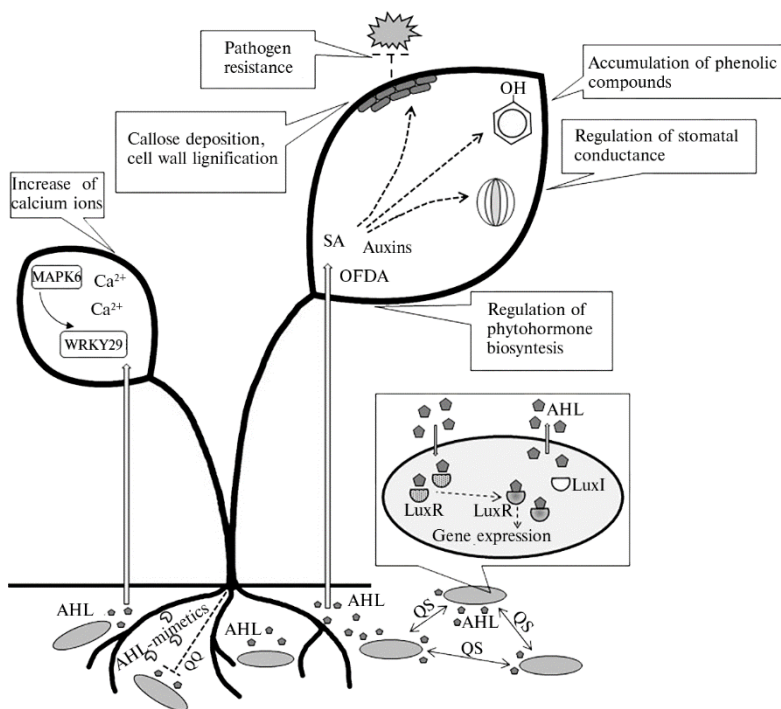


Fig. 2. Physiological and biochemical processes induced by acyl homoserine lactones (AHL). In the quorum sensing (QS) system, bacterial cells produce AHL molecules. The synthesis of these molecules involves two types of AHL synthases — LuxI and LuxR. The LuxI protein is directly involved in the synthesis of AHL. LuxR acts as a binding promoter protein. The concentration of AHL increases with the increasing density of the bacterial population. After the threshold level is reached, AHL penetrate into the cell, bind to proteins of the LuxR type, and activate the expression of target genes. AHL

molecules, penetrating into root cells, cause various plant responses. Among them are triggering a signaling cascade with the participation of mitogen-activated protein kinase (MAPK), expression of WRKY-type transcription factors, an increase in the concentration of Ca^{2+} ions in the cytosol, activation of the oxophytodienic acid cascade (OFDA), synthesis of phytohormones – salicylic acid (SA), auxins. Various protective reactions are formed, including callose deposition, accumulation of phenolic compounds, regulation of stomatal conductance. The quorum quenching (QQ) system of plant intervention allows manipulating QS signaling, selectively inhibiting AHL synthesis, competing with AHL for binding to receptor proteins, and inhibiting the activity of QS-controlled genes. AHL mimetics, plant metabolites produced by root cells and regulating bacterial QS, are considered as one of the components of QQ. The information presented was adapted from A. Schikora et al., 2016 (19).

QS regulates a variety of physiological processes in gram-negative bacteria. Many plant and animal pathogens use QS to manage virulence, which is very promising for practical purposes [52]. Recently, biotechnological developments have appeared aimed at obtaining AHL antagonists [53]. Such substances are used to protect crops from phytopathogens, and in medicine and veterinary medicine, they are used as antimicrobial drugs. With the help of QS, the synthesis of antibiotics is regulated, which makes it possible to activate bacterial culture growth in a production environment. However, the use of QS and AHLs as specific regulators of the bacterial processes is limited by insufficient knowledge of their action mechanism and autoinducer effects. Some literature reports that the study of intercellular communication *in vitro* does not provide a complete understanding of the mechanisms of the QS system and AHL functioning and effects and that further investigation of this phenomenon requires new approaches in the *in situ* system [54].

Role of the host plant in the regulation of bacterial QS. Since bacteria and eukaryotic organisms have co-evolved over millions of years, eukaryotes have developed mechanisms that allow them to perceive QS signaling, manipulate and respond to bacterial interactions [55]. The molecular mechanisms of these processes are not fully understood [13]. In contrast to the QS system, which regulates the behavior of bacterial cells when interacting with the host, the “quorum quenching” (QQ) intervention system functions in plants to manipulate bacterial signaling [56]. The QQ molecular mechanisms of plant metabolites include suppression of AHL synthesis, competition with AHLs for binding to receptor proteins, and a decrease in the QS-controlled gene expression [57-60]. Plant metabolites interacting with AHL receptors and activating transcription of target bacterial genes are of particular interest (see Fig. 2). The ability of such compounds to replace AHLs when interacting with receptor proteins is called AHL mimicry [61].

AHL mimicry is one of the mechanism affected bacterial QS. Plant root exudates contain low molecular weight signaling compounds (so-called AHL mimetics) which regulate bacterial QS and act as antagonists or synergists [63, 63]. It is assumed that AHL mimetics can regulate the functions and composition of the rhizosphere bacterial population, forming the plant rhizomicrobiome [64]. The root exudate of *Medicago truncatula* contains up to 20 signal-mimic compounds which can affect bacterial QS. The secretion intensity and such compound composition changed with the plant age, as well as after the seedling treatment with AHL solutions [62]. Notably, the synthesis of AHL mimetics is activated in plants under the rhizobacteria QS signal influence. The chemical structure of plant molecules affecting AHL-mediated bacterial QS is not understood. It would seem logical that AHL mimetics should have a structure close to AHL; however, the studies performed indicate differences in the structure of these compounds [65, 66].

The first identified eukaryotic AHL mimetic structurally different from AHLs but capable of activating the bacterial QS system was luminochrome [67].

It probably directly interacts with the AHL-binding pocket of the receptor LasRy protein of *Pseudomonas aeruginosa* (AHL receptor LuxR type) [68]. L-canavanine, an analog of arginine found in alfalfa seed exudates, suppressed QS regulation in *Sinorhizobium meliloti* [69]. Rosmarinic acid stimulated the activity of the RhIR transcription regulator in *Pseudomonas aeruginosa* [66]. Coumaric acid secreted by plant roots forms a special QS signal, coumaroyl homoserine lactone, which is perceived by some bacteria [44]. The catechin and naringenin flavonoids of many plants exhibited the QS mimetic activity [70, 71]. AHL-mimetics of rice and bean root exudates regulated the bacterial biofilm formation which indicates the role of these compounds in the plant-bacteria symbiosis formation [65]. *Arachis hypogaea* forms a rhizobial symbiosis with *Bradyrhizobium* spp. synthesizing long-chain AHLs. *A. hypogaea* seed and root exudates containing QS mimetics similar to long-chain AHLs stimulated the growth of bacteria with long-chain AHL-mediated QS-signaling and suppressed the growth and germination of bacteria with short-chain QS AHL-signaling [72].

Another action on bacterial QS is based on the availability and stability of AHL molecules in the rhizosphere. Thus, during the joint cultivation of *Sinorhizobium meliloti* and *Arabidopsis thaliana*, the AHLs synthesized by bacteria decreased, which could be due to both inhibition of the synthesis of these compounds by the host plant and changes in their quantity and availability [73]. The AHL concentration in the rhizosphere depends on soil particle adsorption, the lactone ring hydrolysis, as well as environmental temperature and pH. Another factor in the rhizosphere AHL degradation is their hydrolysis by plant QQ enzymes. Some plants can synthesize enzymes that destroy bacterial AHLs [74, 75]. The degradation of AHLs by plant enzymes is a species-specific process. Thus, AHL destruction within the rhizosphere of dicotyledonous plants proceeded rapidly, while within the monocotyledon rhizosphere, it was slow or absent altogether [74, 76]. The AHL destruction mechanism within the plant rhizosphere is also under-researched. It is thought that by analogy with bacterial degradation, two enzymes can participate in it such as AHL lactonases hydrolyzing the lactone ring or AHL-acylase hydrolyzing the amide bond between the acyl side chain and homoserine lactone, resulting in fatty acid separation from the homoserine lactone. However, the existence of such a mechanism in plants has not yet been established [74, 76]. It is assumed that in the coevolution process, plants have developed specific mechanisms for detecting AHLs in the extracellular environment and strategies for manipulating the bacterial QS systems. Thus, in response to the QQ system of intervention, the host plant can influence AHL-mediated bacterial signaling and determine the nature of relationships between partners in the rhizosphere.

Molecular action of AHLs. AHL priming activates the signaling mechanisms of the plant defense response, resulting in the modulation of salicylate-dependent and oxylipin-induced stress responses, the MAP kinase cascade, stomatal closure, cell wall thickening, and synthesis of phenolic metabolites [18, 20, 77]. PGPR group bacteria induce the surface-active metabolite secretion and the volatile compound synthesis that activate protective signaling pathways and help plants resist the attack of pathogens [78]. Thus, the treatment of tomato roots with AHLs produced by the *Serratia liquefaciens* MG1 rhizobacterium induced resistance to *Alternaria alternata*. The signaling pathway activated by salicylic acid (SA) was involved in the formation of resistance to the pathogen action. Treatment of tomato roots with butanoyl homoserine lactone (C₄-AHL) and hexanoyl homoserine lactone (C₆-AHL) caused the expression of the pathogen-associated protein 1a (PR1a) gene and the genes of two chitinases – components of the SA/ethylene-dependent pathway in tomato leaves. The results obtained showed that

short-chain AHLs in tomato plants acted as a trigger for the SA-dependent signaling pathway [77]. After treatment of Arabidopsis roots with tetradecanoyl homoserine lactone (C₁₄-AHL), the pathogen-associated molecular pattern increased the activity of AtMPK3 and AtMPK6, which expressed WRKY22 and WRKR29 transcription factors involved in PR1 synthesis [18, 79]. It is in response to the action of a pathogen that plants actively synthesize the pathogen-dependent PR1 protein [80, 81]. AHLs stimulated the synthesis of oxophytodienic acid (OFDA) and SAs which are involved in the defense reaction forming. Arabidopsis plants treated with C₁₄-AHL accumulated SA and oxophytodienic acid in leaves, which increased the expression of *HSP70*, *HSP17*, and *CYP81D11* genes which synthesize heat shock proteins and cytochrome P450 [49].

Absciscic acid (ABA) and SA induce stomatal closure, playing a key role in adaptation to stress [82]. Priming of C₁₄-AHL Arabidopsis plants infected with *Pseudomonas syringae* activated stomatal closure; however, priming did not affect the activity of the RD22, RD29, and RAB18 genes involved in ABA synthesis [49, 83]. The mechanism of movement of stomatal guard cells under stress is associated with the ABA accumulation and the specific ion channel activation [84]. Open Stomata 1 (OST1) Ca²⁺-independent protein kinase and Ca²⁺-dependent protein kinases (CPKs) are key enzymes for ABA-induced activation of the SLAC1 slow-type anion channel and stomata closure [82, 85, 86]. It is thought that SA signaling is integrated with ABA signaling [82]. Blocking of SA-induced closure of the stomatal apparatus and SA-activation of the slow-type anion channel was found in the *cpk3* and *cpk6* Arabidopsis mutants with impaired CPK synthesis, and was not found in the *ost1-3* mutant with impaired OST1 synthesis [87]. The SLAC1 phosphorylation sites in ABA signaling, serine-59, and serine-120 are key for SA signaling [88]. Superoxide anion chemiluminescent identification showed that SA signaling did not require activation of *cpk3* and *cpk6* for the reactive oxygen species (ROS) formation. SA activates the peroxidase-mediated ROS signal induced in the Ca²⁺/CPK-dependent branch of ABA signaling, rather than the OST1-dependent signaling branch in stomatal guard cells [82]. After treatment with AHL, stomatal conductance and leaf transpiration increased, which increased the nutrient supply to the bean root colonizing bacteria [89].

In genetically modified tobacco plants with bacterial genes for AHL synthesis and degradation, effective protection against pathogens was formed in AHL-synthesizing plants, while plants with AHL-degrading genes could not protect against pathogen damage. Exogenous treatment with C₁₄-AHL enhanced the systemic resistance of Arabidopsis to the *Golovinomyces orontii* and *P. syringae* pathogens and barley — to *Blumeria graminis* f. sp. *hordei* [18]. After treatment with a C₁₄-AHL solution, callose deposition, phenolic compound accumulation, cell wall lignification, changes in oxylipins, and closure of plant stomata were recorded [7, 49]. The primary root elongation in the *Arabidopsis thaliana* wild genotype, induced by treatment with solutions of C₆-AHL and octanoyl homoserine lactone (C₈-AHL), was not observed in AHL-insensitive mutants [90]. Treatment of Arabidopsis plants with C₄-AHL led to a temporary increase in Ca²⁺ in the cytosol, while treatment with C₆-AHL induced an increase in calcium-binding protein (calmodulin). These results indicate the involvement of calcium ions in the perception of bacterial AHL signaling [32, 91]. It is thought that Cand2 and Cand7 G-proteins (G-protein coupled receptors) are involved in the perception of AHL signals, which are known as the trigger of cell proliferation, formation of defense reactions, light perception, stomatal conductance, regulation of ion channels, seed germination, and synthesis of gibberellin, brassinosteroids, abscisic and jasmonic acids, auxins and ethylene (see Fig. 2). This assumption was based on the

polymerase chain reaction analysis of plants primed with C₆-AHL and C₈-AHL, in which the expression of genes synthesizing Cand2 and Cand7 was observed [92].

Phytostimulating and phytoprotective effects of priming and foliar treatment with AHL solutions. Climatic conditions significantly affect the crop quality and unfavorable changes in temperature and water regime reduce plant resistance to bacterial and fungal invasions. One of the effective technologies for increasing resistance to biotic and abiotic stressors is seed pre-sowing priming, which improves germination and activates defense mechanisms [6–8]. Plant growth and development are also stimulated by bacterial inoculants. They improve mineral nutrition and reduce the pathogen influence in response to competition and stimulation of defense systems [19, 78]. For example, the *Serratia marcescens* bacterium induces systemic resistance of *Solanum lycopersicum* to the *Alternaria alternata* fungal pathogen [93], and root treatment with solutions of synthetic AHLs enhances the expression of genes involved in the defense mechanism formation in tomato leaves [77]. After the presowing priming of C₆-AHL, the number of germinated grains increased by 1.2 times, and the size of the winter wheat coleoptile and root increased by 1.4 times [8]. Field studies have shown an increase in plant biomass at the tillering stage by 1.4 times, productivity by 1.5 times, and grain quality by 1.3 times. In the F₁ generation grown from seeds from primed parental plants, an increase in yield indicating the priming effect preservation was also recorded [8].

Rhizosphere and phyllosphere associated microbial communities have a positive effect on plant growth and resistance to pathogens [94, 95]. AHLs in the soil with primed seeds stimulate the growth of PGPR of bacteria of the genera *Bacillus* and *Pseudomonas*, which colonize the root surface and inhibit the pathogen activity [96]. Under the action of AHLs in the rhizosphere zone, the amylolytic bacteria involved in the degradation of dead root cells increase, the growth of roots increases, and the supply of sugars necessary for other rhizosphere bacteria increases. AHLs affect the formation of a plant defense reaction, initiate systemic resistance, and improve the recognition of pathogenic microorganisms [97]. The priming of *Cicer arietinum* seeds with C₄-AHL enhanced plant growth and increased stress resistance [26]. The primed seeds germinated well under simulated oxidative (5 mM H₂O₂) and salt (200 mM NaCl) stresses. In seedlings, the biomass, chlorophyll, and proteins, and the resistance to damage by *Fusarium oxysporum* f. sp. *ciceri* increased [26]. C₆-AHL can stimulate growth and root formation processes [90, 98, 99] and induce systemic resistance to a wide range of pathogens in many plant species [100]. Thus, various studies have demonstrated that bacterial AHLs can be used to improve plant growth and productivity, reduce dependence on fungicides and fertilizers, and fight pathogens and stressors [101].

So, in the remote transduction of signals in the phytospheric bacterial biocenosis—plant system, AHLs, mediator molecules of bacterial origin, are involved. Among the AHL molecules synthesized by bacteria of different species, there are short-chain ones with 3–6 carbon atoms in the acyl group and long-chain ones with 10–18 carbon atoms. Differences in the structure of molecules ensure that bacteria recognize their own AHLs and separate foreign ones. AHL molecules play a key role in the cooperative activity of bacterial populations, their colonization of new ecological niches, and the regulation of partner interactions within the rhizosphere. AHLs, synthesized by bacteria associated with plants, affect the composition of microbial communities and plant life processes. Plants receive and respond to bacterial AHL signals by adapting to changing conditions and releasing their own AHL mimetics. The AHL transfer from a bacterium to a

host plant is carried out by membrane vesicles. Most phytopathogenic bacteria use QS systems that are directly related to virulence. The expression of virulence factors regulated by QS becomes one of the causes of infection. Phytopathogenic bacteria signaling molecules and QS systems have been studied in sufficient detail. The study of gene expression of QS-regulated virulence functions has led to the development of a QS intervention strategy to fight bacterial plant diseases. A well-known example is the QQ system of bacteria of the genus *Bacillus*, which synthesize lactonase and acylase that break down N-AHLs. Bacteria use volatile organic compounds to interfere with the QS systems of competitive species. In turn, plants synthesize compounds like AHLs (chemical mimicry) that can inhibit or stimulate bacterial QS systems. In recent years, genetics, genomics, biochemistry, and signaling diversity of QS molecules have been actively studied. Rhizosphere is the most dynamic site of the plant and the associated microflora interaction with the AHL participation. The regulation of its functions is the task of particular importance in the development of new biotechnological approaches aimed at increasing the agricultural crop yield and stress resistance. AHLs induce an increased growth, and elevation of photosynthetic pigments causes changes in the ratio of phytohormones in organs and tissues affects the formation of defense mechanisms, which increases the yield of crops. Since AHLs meet the requirements of intensive organic farming, they are considered as promising ecological phytostimulants and phytomodulators.

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SEQUENCING OF THE *U6* PROMOTERS IN CASTOR BEANS AND VECTOR CONSTRUCTION FOR CRISPR/Cas9 GENOMIC EDITING ON THEIR BASIS

O.S. ALEXANDROV ✉, G.I. KARLOV

All-Russian Research Institute of Agricultural Biotechnology, 42, ul. Timiryazevskaya, Moscow, 127550 Russia, e-mail
olegsandrov@gmail.com (✉ corresponding author), karlovg@gmail.com

ORCID:

Alexandrov O.S. orcid.org/0000-0002-7146-4094

Karlov G.I. orcid.org/0000-0002-9016-103X

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Abstract

Castor bean is an important crop in many countries. It is mainly used to obtain the castor oil, which is widely applied in various industries. The rich protein cakes and meals are remains after the oil is pressed. They are promising for use as protein additives in the fodder production. However, it is limited due to the presence of toxins. These are ricin protein and ricinine alkaloid. One of such methods can be genomic editing using the CRISPR/Cas9 system. The technology consists in cutting the target region of the intact DNA by the Cas9 enzyme with the assistance of a short guide RNA fragment. The delivery of the Cas9 and guide RNA genes into the cell of the edited plant is often carried out by plasmid vectors. For efficient synthesis of the guide RNA in such vectors, the promoters of small nuclear RNA genes are usually used. In dicotyledonous plants editing, the promoter of the *Arabidopsis* *U6* gene is most often used. In this work, the amplification, sequencing and analysis of the castor bean *U6* promoters were carried out at the first time. The obtained sequences were analyzed and used in the construction of CRISPR/Cas9 vectors for the ricin gene editing. Our aim was to study castor bean *U6* promoters with help by bioinformatics and molecular genetic approaches. Zanzibar Green and Gibzonskaya varieties of castor bean plants were used in this work. DNA was isolated from young leaves. The preparation of sequences, alignments and homology level calculation were carried out by GenDoc program (<http://www.nrbsc.org/gfx/genedoc/index.html>). Primers for amplification of castor bean *U6* promoters were designed by Primer3 program (<http://bioinfo.ut.ee/primer3-0.4.0/>). PCR was performed using a C-100 PCR machine (Bio-Rad Laboratories, Inc., USA). PCR products were separated in 1.5% agarose gel at 6 B/cm in the Sub-Cell GT electrophoresis camera (Bio-Rad Laboratories, Inc., USA). The amplicons were purified with the GeneJET PCR Purification Kit (Thermo Fisher Scientific, Inc., USA). The purified amplicons were cloned into the pAL2-T vector (Evrogen, Russia) according to protocol of manufacturer. In the CRISPR/Cas9 vector construction, the pRGE31, HindIII and SbfI restriction enzymes (Sibenzyme, Russia) and nucleotides were used. Purification of the digested products was carried out with the GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Inc., USA). Bioinformatic search were conducted in the GenBank base, and 12 scaffolds with the castor bean *U6* gene were found. Six promoters with intact USE and TATA-box elements of regulation were used in the primer design for amplification with cv. Zanzibar Green and cv. Gibzonskaya DNA matrices. One fragment PCR products were cloned and sequenced. The analysis of the obtained amplicon sequences revealed that promoter regions of two studied varieties were similar. The level of promoter identity from different amplicons ranged within 51-77%. In comparison of these promoters with ones from other plants, the level of homology was 42-64%. The promoter sequences with intact USE and TATA-box motifs were used in construction of the CRISPR/Cas9 vectors which can be used for efficient editing of the castor bean genes involved in the ricin and ricinine synthesis.

Keywords: castor beans, promoter, *U6* gene, sequencing, genome editing, CRISPR/Cas9, vector construction

Castor bean (*Ricinus communis* L.) is an agricultural crop in the spurge family (*Euphorbiaceae*) that is farmed in many countries of the world. It is cultivated mainly for seeds that castor oil, a valuable product used in many sectors of the economy, is produced from [1]. Castor oil virtually never dries and preserves its properties in a broad range of operating temperatures, which is why it is used as a lubricant in food machinery and in aircraft modeling engines; it is also a component of plastic lubricants [2]. Castor oil contains substances valuable for chemical synthesis: undecylenic acid and sebacic acids, components of epoxy and alkyd resins, etc. Castor oil has long been used in medicine as an effective laxative [3, 4].

Once castor beans have been pressed for oil, they retain residues rich in protein — meals and cakes, a promising source of protein in feed production. However, these residues contain toxins: ricin and ricinine (a protein and an alkaloid, respectively) [5]. Ricin is a cumulative poison that has a strong local cauterizing effect that triggers hemorrhagic gastroenteritis. Once absorbed into the blood, it causes erythrocyte agglutination and destruction, increases the risk of thrombosis, and triggers ulceration of vascular walls. Besides, ricin has a negative effect on the central nervous system, where it causes convulsions, paresis, and paralysis [6]. Ricinine is far less toxic; however, it is not a desirable feed component either. Castor bean meals and cakes are usually detoxified by destructive thermal and chemical treatment, which jeopardizes protein quality. Such treatment renders proteins less digestible and causes lysine and other scarce amino acids to hydrolyze [5]. This is why finding an alternative method to detoxify castor bean meals and cakes without conventional treatment remains a relevant research challenge.

It would be of undoubted theoretical and practical interest to obtain castor bean plants devoid of ricin and ricinine. To that end, genes involved in the synthesis of either toxin must be knocked out. CRISPR/Cas9 is an effective state-of-the-art genome editing technique that can perform such knockout. Its components are the enzyme Cas9 and a guide RNA, or gRNA [7]. Cas9 and gRNA genes are injected into a cell by plasmid vectors with corresponding promoters. *U3* and *U6* small nuclear RNA (snRNA) promoters are normally used with the gRNA gene [8]. Genome editing of monocotyledons normally uses sequences of *U3* promoters of rice; dicotyledons are edited with *U6* promoters of *Arabidopsis*. However, several papers have shown that editing is more efficient when promoters are of the edited species, and adding them to CrISPR/Cas9 vectors has become a major trend [9–13]. Research into *U6* promoters in various species shows that they contain two RNA polymerase III recognition sites: Upstream Sequence Element (USE) and TATA box [11, 13, 14]. USE has a consensus sequence RTCCCACATCG. Marshallsay *et al.* [14] proposed a system of primers for *U6* promoter amplification; their system is based on the aforementioned sequence and the *Arabidopsis U6* sequence. These primers seem suitable for analyzing *U6* promoters in other species, the genomes of which are yet to be sequenced.

To date, genomes have been sequenced for several *Euphorbiaceae* species (*Jatropha curcas*, *Manihot esculentum*, *Hevea brasiliensis*, *Euphorbia esula*), including castor bean [15–19]. Ricin-coding sequences have also been sequenced and studied [20–23]. They do not contain introns, which is why a large precursor (pre-ricin) is translated from them; this precursor contains a single peptide and ricin A and B chains separated by a linker peptide of 12 amino acids [20]. Parts encoding the signal peptide or the beginning of the A chain are the most suitable targets for editing [23, 24].

This is the first research project to have amplified, sequenced, and analyzed castor bean *U6* promoters. The identified sequences were analyzed and used to construct CRISPR/Cas9 vectors for further editing of ricin genes.

The goal was to study castor bean *U6* promoters by bioinformation, molecular, and genetic methods.

Materials and methods. The researchers used the castor bean varieties Zanzibar Green (Gavrish, Russia) and Gibzonskaya (Gavrish, Russia).

DNA was extracted from young leaves by the method proposed by Doyle *et al.* [25], although slightly modified [26]. Sequence processing, alignment, and homology quantification were performed in GenDoc (<http://www.nrbcs.org/gfx/genedoc/index.html>).

Primers to amplify castor bean *U6* promoters were found by means of Primer3 (<http://bioinfo.ut.ee/primer3-0.4.0/>). Polymerase chain reaction (PCR) was run on a C-100 amplifier (Bio-Rad Laboratories, Inc., USA) with the following parameters: 5 min at 95 °C; 30 s at 95 °C, 30 s at 50 °C, 1 min at 72 °C (35 cycles); 10 min at 72 °C (terminal elongation).

PCR products were separated in 1.5% agarose gel at 6 V/cm in a Sub-Cell GT horizontal electrophoresis chamber (Bio-Rad Laboratories, Inc., USA). Electrophoresis results were visualized by the gel documentation system Gel Doc™ XR+ (Bio-Rad Laboratories, Inc., USA). Amplicons were purified using the GeneJET PCR Purification Kit (Thermo Fisher Scientific, Inc., USA).

The purified amplicons were cloned using the pAL2-T vector (Evrogen, Russia) according to the manufacturer's instructions.

To construct CRISPR/Cas9 vectors, the plasmid pRGE31, HindIII and SbfI restriction endonucleases (Sibenzyme, Russia), and the required oligonucleotides were used. To cut restriction products out of the gel, the GeneJET Gel Extraction Kit (Thermo Fisher Scientific, Inc., USA) was applied.

Results. Twelve scaffolds containing the castor bean *U6* sequence were found in GenBank: AASG02000063, AASG02000163, AASG02000719, AASG02001323, AASG02002949, AASG02003223, AASG02003842, AASG02006600, AASG02019053, AASG02021200, AASG02020516, AASG02025904. However, the characteristic USE and TATA elements were found in the promoter region of the *U6* gene only in six of these scaffolds: AASG02021200, AASG02020516, AASG02006600, AASG02000719, AASG02025904, AASG02002949. Comparison to the results of the search for USE and TATA-featuring *U6* promoters in the genomes of other Euphorbiaceae species showed the number of such could vary. The genomes of *Jatropha curcas* and *Manihot esculentum* (variety W14) contain 6 such promoters, as does castor bean; however, *Hevea brasiliensis* and *Euphorbia esula* have 9 and 23, respectively.

Sequences of the scaffolds AASG02021200, AASG02020516, AASG02006600, AASG02000719, AASG02025904, AASG02002949, and that of a single USE and TATA-less scaffold AASG02000063 were used to select primers (Table 1) and further amplify the fragments containing the upstream region of the respective *U6* genes, see Figure 1. In all cases except AASG02000063, single fragments of expected length were amplified (amplicons Rc200, Rc516, Rc600, Rc719, Rc904, and Rc949). Since the case of AASG02000063 saw the amplification of two more fragments, this upstream region was subject to no further analysis. Single fragments of other variants were cloned and sequenced.

1. Primers for amplification of the *U6* gene promoter region in the analyzed castor bean (*Ricinus communis* L.) scaffolds

Primer	Nucleotide sequence	Scaffold	Amplicon	Size, bp
RcU6-200f	5'-TGGATAAGAGGAGATTCTTGAATTG-3'	AASG02021200	Rc200	392
RcU6-200r	5'-AGGGGCCATGCTAATCTTCT-3'			
RcU6-516f	5'-GTTGGCAGCCTTCAGATTTC-3'	AASG02020516	Rc516	590
RcU6-516r	5'-AGGGGCCATGCTAATCTTCT-3'			

				Continued Table 1
RcU6-600f	5'-CTCCGAATTATATTTGGGGTTTT-3'	AASG02006600	Rc600	280
RcU6-600r	5'-AAAAATTTGGACCATTTCTCG-3'			
RcU6-719f	5'-ACCTGTGAGGTGGCTTTCTG-3'	AASG02000719	Rc719	346
RcU6-719r	5'-CGGTGTCTGTTTGCCCTAAT-3'			
RcU6-904f	5'-AGCCCATTTTGGGGTGTAT-3'	AASG02025904	Rc904	512
RcU6-904r	5'-AGGGGCCATGCTAATCTTCT-3'			
RcU6-949f	5'-TTCTGGGAGGTATGCATCAA-3'	AASG02002949	Rc949	401
RcU6-949r	5'-AAAAATTTGGACCATTTCTCG-3'			
RcU6-063f	5'-CAACCCGACTCCTTCATCAT-3'	AASG02000063	Rc063	584
RcU6-063r	5'-AGGGGGCCCTACTTTGATACC-3'			

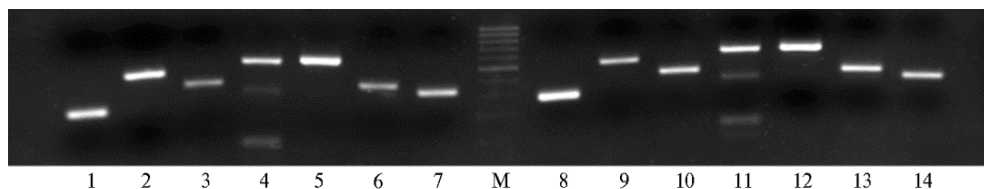


Fig. 1. PCR amplification of the *U6* gene promoter region from the template DNA of castor bean (*Ricinus communis* L.) Zanzibar Green (1-7) and Gibzonskaya (8-14) cultivars using selected primers (see Table 1): 1 and 8 — Rc600f/Rc600r, 2 and 9 — Rc904f/Rc904r, 3 and 10 — Rc949f/Rc949r, 4 and 11 — Rc063f/Rc063r, 5 and 12 — Rc516f/Rc516r, 6 and 13 — Rc200f/Rc200r, 7 and 14 — Rc719f/Rc719r; M — molecular weight marker (100 bp DNA Ladder M-214S, Jena Biosience GmbH, Germany).

2. Homology (%) between the nucleotide sequences of six selected *U6* gene promoters of castor bean (*Ricinus communis* L.)

Promoter	Rc200ZG	Rc516ZG	Rc600ZG	Rc719ZG	Rc904ZG	Rc949ZG
Rc200ZG	100					
Rc516ZG		100				
Rc600ZG			100			
Rc719ZG				100		
Rc904ZG					100	
Rc949ZG						100

Amplicon sequences obtained from Zanzibar Green (Rc200ZG, Rc516ZG, Rc600ZG, Rc719ZG, Rc904ZG, and Rc949ZG) and Gibzonskaya (Rc200Gib, Rc516Gib, Rc600Gib, Rc719Gib, Rc904Gib, and Rc949Gib) were analyzed; this analysis found these varieties to have fully matching promoter regions which also matched the corresponding sequences in the scaffolds AASG02021200, AASG02020516, AASG02006600, AASG02000719, AASG02025904, AASG02002949. However, two polymorphic sites were found outside regulatory element-containing promoter regions, the G/A substitution at a position –228 from the start site of the *U6* gene in Rc200Gib and T/A substitution at a position –351 from the start site of the *U6* gene in Rc904Gib. Apparently, the tested promoter regions are rather conservative in the varieties of the same species.

A similar pattern was observed in the case of cassava varieties AM560-2 and KU50. *U6* promoter regions from their scaffolds LTYI01019634 (nucleotides 16035-16336) and JPQF01078381 (nucleotides 1216-1517), as well as LTYI01021841 (nucleotides 27091-27392) and JPQF01070438 (nucleotides 16559-16860), were found to fully match.

Comparing sequenced castor bean *U6* promoters against each other showed medium homology varying within 51-77% (Table 2). Similar homology of 42-64% was observed when those were compared against randomly sampled *U6* promoters of other randomly picked species from different families (Tables 3 and 4).

3. Nucleotide sequences of randomly selected promoters of plant species from different families used to assess the degree of homology between them and six *U6* gene promoters of castor bean (*Ricinus communis* L.)

Code	Nucleotide sequence	GenBank acc. no.	The first nucleotide	The end nucleotide
abrus1	5'-GCCCCACATCGAACAGTATTATCAAAGCATGACACAATATATAGCAAAAGAAACACGCAGAGAGT-3'	XM_027482675	708	644
arachis1	5'-GTACCACATCGAGTAGCATCATATAAAGTCTGACAAATATATATAGCAGAGGGTGCAAGAAAGGCTC-3'	XM_025790263	575	511
arachis2	5'-GTCCCACATCGCTTAGTATCAGACCCTCTGACAGAATATATATCAAAGGAAACACAAAAGGCTC-3'	XR_003810923	117	181
arachis4	5'-GTCTCACATCGCCCGAGTTTGTAGAAACCAATACTTATATATCAGAGGGCGAAGCAAGGCTC-3'	XM_025845620	218	281
bras_ol1	5'-GTCCCACATCGCTCAGGTGAAGGAGAGGAGCTGCGTTTATATAGCGATGAAGTCACGAAAGTGATT-3'	LR031877	48176336	48176271
bras_rap1	5'-CTCCCACATCGCTCAGCGAAGCAAAAGAAGCTCCTGTTTATATACTTTCAGAGTCAAGAAAGATGATT-3'	LR031575	1103139	1103205
bras_rap2	5'-CTCCCACATCGTTTATCAGAGAAGCAGAAGCCGAGTTTATATAGGGACGGAGTGACGAAGGAGATT-3'	LR031575	1143184	1143119
cicer1	5'-GTCCCACATCGAATACATGTATCCCATTTTCCATATTTATATAACGCAGGTTAACCATGTCAGTAT-3'	CP039335	30486401	30486452
cicer2	5'-GTCCCACATCGAATACATTTATCCCTTTTCCGTTATTTATATAACGCAGGTTAACCATGGAGTTT-3'	CP039335	30494676	30494740
cicer3	5'-GTCCCACACCGAATCATCTATCATTTTTCGCTTTTATATAACCCATGTTAATCATTAGGTTT-3'	CP039335	30512524	30512587
cicer4	5'-GTCCCACATCGTCTAAATATTCGAATATTTAATATTTATATAACAATGTTTCGAGCAGTATAGTAT-3'	CP039333	18318744	18318807
cicer7	5'-GTCCCACACCGGTACGCATAACATGTGTTTCAGTGTTTATAATACCCTCGCACACATCATCAAC-3'	CP039333	31682334	31682398
cicer8	5'-GTTCCACATCGTCTACATCTATCATTTTTCGCTTTTATATTAACCCATGAGCCATAAGGCTT-3'	CP039333	39009683	39009747
cichorium1	5'-CGTCCATACCCGACAGTAAAGTACTTCCCGTCGCTTATATAGCGCAGCTCGGCGACTATCATC-3'	MK455779	235	299
cichorium2	5'-GTCCCATACCGACAGTAAAGTACTTCCCGTCGCTTATATAGCGCAGCTCGGCGACTATCATC-3'	MK455779	236	299
cichorium3	5'-TTCCCACATCGCTCTTTGAAGCAACATCGCCATGCTTTATATAGCTTGGCTTCCAAACATATATC-3'	MK455773	235	299
cichorium4	5'-TTCCCACATCGATGATTGAAACGATTCTCCGTTGTTTATATAGCCTGGCTTCCAAATCAATATC-3'	MK455776	238	302
cichorium5	5'-CTCCCACATCGATGATCGGAACGGTTGTTTCGTCGTTTATATAGCTCGGGTTCCAACCATTTATC-3'	MK455775	238	302
citrullus1	5'-GTCCCACATCGGTAAGTTTGTATTCTAGTTTACGCTTTATATACTAAGACTGCAGTACAAGGCTT-3'	VOOL01000005	2250542	2250477
cynara1	5'-AATCCCACATCGCCTTTAACGATATCCAGTGCTAGCTTTATATGGCGGAGGTCGGCAGCTAAGATC-3'	XR_003069239	1	65
goss_raim1	5'-ATCCCATATCGCTAAAGAACTATAACACAGGAGCGTTTATATAAGCGAAAGAAGCAGCAAATGATT-3'	CP032562	3130166	3130101
goss_raim2	5'-ATCCCATAGCTAAAGAAATTAGGAAAAATTTATTGTTTATAAAGGCAAGGAAGAACTATTATT-3'	CP032562	3328533	3328468
goss_raim3	5'-ATCCCGCATCGCTAAAGAAATTGAAAAAATTTATTGTTTATATAGGAAAAACAAGCTGACTATGATT-3'	CP032562	3351518	3351452
goss_raim4	5'-ATCCCGCATCGCTAAAGAAATTGAAAAAATTTATTGTTTATATAGGAAAAACAAGCTGACTATGATT-3'	CP032562	3351518	3351452
gossipioides1	5'-ATCCCACATCGCTAAAGAACTAAAAATGCGGAAGTATTTATATAAGCGAAAGAAACAGCATTAGTGT-3'	CP032252	2333488	2333423
gossipioides2	5'-ATCCCACATTGCTAAAGAAATTAACAAATACTATTGTTTATATAGGCAAAAGAAACACCGTAGCAGT-3'	CP032252	2341665	2341600
gossipioides3	5'-ATCCCACATCTCTAAAGAAATTAACAAACACTATTGTTTATATAGGCAAAAGAAAGCACCCTTGTATT-3'	CP032252	2343934	2343869
ipomea1	5'-CTCCCACATCGGGCGATGAAGCAGCTCTCTCCAGTACACATACTCCGCCATTGGAAGAAGAAGAAC-3'	CP025668	7620112	7620047
ipomea2	5'-CTCCCACATCGGCAATGAGCCATCTTACTTCCAGTACATATACTCCGCCATGGAAGCTCTTATC-3'	XR_004100417	33	97
ipomea3	5'-CTCCCACATCGGCTGATGAAACAACCTTGCTCCAGTATACATACTCTATCATGGAAGCACTGAGC-3'	XM_031272161	1	65
ipomea4	5'-GTCCCACATCGGCAATGAGCCATCTTACTTCCAGTACATATACTCCGCCATTGGAAGCACTTATGC-3'	CP025668	7980996	7981060
ipomea5	5'-TTCCCACATCGGGCGATGAAGCAGCTCTCTCCATTACACATACTCCGCCATTGAGGAAGGAGAAT-3'	CP025668	7984209	7984274
lotus1	5'-GTCCCACACCGGATAAACATACAGAAATATGAGTGTTTATAAGCAATAGTCAGCAATAAGGTTT-3'	AP010923	70411	70347
lotus2	5'-GTCCCACATCGGCTAGTTGATTAAGATTTTATAGTTTATATCTACTACAGAACGCAAGTATT-3'	AP010923	68417	68353
tea1	5'-GTCCCACATCGAACTTCGACGTTATAGACATGGAAGTTTATAAGAAAGAAAGAGAGAACGACGTT-3'	XR_003649102	150	215
tomato1	5'-CTCCCTCATCGCTTACAGAAAAAGCTATATGCTGTTTATGCGAAATCTAACAGTGTAGTTT-3'	XM_004230407	25	89
tomato2	5'-CTCCCTCATCGCTTACAGAAAAAGCTATATGCTGTTTATATTGCGAATCTAACAGTGTAGTTT-3'	XS1447	198	261
vigna1	5'-GTCCCACATCGTCCAAACATGTCAACCTTCCATGTTTAAAAACGCACGCCTACTCGCTGCTGTT-3'	CP039354	53496613	53496677
vigna2	5'-GTCCCACACCGTATACTTTCACTAGAGGTTTAGTGTTTATATAGATACAGACTGCATCCAAGCTT-3'	CP039350	33742544	33742608

4. Homology (%) between nucleotide sequences of six selected *U6* gene promoters of castor bean (*Ricinus communis* L.) and randomly selected *U6* gene promoters of plant species from different families

Code	Promoter					
	Rc200ZG	Rc516ZG	Rc600ZG	Rc719ZG	Rc904ZG	Rc949ZG
abrus1	48	53	56	46	60	62
arachis1	52	53	57	47	55	49
arachis2	54	57	57	51	56	58
arachis4	54	54	63	52	49	54
bras_ol1	53	54	57	48	52	53
bras_rap1	54	55	57	56	54	52
bras_rap2	45	54	54	46	52	54
cicer1	56	54	53	53	55	52
cicer2	58	52	53	57	55	49
cicer3	59	53	50	54	51	46
cicer4	50	56	59	53	56	55
cicer7	47	64	53	50	50	55
cicer8	51	57	58	54	55	55
cichorium1	42	47	55	45	47	50
cichorium2	43	48	55	46	48	51
cichorium3	55	53	50	52	49	52
cichorium4	49	48	50	51	50	45
cichorium5	55	51	54	55	47	44
citrullus1	57	60	59	58	56	59
cynara1	52	55	63	55	56	51
goss_raim1	48	62	57	51	61	59
goss_raim2	47	58	53	50	58	56
goss_raim3	51	61	50	52	65	63
goss_raim4	50	46	50	52	65	63
gossipioides1	49	55	57	54	62	58
gossipioides2	51	60	54	51	62	61
gossipioides3	52	60	57	52	60	60
ipomea1	42	52	47	46	44	47
ipomea2	48	58	52	44	50	49
ipomea3	46	58	54	43	52	52
ipomea4	50	58	51	48	48	49
ipomea5	45	52	47	43	47	47
lotus1	46	55	58	52	63	59
lotus2	59	59	62	59	56	58
tea1	57	53	56	58	57	58
tomato1	48	47	53	48	53	51
tomato2	48	47	53	47	52	50
vigna1	52	60	57	46	53	52
vigna2	55	66	56	53	61	55

To a considerable extent, such homology was due to the presence of fairly conservative USE and TATA elements, whereas other regions had low homology (Fig. 2).

USE sequence analysis of these promoters showed castor bean to have several variants of this regulatory element: A (GAACCACATCG) of the amplicons AASG02021200ZG and AASG02021200Gib, B (A-CCCACATCG) of the amplicons AASG02020516ZG and AASG02020516Gib, and D (AT-CCACATCG) of the amplicons AASG02000719ZG and AASG02000719Gib. In the other three cases, USE motives (C, E, and F, see Fig. 2) matched the *Arabidopsis* consensus sequence RTCCCACATCG.

The identified castor bean *U6* promoters were then used to design vectors to edit the ricin gene by means of CRISPR/Cas9.

To construct CRISPR/Cas9 vectors, the research team used the plasmid pRGE31 containing the *Cas9* gene under the CaMV 35S promoter, as well as a gRNA synthesis structure: *U3* promoter of rice (*OsU3*), a motif of two divergent sites of the Bso31I restriction endonuclease (to embed the target site once the plasmid has been hydrolyzed by the enzyme, 2xBso31I), and a sequence encoding the gRNA part interacting with the Cas9 protein (gRNAgene).

First, we searched for restriction endonuclease sites in the original plasmid pRGE31 such that one site was located before the *U3* promoter beginnin, and another site after that. Still, both sites had to be absent from the structure to ligate with pRGE31. HindIII and SbfI sites were picked as a result. Since the region between them contained the entirety of the OsU3-2xBso3II-gRNAgene structure, it was necessary to synthesize an insertion sequence that would contain 2xBso3II-gRNAgene under the corresponding castor bean promoter.

Fig. 2. Alignment of castor bean (*Ricinus communis* L.) *U6* gene promoter sequences: A — promoter from the AASG02021200ZG and AASG02021200Gib amplicons, B — AASG02020516ZG and AASG02020516Gib amplicons, C — AASG02006600ZG and AASG02006600Gib amplicons, D — AASG02000719ZG and AASG02000719Gib amplicons, E — AASG02025904ZG and AASG02025904Gib amplicons, F — AASG02002949ZG and AASG02002949Gib amplicons. The USEs with a consensus sequence RTCCACATCG of *Arabidopsis* are highlighted in red. The part of the *U6* gene that is amplified from the Oligo3 primer [14] is highlighted in green.

5. Oligonucleotides used for constructing and testing CRISPR/Cas9 vectors with castor bean (*Ricinus communis* L.) *U6* gene promoter s

Promer	Nucleotide sequence
Oligos for synthesis of double strand constructs RcU6(n)-2xBso3II-gRNAgene	
forward-A	5'-GAACCACATCGATTTCGTTGTAGCTTTTAGAATTCTTTATATGAATTAGGGCAAACAGCTGGTTGGGGAGACCGAGGTCTCGGTTTTAGAGCTAGAAATAGC-3'
forward-B	5'-AACCCACATCGTCTAGTTACGCATAACTTTAGAGTTTATAAACGCCTACAAGCAAGCACCATGCTGGAGACCGAGGTCTCGGTTTTAGAGCTAGAAATAGC-3'
forward-C	5'-GTCCACATCGTCTGGTTACGTGAGACTAATGATCCTTATATGCAAACGGGAGGCAGCATGATGCTGGAGACCGAGGTCTCGGTTTTAGAGCTAGAAATAGC-3'
forward-D	5'-AATCCACATCGGTTATTTGTCGGTCTTTAGAAGTCTTTATATGAATTAGGGCAAACAGACACCGTTGGAGACCGAGGTCTCGGTTTTAGAGCTAGAAATAGC-3'
forward-E	5'-ATCCACATCGTTTAGTTACATAAAATATTAGACTTTATAAGAAAATCAAGCTAGCTTCTGAAGTGGAGACCGAGGTCTCGGTTTTAGAGCTAGAAATAGC-3'
forward-F	5'-ATCCACATCGACAAGGAATACAAATTTAAAACGGTTTATAAGTAAACAAAAACGACATGCTACTGGAGACCGAGGTCTCGGTTTTAGAGCTAGAAATAGC-3'
reverse	5'-AAAACAAAAAGCACCGACTCGGTGCCACTTTTTCAAGTTGATAACGGACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAAC-3'
Oligos for introducing restriction enzyme sites into RcU6(n)-2xBso3II-gRNAgene constructs	
HindIII-A	5'-AAGCTTGAACCCACATCGATTTC-3'
HindIII-B	5'-AAGCTTAACCCACATCGTCTA-3'
HindIII-C	5'-AAGCTTGTCACATCGTCTG-3'
HindIII-D	5'-AAGCTTAATCCACATCGGTTA-3'
HindIII-E	5'-AAGCTTATCCACATCGTTTA-3'
HindIII-F	5'-AAGCTTATCCACATCGACAA-3'
SbfI-reverse	5'-CCTGCAGAAAAAAGACAC-3'
Oligos for testing vectors	
35Spr-F	5'-CTATCCTTCGCAAGACCCTTC-3'
Amp-R	5'-ATAATACCGCGCCATAGC-3'
pBR322ori-F	5'-GGGAAACGCCTGGTATCTTT-3'

HindIII and SbfI restriction sites were incorporated into the insertion sequence by means of PCR. Amplification involved primers selected for the borders of RcU6(n)-2xBso31I-gRNA gene constructs and containing corresponding restriction sites in the 5'-region (Table 5). PCR products were purified, cloned in the pAL2-T AT vector, and sequenced to verify the absence of synthesis errors. Once the plasmid pRGE31 and the pAL2-T plasmids featuring RcU6(n)-2xBso31I-gRNA gene structures were processed with HindIII and SbfI restriction endonucleases, the products were separated by electrophoresis, and target fragments were cut out. Purified fragments were ligated and used to transform competent *Escherichia coli* cells. Clones were selected by PCR with primers selected for the borders of RcU6(n)-2xBso31I-gRNA gene constructs. Plasmids extracted from the selected clones were tested with HindIII (linearizes the target plasmid) and Bso31I (does not break down the target plasmid), then sequenced using the insertion primers (SbfI-reverse) and primers for the target regions: Cas9 gene (35Spr-F), the ampicillin resistance gene (Amp-R), ori (pBR322ori-F) (see Table 5).

The created vector constructs were further compared against each other and against vectors containing the *Arabidopsis U6* gene in terms of effectiveness. Such experiments seem advisable, since, for example, potatoes have been shown to produce twice as many mutant forms where the gRNA gene promoter, AtU6 (*U6* promoter of *Arabidopsis thaliana*, GenBank accession no. X52527.1) was substituted with StU6 (*U6* promoter of *Solanum tuberosum*, GenBank accession no. Z17290.1) [9].

The same conclusions were made by Long et al. [11] when they researched into the optimization of CRISPR/Cas9 for editing the cotton genome. gRNA expression grew by a factor of 6-7 once the endogenous cotton promoter, GhU6.3 (*U6* promoter of *Gossypium hirsutum*) replaced AtU6-29 (*U6* promoter *Arabidopsis thaliana*) in the CRISPR/Cas9 vector structure. As a result, the mutagenic effectiveness of CRISPR/Cas9 rose by a factor of 4-6 [11]. In turn, Sun et al. [10] obtained similar results in their comparison of endogenous and exogenous promoters in CRISPR/Cas9 cassettes for soy genome editing. gRNA expression under GmU6 (*U6* promoter of *Glycine max*) was twice as high as under an exogenous promoter, AtU6-26 (*U6* promoter of *Arabidopsis thaliana*). The effectiveness of gene editing varied within 14.7% to 20.2% in the first case and within 3.2% to 9.7% in the second case [10]. These experiments were carried out on non-related plant species from different families; their results clearly show the benefits of using endogenous promoters in CRISPR/Cas9 vectors. However, it is important to compare not only endogenous vs. exogenous promoters but also endogenous promoters against each other. This research identified various castor bean *U6* promoters with USE and TATA regulatory elements which are necessary for normal functioning; it would be logical to compare them against each other in editing experiments on castor bean, as endogenous promoters, as was reported earlier, might vary in the effectiveness of controlling gene expression [27-30].

Thus, the castor bean genome contains six *U6* promoters with USE and TATA elements. Sequencing these promoters in Zanzibar Green and Gibbonskaya varieties showed them to be highly conservative. The promoters are medium-homologous to each other and to the promoters of other species. The promoters were found to contain USE elements; in three promoters, those matched the consensus sequence of the USE element in *Arabidopsis*; in three others, they did not. CRISPR/Cas9 based on the identified castor bean *U6* promoters will constitute a valuable tool for further attempts to edit the genome of this species and to evaluate the effectiveness of various promoters with respect to gRNA synthesis.

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VARIABILITY OF GENOMIC RGA-LOCI OF MODERN RUSSIAN POTATO CULTIVARS: NBS-PROFILING DATA

E.A. DYACHENKO ✉, A.V. KULAKOVA, E.Z. KOCHIEVA, A.V. SHCHENNIKOVA

Institute of Bioengineering, Federal Research Center Fundamentals of Biotechnology RAS, 33/2, Leninskii prospect, Moscow, 119071 Russia, e-mail dyachenko-el@yandex.ru (✉ corresponding author), kulakova_97@mail.ru, ekochieva@yandex.ru, shchennikova@yandex.ru

ORCID:

Dyachenko E.A. orcid.org/0000-0002-0570-9751

Kochieva E.Z. orcid.org/0000-0002-6091-0765

Kulakova A.V. orcid.org/0000-0002-3124-525X

Shchennikova A.V. orcid.org/0000-0003-4692-3727

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Abstract

The plant immunity is aimed at protecting against biotic and abiotic stresses and, therefore, at adapting to adverse environmental conditions. At the first protection step, a wide range of phyto-pathogen receptors encoded by resistance *R*-genes is employed. The presence of a conserved NBS-domain in the receptors makes it possible to profile the plant genome by amplification of *R*-gene analogs. The multilocus NBS-profiling method makes it possible to efficiently characterize the plant genome in terms of the representativeness and variability of the NBS-domain containing *R*-genes. This method is used to study the diversity of *R*-gene loci in crops and related wild species, as well as introgressive hybridization and the *R*-gene evolution in plant species with varying degrees of pathogen resistance. NBS-profiling is also applied for genotyping GenBank collections, developing markers and saturating genetic maps. The requirement for cultivar genotype certification and profiling, along with a limited number of similar studies in Russia, makes research on the molecular profiling of domestic and foreign cultivars farmed in the Russian Federation relevant. In the present work, NBS-profiling was used for genotyping 65 potato *Solanum tuberosum* cultivars of mainly modern domestic breeding, as well as the related species *Solanum stoloniferum* (as an outgroup). Using two primer/enzyme combinations (NBS7/MseI and NBS9/MseI), 204 NBS fragments were generated, of which 144 (70.6%) were polymorphic and one fragment was unique to cv. Gala. For each cultivar, a specific spectrum of NBS fragments was determined. Analysis of genetic distance matrix revealed a high level of polymorphism (GD = 0.18–0.45 with an average value of 0.33) among the studied cultivars. Genetic distances within the analyzed cultivars varied more than between the cultivars and *S. stoloniferum* accession (GD = 0.27–0.40). The most related cultivars were Solnechny/Pamyati Rogacheva (GD = 0.18) and Velikan/Vympel (GD = 0.19) originated from Lorch Potato Research Institute, and the most distant cultivars were Charoit/Red Scarlett (GD = 0.45). Statistical analysis of NBS-profiling data clustered studied potato cultivars in accordance with different traits and resistance to phytopathogens. On the dendrogram and graphs generated using the PAST and Structure 2.3.4 software, a pronounced tendency to group cultivars by traits of resistance to the Potato virus Y (*Potyvirus*, *Potyviridae*) and the Potato leafroll virus (*Polerovirus*, *Luteoviridae*) was shown. The primer/enzyme systems used in this study for NBS-profiling can be applied to study the mechanisms of potato resistance to biotic stresses.

Keywords: *Solanum tuberosum*, Russian cultivars, foreign cultivars, genomic polymorphism, NBS-LRR-profiling, RGA-analysis

Plant immunity is designed to protect against biotic threats and provide adaptation; it contains a broad range of *R*-genes (resistance genes) that encode phytopathogen receptors of several classes, NLR (nucleotide-binding leucine-rich repeat proteins) being the most common class [1, 2]. As the products of *R*-genes

contain conservative domains, plant genome can be analyzed by amplifying RGAs, or resistance gene analogs. Plant genome can contain hundreds of these genes due to tandem and ectopic duplications followed by local gene rearrangements and conversion [2]. NBS-LRR receptors contain two main conservative domains: the central domain NBS (nucleotide-binding site) and C-terminal domain, or LRRs (Leucine-Rich Repeats) [1, 3]. Gene families encoding NBS-LRR receptors have been identified by whole genome sequencing in several plant species: 57 genes in *Cucumis sativus*, 149 in *Arabidopsis thaliana*, and 653 in *Oryza sativa*. The genome of *Solanum tuberosum* L. was found to have 435 genes and 179 pseudogenes of NBS-LRR family [1].

Whilst the LRR domain sequence is relatively polymorphic, the NBS domain consists of several highly conservative and strictly ordered motifs: P-loop, Kinase-2, Gly-Leu-Pro-Leu (GLPL); mutations affecting them can disrupt receptor functioning [1]. Multilocus NBS profiling helps effectively describe plant genome in terms of the representation and variability of *R*-genes, the product of which contains an NBS domain. The method is based on amplifying genomic DNA sequences flanking the region that encodes the NBS domain of *R*-genes; it uses degenerate primers complementary to the highly conservative regions of the NBS domain [4].

NBS profiling is commonly used in research of the RGA locus diversity in crops such as potatoes, tomatoes, wheat, lettuce, etc. [5-7], as well as in related wild species [2, 4, 8]. NBS profiling also makes it possible to study introgressive hybridization between cultivated and wild species [6]. Besides, NBS profiling is used to study the evolution of *R*-genes in species that vary in their pathogen resistance; the method is employed to genotype gene bank collections, develop co-dominant markers, and saturate genetic maps [4, 6, 7].

This paper presents NBS profiling of potato cultivars and promising breeding clones; most have been bred in Russia.

The goal was to evaluate the NBS-LRR *R*-genes polymorphism and to find a possible correlation between clustering based on NBS analysis and resistance traits to various potato-affecting phytopathogens.

Materials and methods. For NBS-LRR profiling, 60 *Solanum tuberosum* cultivars and five promising breeding clones bred in Russia and abroad were used; for control, we used a related species, *Solanum stoloniferum*, which, among its other uses, is often utilized by breeders as a source of resistance to various phytopathogens. These cultivars were picked as they were (and are) part of research efforts covered by Russia's Federal Potato Breeding and Seed Farming Research Program. Most of these cultivars (59 out of 65; 90.77%) are in the Russian Public Register of Selected Breeds and Cultivars Allowed for Use in 2020 (<http://reestr.gossortrf.ru/reestr/culture/159.html>). Tubers were provided by the Russian Potato Research Center (Lorch Institute) and grown in a standard greenhouse environment (23 °C/25 °C, 16 h/8 h day/night).

Genomic DNA was extracted from tissues of 5-day sprouts by the CTAB protocol [9, 10] in two analytical replicates.

NBS profiling followed the standard protocol [4]. For analysis, 350 ng of genomic DNA from the accessions was digested with the *Mse*I endonuclease (Thermo Fisher Scientific, USA). The resulting fragments were ligated to appropriate adapters and then used in two-round amplification at a T100™ amplifier, ThermoCycler, Bio-Rad, USA. The first polymerase chain reaction (PCR) round had 30 cycles (denaturation 30 s at 95 °C, primer annealing 1 minute at 55-60 °C, elongation 2 minutes at 72 °C) using NBS7, NBS9 primers [4, 11]. An aliquot of the PCR mix (0.5 µl) was used in the second PCR round (same parameters) with an IRD 700/800-labeled NBS primer and a *Mse*I-adapter [4] primer. Amplification products were separated in a 6% denaturing polyacrylamide gel and visualized

550 bps, which could presumably be associated with the specific pathogen resistance in the analyzed cultivars.

A total of 204 NBS fragments were sampled, including 144 polymorphic fragments (70.6%) and a single unique fragment for cv. Gala, see Table 2. Potato accessions that have the most divergent *R*-genes spectra could be considered potential donors of resistance genes for breeding programs.

2. Polymorphism revealed by NBS profiling in 65 potato cultivars, breeding clones, and *Solanum stoloniferum*

Primer/enzyme combination	Number of NBS fragments			
	total	polymorphic		unique
		total	%	
NBS7/MseI	108	70	64.8	1
NBS9/MseI	96	74	77.1	0
Total	204	144	70.6	1

Statistical analysis of NBS spectra. NBS-LRR profiling data was used to obtain binary matrices for cluster analysis.

GD values of the tested cultivars varied within 0.18 to 0.45 ($GD_{mean} = 0.33$). Profiling found Lorch Institute-bred cultivars to be the closest: Solnechny and Pamyati Rogacheva ($GD = 0.18$), Velikan and Vympel ($GD = 0.19$). Maximum genetic distances were found between Charoit and Red Scarlett ($GD = 0.45$). Notably, GD between potato cultivars and *S. stoloniferum* accession varied insignificantly ($GD = 0.27$ - 0.40). This might be due to the fact that *S. stoloniferum* is commonly used in potato breeding as a donor of valuable resistance genes alleles that provide resistance foremost to viral pathogens [20]. Most of the tested cultivars were modern varieties, thus likely using in breeding programs interspecies hybridization involving several wild species as shown in [21].

There is only limited evidence on the *R*-genes variability in *Solanum* (including potato cultivars), which is why it seems interesting to compare the obtained data with the other plant species. Thus, NBS profiling of 32 Russian and non-Russian apple (*Malus domestica* Borkh.) cultivars showed their *R*-gene polymorphism (49% polymorphic fragments, $GD_{mean} = 0.14$) [22] being far below that of the tested potato cultivars ($GD_{mean} = 0.33$). NBS genotyping of wheat cultivars from Turkey, Kazakhstan, and Europe detected a comparable level of *R*-gene polymorphism ($GD_{mean} = 0.30$) [23]. Analysis of pepper cultivars (*Capsicum annuum* L.) results in an inter-variety polymorphism (GD of up to 0.26) at half the value detected in wild accessions of the same species (GD of up to 0.58) [8]. So it may be assumed that the resistance genes polymorphism in cultivars depends on both the variability of *R*-genes in the species genome and on the involvement of wild material in breeding.

Today, cultivar- and species-specific NBS profiling sees increasing use not only in genotyping, but also for the purpose of identifying new resistance genes to various diseases [2, 24]. The above-described primer systems for NBS analysis further could find use in studying biotic stress resistance mechanisms in potato cultivars.

A dendrogram was plotted on the basis of the obtained NBS spectra; the tested accessions form a high-polymorphism cluster split into groups with low bootstrap support (Fig. 2).

It seemed interesting to assess how accession clustering correlated with specific traits and with the breeding center (Table 1). No clear clustering by affiliation with the breeding center was found, that may be due to the intensive exchange of breeding material. Six non-Russian cultivars clustered into pairs (Red Impala and Saturna, Newton/Lady Clair, Gala/Impala); however, these pairs were not breeding center-specific (Fig. 2). Nevertheless, the dendrogram shows four clusters that mainly contain Lorch Institute-selected cultivars (see Fig. 2).

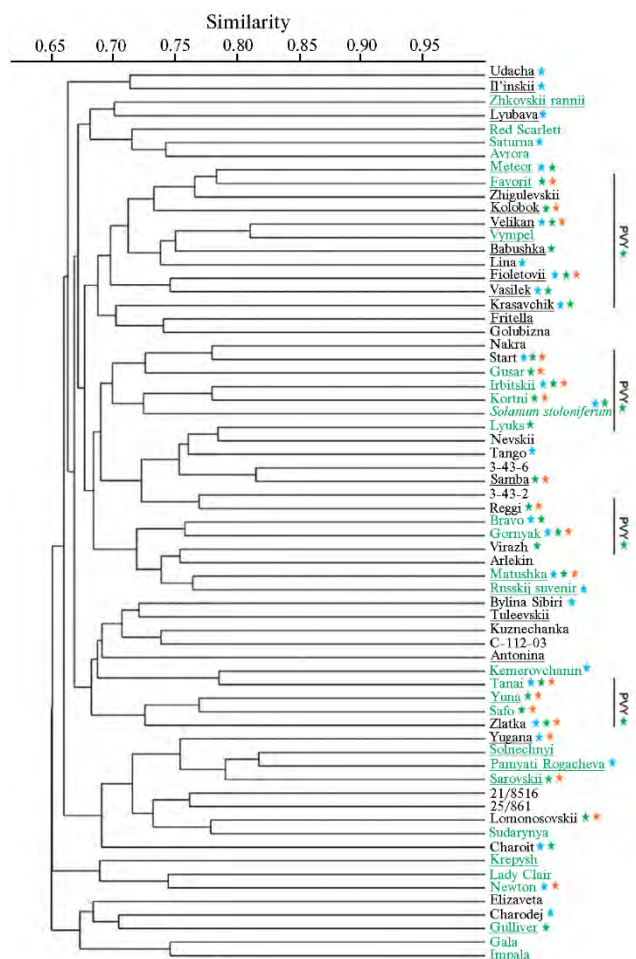


Fig. 2. Dendrogram of genetic variability in 65 tested cultivars and breeding clones of potatoes and *Solanum stoloniferum*, plotted by neighbor-joining (NJ, PAST software) on NBS profiling data. The highlighted cultivars are resistant to nematode (green font), Phytophthora (blue asterisk next to name), PVY (Potato virus Y, green asterisk), and PLRV (Potato leafroll virus, orange asterisk). Cultivars of Lorch Institute breeding are bold and underlined. For cultivar description, see Table 1.

Possible clustering of samples by resistance to phytopathogens was analyzed. Several small clusters were found that featured resistance to *Globodera rostochiensis* (Fig. 2); this could be due to the involvement of various donors including several wild species such as *Solanum acaule*, *S. spegazzinii*, or *S. vernei* [25]. Of all the five known *G. rostochiensis* pathotypes [26], only one is common in Russia: Ro1 [27]. Resistance to this pathotype is determined by the dominant alleles of the following genes: *H1* (Chromosome 5) and *Gro1* (Chromosome 7) [17, 28-32]; their loci contain numerous RGA copies [33, 34]. *H1* has been introduced by introgression from singular nematode-resistant *S. tuberosum* subsp. *andigenum*, *S. vernei* accessions [26, 35], whereas *Gro1-4* originated from *S. spegazzinii* [28].

Solanum demissum accessions are the core donors of resistance to phytophthora [25]. As far as this trait is concerned, the tested cultivars are scattered all over the dendrogram without apparent clustering (see Fig. 2). This might be due to the fact that BAK1/SERK3 proteins, which are involved in the protective response to phytophthora pathogens in plants, are leucine-rich repeat receptor-like kinases (LRR-RLK) that lack an NBS domain [36, 37].

PVY (Potato virus Y, *Potyvirus*, *Potyviridae*) is one of the most economically significant pathogens [38]. Upon infection, NBS-LRR gene Ny-1 (Chromosome

9) recognizes the viral effector [38, 39]. In the dendrogram, PVY-resistant cultivars fall into 4 groups (Fig. 2).

PLRV (Potato leafroll virus, *Polerovirus*, *Luteoviridae*) is considered the second most critical pathogen. Three PLRV resistance-associated quantitative trait loci are localized on Chromosomes 11 and 5 [40, 41]. Interestingly, clustering by PLRV resistance was found to overlap with clustering by PVY resistance (Fig. 2). Main donors of resistance to both viruses are wild species *S. demissum*, *S. acaule*, *S. chacoense*, and *S. stoloniferum* [25]. This may be the reason that *S. stoloniferum* accession grouped together with both PVY-resistant and PLRV-resistant cultivars in the dendrogram (Fig. 2).

Two cultivars, Fioletovy and Vasilek, had very peculiar clustering. Those have higher anthocyanin concentrations, making them the only cultivars in the tested population to have bluish-violet skin (Fioletovy also has similarly colored flesh). Both cultivars are Phytophthora and PVY-resistant. However, they have different breeding records. Although the intensity of anthocyanin accumulation has already been shown to correlate with the strength of response to phytophthora [42], this grouping indicates rather similarity in *R*-gene patterns and not with coloration. Interestingly, cultivar resistance description that the originators published in the Public Register do not always match molecular profiling data [30-32, 43, 44], which might be due to the difficulty of determining infection response symptoms by visual cues.

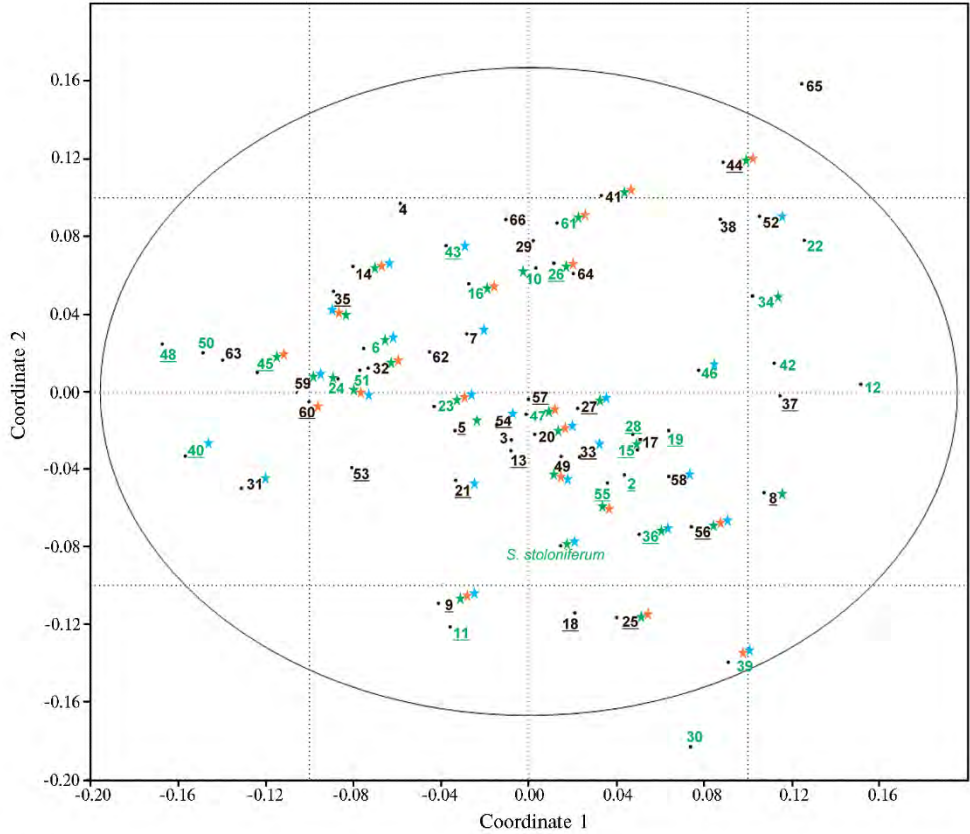
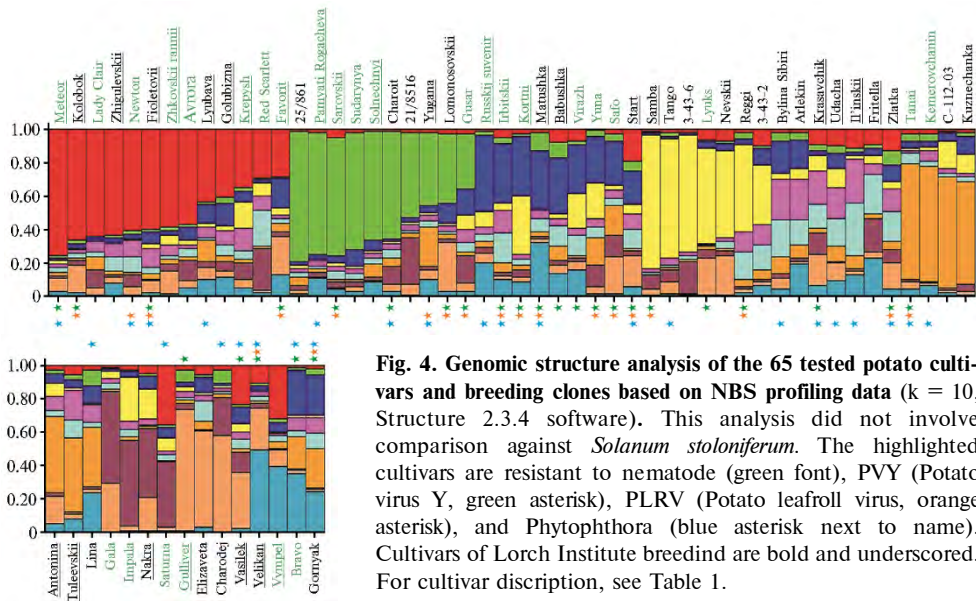


Fig. 3. PCA analysis of NBS profiling data for 65 studied potato cultivars, breeding clones, and *Solanum stoloniferum*. Cultivars resistant to nematode (green numbers), Phytophthora (blue asterisk next to number), PVY (Potato virus Y, green asterisk next to number), and PLRV (Potato leafroll virus, orange asterisk next to number) are indicated. Cultivars of Lorch Institute breeding are underscored. For cultivar description, see Table 1.

Cluster analysis was complemented with PCA that confirmed there was a single polymorphic group of cultivars and lines selected in Russia and other countries (Fig. 3). The PCA diagram shows a slightly different grouping from the dendrogram. Accessions tended to converge when they shared nematode and PVY/PLRV resistance or were from the same breeding center, although this tendency was weak. Non-Russian cultivars mainly occupied the right side of the diagram, whilst *S. stoloniferum* was in the center.

Also, according to the NBS-marking data, the analysis of the genomic structure of 65 analyzed potato accessions was carried out using the Structure 2.3.4 program. Variants of division into subgroups from $k = 2$ to $k = 15$ were tested, and the best result was obtained for $k = 10$ (LnLike = -75339.2). In Figure 4, the NBS labeling data of the analyzed samples are presented in the form of different ratios of blocks with sets of resistance genes.



The analysis made it possible to identify 10 clusters of accessions, in five of which one of the blocks in the genome prevailed. Also one group was identified where there were no major blocks, and the samples of this group showed the greatest polymorphism. The analysis performed did not reveal a single potato accession that would be represented by one or two blocks of resistance gene sets (Fig. 4).

Thus, as a result of multilocus analysis using the NBS-marking method, a collection of 60 cultivars and five breeding clones (*Solanum tuberosum*) of Russian and foreign breeding was characterized for the first time. In general, a high level of NBS-LRR gene polymorphism was revealed in Russian potato cultivars. We identified groups of accessions with the most similar and different patterns of NBS-LRR genes. Accessions tended to converge when they shared nematode and PVY (Potato virus Y)/PLRV (Potato leafroll virus) resistance or were from the same breeding center, although this tendency was weak. Primer/enzyme systems used in this study can be further used for NBS genotyping as part of primary screening for resistance to various biotic stresses, or in research of biotic stress resistance mechanisms.

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EFFICIENCY OF ETHYLENE APPLICATION ON SEED POTATO TUBERS

S.V. MALTSEV ✉

Lorkha Russian Potato Research Centre, 23-B, ul. Lorkha, pos. Korenevo, Lyuberetsky District, Moscow Province, 140051 Russia, e-mail stanmalcev@yandex.ru (✉ corresponding author)

ORCID:

Maltsev S.V. orcid.org/0000-0001-7211-315X

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Abstract

Potato (*Solanum tuberosum* L.) is one of the most important agricultural crops in Russia. Potato tubers contain protein of high biological value, starch and vitamins necessary for humans. There are various ways to increase the yield of the crop, currently, it is mainly due to application of mineral fertilizers. However, this method has limitations, since an increase in doses of fertilizers is associated with deterioration of consumer quality and culinary indicators of tubers. One possible alternative is the gassing of seed tubers with ethylene. This phytohormone is widely used on various crops, but its effect on potatoes has not yet been fully studied. It is known that depending on the treatment modes it can act as both a stimulator and an inhibitor of tuber germination. We have developed a new method for increasing potato yield based on the property of phytohormone ethylene to suppress apical dominance at the beginning of tuber germination and thereby promote the formation of a larger number of lateral sprouts from which additional stems are formed. The aim of the research was to determine the effect of seed potato tubers treatment with ethylene on their biochemical parameters, as well as on the growth, development and tuber yield of the crop. The experiments were conducted on the basis of storage facilities of JSC Ozyory (Ozyorsky District, Moscow Province). For gassing, the adapted technology of the company «Restraining Company Ltd» (Great Britain) was used. The storage temperature for seed potatoes was 4 °C; the period of ethylene gassing was from November to the end of April. The treatment with ethylene was stopped 3-5 days before planting. Ethylene-treated potatoes were planted in 2015-2017 on the territory of the Moscow region. A two-factor small-plot field trial was conducted in the Lyuberetsky District at the Korenevo experimental base, the soil was sod-podzolic sandy loam. Seed tubers of potato varieties Lady Claire (early), Gala (mid-early) and Saturna (mid-late) were treated with ethylene. In the control, ethylene was not applied. A three-factor large-scale (40 ha) field trial was performed during commercial potato growing of the same varieties (JSC Ozyory, Ozyorsky District, Moscow Province, sod-podzolic loamy soil). We used the same treatment as in experiment 1 added with 2-3 irrigation (200 m³/ha). In both experiments, the potatoes were planted in the first decade of May (45 thousand tubers per hectare, row spacing width of 75 cm; N₆₀P₁₂₀K₁₂₀ applied locally). Biochemical, biometric parameters, and potato yield were measured. It was found that the treatment of potato seed tubers with ethylene at 4 °C changes their biochemical parameters, i.e., dry matter content decreases by 0.2-0.5 % while sucrose increases by 0.03-0.08 %, which indicates the breakdown of tubers' dormancy. There was a suppression of apical dominance and the formation of more lateral sprouts on tubers, a greater number of stems per plant (by 19.9-36.0 %), and an increase in number of tubers per plant (by 6.3-19.0 %, especially in the Gala variety). Potato yield increased by 9.9-19.0 % depending on the variety, growing area and irrigation. The produced potato tubers were more uniform and marketable. Depending on the common agricultural level and the technology used at a farm, a differentiated approach is proposed in choosing the planting rate. If low agricultural technology and no irrigation, the planting rate of ethylene-treated tubers reduced by 10-15 % allows for the same yield as that for high technology and irrigation.

Keywords: potato, variety, ethylene, phytohormone, phenological phases, number of stems, yield, crop structure, Restraining technology

The phytohormone ethylene can act as both a stimulant and an inhibitor of potato germination. The stimulating properties of ethylene, which determine the release of tubers from the state of natural dormancy, have been known since 1925 [1], and its inhibitory properties were revealed in 1932-1933 [2, 3]. In the

work of Ryłski et al. [4], the double effect of ethylene was shown. Its short-term exposure (72 h in the range of 0.02–20 ppm) stimulated the onset of germination of tubers, and continuous action during long-term storage of potatoes suppressed the development of sprouts. There is information [5–8] that ethylene promotes an increase in the number of sprouts, but at the same time prevents their growth in length (elongation).

The perception of ethylene begins with its binding to receptors localized in all higher plants in the membrane of the endoplasmic reticulum and the Golgi apparatus [9]. The unusual intracellular localization of receptors does not interfere with the perception of the hormone, since gaseous ethylene freely diffuses in lipid and aqueous media [10, 11]. It is assumed that the binding of ethylene to receptor histidine kinases changes their conformation, inactivates the complexes of receptors with CTR1 (Ser/Thr-protein kinase, similar to protein kinases of the Raf family). This leads to dephosphorylation of the EIN2 protein, similar to the metal ion transporter Nrap, and the cleavage of its C-terminal domain, which is transferred into the nucleus and initiates the transcriptional response of ethylene-dependent genes due to the sequential activation of transcription factors of the EIN3/EIL1 and ERF families [12]. It is known that with continuous prolonged exposure to ethylene in tubers, the ratio of polyamines involved in the regulation of cell division and growth changes [13, 14]. Such changes are variety-specific and associated with potato germination [15, 16].

Previously, various chemical compounds, primarily 2-chloroethylphosphonic acid, were used as “ethylene producers”; however, as applied to potato culture, other ethylene supply systems are more technologically advanced [17, 18].

Currently, two UK companies supply ethylene management systems for potato storage. Both use the same standards for permitted ethylene concentrations (up to 50 ml/m³, the dosage is specified by the manufacturer, depending on the purpose of the potato). One of the companies (BioFresh Ltd.) supplies pure ethylene in cylinders (which is potentially explosive); the gas concentration in the storage is monitored by a chemiluminescent sensor. Another company (Restrainer Company Ltd., represented in Russia by Peya Agro LLC) produces a system in which ethylene is made of ethanol [19].

There are many techniques and methods aimed at increasing the yield of potatoes, e.g., the use of virus-free seeds of high reproduction, pre-planting treatment of tubers by chemical and physical methods, protection of plantings from weeds, diseases, and pests, application of fertilizers, primarily mineral fertilizers [20]. However, the use of mineral fertilizers, even with an optimal NPK ratio, taking into account soil and climatic conditions, often leads to a decrease in the accumulation of dry matter, starch in the tubers, a deterioration in consumer (pulp resistance to darkening) and culinary parameters (taste, friability), as a result of which the suitability of potatoes for processing, such as vacuum packaging and quick freezing, is reduced [21].

Here, a new method are reported for increasing potato productivity, based on the property of the phytohormone ethylene to suppress apical dominance at the beginning of tuber germination and thereby promote the formation of a larger number of lateral shoots from which additional stems are formed.

The aim of the research is to determine the effect of ethylene treatment of seed tubers of potatoes (*Solanum tuberosum* L.) on their biochemical parameters, as well as on the growth, development, and yield of the crop.

Materials and methods. The experiments were carried out on the basis of the storage facilities of Ozyory JSC (Ozyorsky District, Moscow Province). The storage capacity was 2000 tons. For gassing, the adapted technology of the Restrainer

Company Ltd. (Great Britain) was used. The adaptation of the technology to Russian conditions consisted in the fact that the storage temperature of seed potatoes was not 6-8 °C, as is recommended for the climatic conditions of Western countries, but 4 °C; the period of gassing with ethylene was 1 month longer (from November to the end of April, and not to the end of March). Since the winters in the Central region of Russia are colder and longer, and the planting of potatoes begins 1 month later, it was risky to maintain an elevated temperature in the storage, since in this case, premature germination of tubers is possible. In order for the tubers to start germinating in a timely manner, the ethylene supply was stopped 3-5 days before planting.

Potatoes treated with ethylene were planted in 2015-2017 in the Moscow Province. A two-factor field experiment (experiment 1; factor A was a potato variety and factor B was ethylene treatment) was carried out in the Lyuberetsky District at the Korenevo experimental base (a small plot experiment, sod-podzolic sandy loam soil). Seed tubers of potatoes of foreign breeding varieties Lady Claire (early), Gala (mid-early), and Saturna (mid-late) were treated with ethylene (gassing at a dose of 15 ml/m³; no treatment was carried out in the control). The experiment was repeated 3 times. A three-factor experiment (experiment 2; factor A was a potato variety, factor B was treatment with ethylene, factor C was irrigation) was performed in the Ozyorsky District during large-scale production of potatoes on the basis of the Ozyory JSC farm. The soil was sod-podzolic loamy; the total area of the experiment was 40 hectares. The cultivars and the tillage were the same as in experiment 1. Irrigation of crops (2-3 irrigations of 200 m³/ha, control without irrigation) was carried out using sprinkler machines of the Fregat type (Plant Fregat PJSC, Ukraine). In the experiment, there were two variables (with and without irrigation). The experiment was repeated 3 times.

In both experiments, potatoes were planted in the first decade of May. Planting density was 45 thousand tubers/ha, row spacing 75 cm, background mineral nutrition N₆₀P₁₂₀K₁₂₀ with local fertilization.

Biochemical (the content of dry matter, sucrose, glucose, reducing sugars, nitrates, vitamin C) and biometric (the number of stems and tubers per bush) indicators, as well as the yield of potatoes, were determined according to GOST 29270-95 [22] and the methodology of the Federal State Budgetary Scientific Institution of the Federal Research Center of Potatoes named after A.G. Lorch (Russian Potato Research Centre) [23]. Mathematical data processing by the method of analysis of variance was carried out according to Dospekhov [24] using the AgCStat software package as an add-on to Microsoft Excel. The mean value of the sample (M) and the standard deviation of the mean ($\pm\sigma$) were calculated. The significance of differences was assessed by Fisher's F -test. With $F_{\text{fact.}} \geq F_{\text{theor.}}$, the null hypothesis was rejected (there are significant differences between the sample means), and the test ended with the calculation of the least significant difference for the 5% significance level (LSD₀₅).

Results. The equipment used in the experiment is shown in Figure 1. Treatment with ethylene during long-term storage of seed tubers led to a change in their biochemical parameters. The amount of dry matter decreased by 0.2-0.5% (LSD₀₅ 0.3%), sucrose increased by 0.03-0.08%, glucose varied within the statistical error (LSD₀₅ 0.05%). This indicates the physiological effect of ethylene on the release of tubers from the state of natural dormancy [25-27], accompanied by a short-term increase in respiration intensity, which is consistent with the literature data [28-30]. The nitrate content did not exceed the maximum permissible concentration (90-100 mg/kg, LSD₀₅ 28 mg/kg). The amount of vitamin C depended on the variety and shelf life of potatoes (19.7-29.0 mg% at LSD₀₅ 1.3-1.5 mg%),

but not on the use of ethylene.

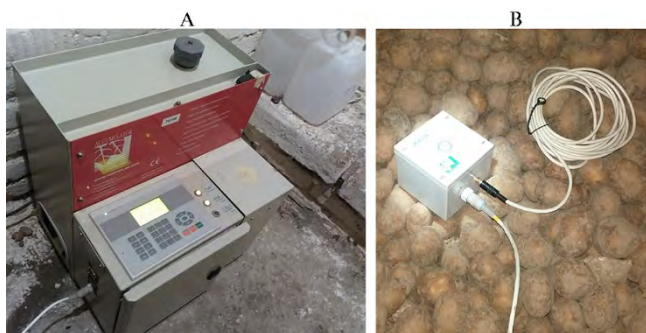


Fig. 1. Equipment used for ethylene treatment of seed tubers of potatoes (*Solanum tuberosum* L.) varieties Lady Claire, Gala and Saturna: A — ethylene generator ACCUMULATOR (Restrain Company Ltd., Great Britain) (installed in the storage and, when heated, catalyzes the catalytic decomposition of 96% alcohol ethanol for ethylene and water), B — an ICA534 ethylene concentration sensor (Restrain Company Ltd., Great Britain), located on the surface of the potato bulk, which controls the operation of the generator.

The growth and development of plants in field experiments were largely determined by the meteorological conditions of the growing season, especially when grown in the Lyuberetsky District of the Moscow Province, where irrigation was not used. Therefore, the analysis of the efficiency of ethylene use is given in close relationship with meteorological conditions (Table 1), separately for each year.

As known, the lack of moisture in the budding-flowering phase can-

not be compensated for by subsequent precipitation (critical phase of development). The rains in May and June determine the number of potato tubers, and the rains in July and August determine the mass of tubers [31].

1. Brief characteristics of meteorological conditions during the growing season of potatoes in the areas of field trials (Moscow Province)

District	2015				2016				2017			
	May	June	July	August	May	June	July	August	May	June	July	August
Lyuberetsky	++	+	-	—	++	-	++	++	+	+	++	++
Ozersky	++	+	++	—	++	++	+	++	+	+	++	++

Note. “++” — very favorable conditions for the growth and development of potatoes, “+” — favorable (slightly higher than the average long-term norm), “-” — unfavorable (slightly lower than the average long-term norm), “—” — very unfavorable (drought).

The year 2015 was the least favorable for the growth and development of potatoes. In the Lyuberetsky District, a drought was observed in July-August (see Table 1). In the Ozyorsky District, it only began in August, therefore, due to the greater water-holding capacity of the loamy soil in comparison with the sandy loam plants of the varieties Lady Claire, Gala, and Saturna, it was possible to accumulate a larger crop than in the Lyuberetsky District by 45%, 50%, and 50%, or 7.6, 8.1 and 7.1 t/ha, respectively. With double irrigation in Ozyorsky District in August, the moisture deficit was almost completely compensated, which ensured an increase in yield (without treatment of tubers with ethylene) in the variant with irrigation compared to the variant without irrigation by 27%, 33%, and 21%, or 6.6, 7.9, and 4.4 t/ha, respectively. In addition, watering helped to increase the yield of commercial fraction tubers (> 50 mm in size) by 4-5%.

In 2015, potato varieties with a longer ripening period (Saturna, Gala), when grown without irrigation in the Lyuberetsky District, were in a less favorable position compared to the early maturing variety Lady Claire, which to a greater extent managed to realize the biological potential of yield before the onset of drought.

Treatment of potato seed tubers with ethylene in 2015 led to an increase in the number of peaked eyes (mainly due to lateral ones with the exclusion of apical dominance) and the emergence of seedlings 3-5 days earlier than in the

control. The plants were 3-5 cm taller and outperformed the control in terms of leaf area. The number of stems per bush was higher in the varieties Lady Claire, Gala, and Saturna by 20.0-22.4, 33.3-40.0, and 16.3-22.2%, respectively. The phase of the onset of tuberization began a few days earlier, and the number of tubers per bush was greater — by 18.6-22.2, 23.5-31.9, and 11.8-19.1%, respectively (Fig. 2).

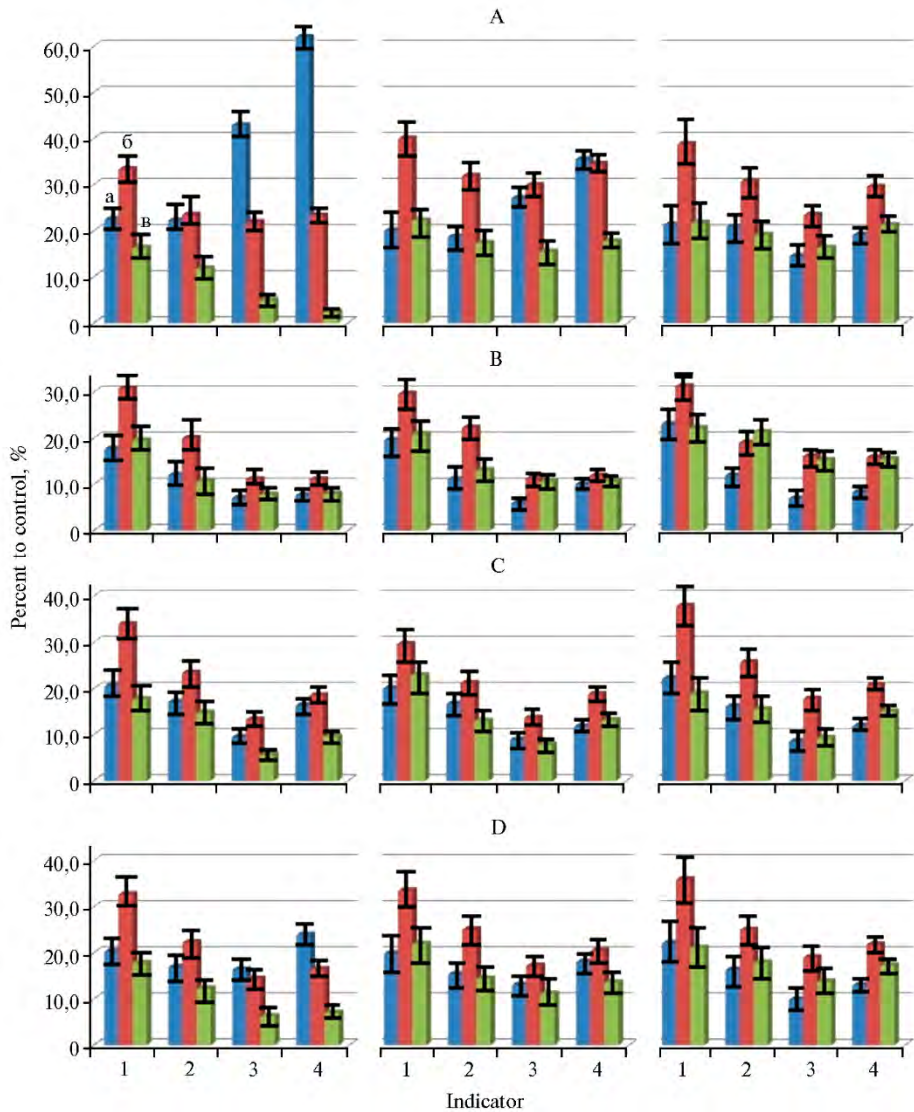


Fig. 2. Increase in growth indicators, development and yield of potatoes (*Solanum tuberosum* L.) varieties Lady Claire (a), Gala (b), and Saturna (c) when treating seed tubers with ethylene in 2015 (A), 2016 (B), and 2017 (C) and on average for three years (D): 1 — number of stems, 2 — number of tubers, 3 — total yield, 4 — marketable yield; Lyuberetsky District, sandy loam soil, without irrigation (left), Ozyorsky District, loamy soil, without irrigation (the center), — Ozyorsky District, loamy soil, with irrigation (right) ($M \pm \sigma$; Moscow Province). The observed differences with the control are statistically significant at $p < 0.05$.

Ultimately, the yield in variants with the treatment of seed tubers with ethylene in 2015 for these varieties increased depending on the area of cultivation and the use of irrigation by 14.5-42.9, 21.9-29.9, and 4.9-16.3%, or 4.5-7.2, 3.5-7.5, and 0.7-4.2 t/ha, respectively. A more homogeneous crop structure was also noted (Fig. 3).



Fig. 3. Potato tubers (*Solanum tuberosum* L.) under a bush of the Lady Claire variety when grown on sod-podzolic loamy soil: A — без обработки этиленом (контроль), Б — с обработкой семенных клубней этиленом (Озерский р-н, Московская обл., 15 августа 2015 года).

The revealed patterns applied to all the studied varieties, but they were especially evident in the early variety Lady Claire when grown in the Lyubertsy District without irrigation. Under these conditions, an increase in the total yield, as well as an increase in marketable yield, which reached 62.3%, or 7.6 t/ha, was due not only to the stimulating effect of ethylene on the formation

of additional tubers but also to a shift in the passage of phenological phases of development by more early timing, which was critical. Varieties of later maturity groups, especially Saturna, under conditions with a shortened favorable growing season were less responsive to the treatment of seed tubers with ethylene. In the structure of their yield, the fine fraction increased, that is, the advantage in additional tuberization was not realized.

2. Biometric indicators and yield of potatoes (*Solanum tuberosum* L.) of different varieties in the control and in the treatment of seed tubers with ethylene, depending on the area of cultivation and the use of irrigation ($M \pm \sigma$, Moscow Province, 2015-2017)

District (soil)	Treatment (irrigation)	Number per bush		Yield, t/ha	
		stems	tubers	total	commercial (tubers of fraction > 50 mm)
Cv. L a d y C l a i r e (e a r l y)					
Lyuberetsky	Control	5.1±0.4	10.0±0.6	23.8±0.9	18.9±0.7
(sandy loam soil)	Ethylene	6.1±0.5	11.7±0.7	27.7±1.0	23.5±0.8
LSD ₀₅		0.3	0.5	0.8	0.6
Ozyorsky	Control (no irrigation)	5.2±0.4	11.3±0.8	28.3±1.4	24.0±1.1
(loamy soil)	Ethylene (no irrigation)	6.2±0.6	13.0±0.9	32.0±1.5	28.3±1.2
	Control (irrigation)	5.3±0.5	12.3±0.7	32.4±1.5	28.8±1.2
	Ethylene (irrigation)	6.4±0.6	14.3±1.0	35.6±1.6	32.5±1.3
LSD ₀₅		0.4	0.7	1.2	1.0
Cv. G a l a (m e d i u m e a r l y)					
Lyuberetsky	Control	5.4±0.5	17.4±0.7	23.9±1.0	19.1±0.7
(sandy loam soil)	Ethylene	7.2±0.6	21.2±0.7	27.3±1.1	22.2±0.8
LSD ₀₅		0.3	0.6	0.8	0.6
Ozyorsky	Control (no irrigatio)	5.8±0.4	18.3±1.0	29.4±1.3	25.3±1.1
(loamy soil)	Ethylene (no irrigatio)	7.7±0.6	22.9±1.1	34.5±1.5	30.5±1.2
	Control (irrigatio)	6.3±0.6	19.2±0.9	33.9±1.4	30.4±1.2
	Ethylene (irrigatio)	8.6±0.7	24.0±1.0	40.3±1.6	37.0±1.4
LSD ₀₅		0.5	0.9	1.2	1.0
Cv. S a t u r n a (m e d i u m l a t e)					
Lyuberetsky	Control	3.9±0.3	9.8±0.5	19.7±0.8	14.6±0.6
(sandy loam soil)	Ethylene	4.6±0.3	11.0±0.6	20.9±0.8	15.7±0.6
LSD ₀₅		0.2	0.5	0.7	0.5
Ozyorsky	Control (no irrigatio)	4.1±0.4	10.6±0.7	23.3±1.1	19.3±1.0
(loamy soil)	Ethylene (no irrigatio)	5.0±0.4	12.2±0.8	25.9±1.2	22.0±1.1
	Control (irrigatio)	4.3±0.4	11.0±0.7	26.4±1.4	22.7±1.1
	Ethylene (irrigatio)	5.2±0.5	13.0±0.8	30.0±1.5	26.7±1.3
LSD ₀₅		0.4	0.6	1.2	1.0

The use of ethylene on seed potatoes in 2016 and 2017, more favorable in terms of moisture supply, had a similar effect on the growth of biometric indicators and yield (see Fig. 2). In relative terms, the effect of ethylene was noticeably lower than in 2015 (for the varieties Lady Claire, Gala, and Saturna, the increase in

yield was 6.9-9.5, 11.1-17.8, and 5.6-15.3%, respectively, compared to the control); however, in absolute values, taking into account the higher yield, there was almost no difference compared to 2015 (1.8-2.8, 3.1-6.2, and 1.2-4.5 t/ha, respectively). The Gala variety was the most responsive to the use of ethylene under favorable growing conditions and on average over 3 years (Table 2).

Similar data using the Restrained technology adapted to Russian conditions on seed potatoes were obtained in 2015-2016 in the Dmitrovsky District of the Moscow Province [32, 33]. The use of the original Restrained technology when growing the same varieties in Great Britain and the Netherlands in combination with more favorable climatic conditions (moisture supply is 2 times higher) and with a high culture of farming provided a comparable increase in plant growth and development (+40% stems per bush; +20% of tubers per bush) and an increase in total yield up to 20% [34].

The increase in the yield of potatoes when treated with ethylene in our experiments was not accompanied by a decrease in the content of starch and dry matter in the tubers, which often happens with the introduction of increased doses of mineral fertilizers. In the control, these indicators over 3 years averaged 17.5 and 23.2% for the Lady Claire variety, 16.4 and 22.0% for Gala, 18.2 and 23.9% for Saturna. When treating seed tubers with ethylene, the values were as follows: Lady Claire 17.6 and 23.3%, Gala 16.5 and 22.1%, Saturna 18.1 and 23.8%. The content of reducing sugars in the post-harvest period for the studied varieties, regardless of the treatment option, was 0.15-0.20%. The resistance of the pulp of tubers to darkening did not decrease (8.0-8.5 points on a 9-point rating scale both in the control and in the variant with ethylene treatment). In combination with achieving a more uniform crop structure, this is extremely important when growing potatoes for processing into crisp, frozen potatoes (taking into account the requirements for the shape of the tubers) and in vacuum packaging. With early harvesting periods, it becomes possible to obtain a higher yield of a uniform seed fraction of potatoes.

In addition, the treatment of seed tubers with ethylene makes it possible to use a differentiated approach in the choice of the seeding rate depending on the level of crop cultivation. In the absence of irrigation and low agricultural technology, the seeding rate can be reduced by 10-15%, based on the fact that the use of ethylene will achieve the same stalk per hectare and no less yield than with the usual seeding rate, that is, one can save on seed material. With a high level of agricultural technology, the availability of irrigation systems, and the use of fertilizers, it is possible not to reduce the seeding rate of ethylene-treated seeds, since potato plants will be able to realize their full biological potential and accumulate high yields.

Thus, the treatment of seed tubers of the potato varieties Lady Claire, Gala, and Saturna with ethylene according to the Restrained technology adapted to Russian conditions contributed to a change in the biochemical parameters of tubers (the dry matter content decreased by 0.2-0.5%, the amount of sucrose increased by 0.03-0.08%), suppression of apical dominance, the formation of a larger number of stems (by 19.9-36.0%) and tubers (by 6.3-19.0%) per bush, the formation of additional lateral shoots, and an increase in yield by 9.9-19.0%, depending on the variety, the area of cultivation, and the use of irrigation. A more homogeneous crop structure was also noted. There was no significant effect on the content of nitrates and vitamin C in tubers. The most responsive of the studied varieties was the medium early variety Gala under irrigation. In general, the treatment of seed tubers with phytohormone ethylene makes it possible to increase the

yield of potatoes without the use of increased doses of mineral fertilizers, which eliminates the associated negative consequences such as a decrease in the content of dry matter, starch, and suitability for processing.

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GENOTYPE AND ENVIRONMENT INFLUENCE ON THE RATE OF GRAIN MOISTURE LOSS IN CORN DURING RIPENING PERIOD

**V.S. SOTCHENKO¹, A.E. PANFILOV² ✉, A.G. GORBACHEVA¹, N.I. KAZAKOVA²,
I.A. VETOSHKINA¹**

¹All-Russian Research Institute of Corn, 14-B, ul. Ermolova str., Pyatigorsk, 357528 Russia, e-mail 976067@mail.ru, gorba4ewa.a@yandex.ru, vetira2014@yandex.ru;

²South Ural State Agrarian University, 13, ul. Gagarina, Troitsk, Chelyabinsk Province, 457100 Russia, e-mail al_panfilov@mail.ru (✉ corresponding author), kni1711@yandex.ru

ORCID:

Sotchenko V.S. orcid.org/0000-0002-0741-4412

Kazakova N.I. orcid.org/0000-0001-7623-4178

Panfilov A.E. orcid.org/0000-0001-5026-1274

Vetoshkina I.A. orcid.org/0000-0002-8040-7040

Gorbacheva A.G. orcid.org/0000-0001-9936-4565

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Abstract

High grain moisture during harvesting is a factor that restrains the expansion of corn (*Zea mays* L.) crops in the Ural region. A decrease in the harvesting moisture content of seeds is achieved by corn breeding for early maturity (early flowering of the ear) and for accelerated moisture loss from grain in the pre-harvesting period. In our study, it was found for the first time that in the conditions of southern Russia under weather conditions that weakly limit moisture losses, the harvesting moisture of corn grain is determined by the rate of pre-harvesting moisture losses while in the Ural region where the weather conditions are periodically unfavorable the moisture losses are mainly due to the early maturity of the studied hybrids. This work aims to identify corn hybrids with an increased rate of moisture loss during grain ripening and to assess this trait under unfavorable hydrothermal conditions. The studies were carried out in 2016-2018 in two contrasting geographical sites, in the foothill zone of the North Caucasus (experimental field of the All-Russian Research Institute of Corn, the settlement Pyatigorskii, Predgornii District of the Stavropol Territory) and in the northern forest-steppe of the Southern Urals (South Ural GAU, village Miasskoye, Krasnoarmeyskiy District, the Chelyabinsk Province). Six ultra-early corn hybrids (*Zea mays* L.) Nur, Ross 130 MV, Obsky 140 SV, Kubansky 141 SV, Mashuk 150 MV, Uralskiy 150 and early ripening Bilyar 160 hybrid were involved in the study. The field experiment was arranged in triplicate using a randomized field plot layout (28 m² plots with a full set of hybrids per plot). The recorded phenological phases were germination, ear flowering and physiological ripeness of grain detected by the corn kernel "black layer" as an indicator of physiological maturity. The corn grain moisture was measured gravimetrically from July 25 to September 11 in the North Caucasus and from August 15 to October 10 in the South Urals. Samples weighting not less than 70 g were dried at 150 °C to a constant weight (a SNOL 58/350 exicator, Elektrotehnika, AB, Republic of Lithuania; a CAS MW-II electronic Weighing Scale, CAS Corporation, Republic of Korea). For analysis, 10 ears of 1.2 to 1.8 kg were collected and completely threshed in three reps. Grain sampling was carried out with a 3-7-day interval providing 8 to 16 control points. Statistical hypotheses were tested by methods of variance, correlation and regression analysis. It was found that in the northern site, the calendar dates of flowering of the ear occurred in the beginning or end of the third decade of July, that is, 22-36 days later than in the south site. In the conditions of the North Caucasus, flowering occurred in the third decade of June—early July, the grain physiological ripeness was reached during the first week of August, that is, in ultra-early maturing hybrids, grain maturation and ripening occurred under favorable hydrothermal conditions. Consequently, environmental conditions did not limit the rate of moisture loss in grain, which provided reliable estimates of the differences between hybrids according to the trait. The research revealed significant differences in the rate of moisture loss both between the sites of trials and the hybrids. In the North Caucasian region, the rate of moisture loss in grain after reaching physiological ripeness varied for hybrids from 0.63 to 0.78 % per day with slight variation over the years. Under higher relative humidity and low air temperature the likelihood of which in the pre-harvest period is high for the Ural region, the rate of moisture loss decreased to 0.21-0.35 % per day, and under favorable hydrothermal conditions it reached only 0.52-0.72 % per day. It has been established that the harvest moisture content of corn grain is associated with two main characteristics

of hybrids, i.e., the early maturity and the ability to quickly lose moisture at the final stages of ontogenesis. The contribution of each factor is due to the conditions of grain ripening. I.e., for the south of Russia, grain moisture is largely due to the ability of a hybrid to accelerate moisture losses. On the contrary, in the conditions of the Southern Urals, the early flowering of the ear is of primary importance. Differences between hybrids in the rate of moisture loss under these conditions appear irregularly and are often leveled by the influence of the environment. Therefore, for the northern zone of corn sowing, the corn breeding for its ability to rapidly lose moisture from grain makes sense only in combination with breeding for a short growing season which should be considered as a priority trait when creating adapted hybrids.

Keywords: corn, hybrids, ultra-early maturity, ontogenesis, vegetation period, grain moisture, moisture loss rate, Southern Urals, Northern Caucasus

One of the tendencies in developing corn sowing in Russia is the expansion of the sowing of this crop in the northern direction, including in the vast and diverse Ural region in terms of the complex of limiting factors. This process is held back by the high level of grain moisture during harvesting. The most severe restrictions on the maximum level of harvesting moisture content (25-30%) arise when harvesting grain with thermal drying, which is intended for long-term storage [1]. The efficiency of producing alternative types of feed from corn (preserved wet grain and high-energy silage) also depends on the moisture content in grain due to the close correlation between this parameter and the rest of the chemical composition of the caryopsis, including starch, the main target component for growing crops [2-5].

The main way to reduce grain moisture in the field conditions is the selection of early maturity corn. Depending on the growing area and the purpose of crops (for silage or grain), the hybrids adapted for the Urals should be characterized by numbers from 110 to 170 units FAO [6, 7]. The creation of hybrids with early flowering is a significant part of Russian programs for the selection of corn [8]. In world breeding, this direction develops locally, mainly to fill northern ecological niches [9-11]. Most foreign publications indicate high potential productivity [12-15], resistance to drought [2, 16-18], and resistance to cold [19, 20] as the main goals of corn breeding. It is noted that the final feature is indirectly related to the problem of reducing the harvest moisture content of grain, since it ensures the stable development of corn in the conditions of low temperatures.

When creating ultra-early samples of corn, recurrent selection for early flowering from industrial hybrids or hybrids, specially created for this purpose, as well as from local populations with high genetic diversity for various traits, can be used [10, 21, 22]. Isolated populations, donors of a wide range of economically useful traits, are of particular value [23]. Formed under the influence of long-term artificial and natural selection, they serve as potential sources of early maturity [24].

The modern development of early maturity selection ensures the guaranteed achievement of physiological ripeness by the corn grain practically up to 56° northern latitude. However, even in ultra-early hybrids of the FAO 130-150 ripening class, adapted for regions with scarce heat resources, the harvest moisture content of grain under the influence of hydrothermal conditions varies widely and acquires the values, at which thermal drying is associated with unreasonably high costs [25].

As a supplementary or alternative direction, providing the regulation of grain moisture content during the ripening period, the selection of corn for accelerated moisture loss in grain is considered [26-29]. The ability of corn grain to quickly yield moisture is a polygenic trait due to a complex of morphological and physiological characteristics of the genotype: endosperm type (odontoid, siliceous, or intermediate), grain size, the density of wrapping leaves of an ear, ear height above the soil surface, core thickness, and tolerance to suboptimal temperatures [29-32]. A high variation in the rate of moisture loss, from 0.5 to 1.3% per day,

was noted both in industrial hybrids and in self-pollinated lines [33-36].

The process of reducing grain moisture in the pre-harvesting period takes place in two stages. Before the onset of physiological ripeness, it is due to the accumulation of organic matter in the endosperm and depends mainly on the air temperature. After physiological ripeness, moisture is lost due to physical evaporation, the rate of which is strongly inversely related to the relative humidity of the air and the amount of precipitation [31, 37, 38]. As a result of the complex interaction between the genotype and the environment, unstable dynamics of moisture loss are observed. Thus, under conditions that do not limit the physiological and physical processes of moisture loss, the intensity of moisture yield in specific samples is reproduced quite stably and varies slightly over the years [29, 31, 39]. However, an unfavorable hydrothermal background can neutralize the manifestation of the genetic characteristics of hybrids [40]. Excessive moisture during this period negatively affects the dynamics of grain moisture, even in hybrids with a potentially high capacity for moisture loss.

The difficulty in breeding corn for accelerated moisture loss in grain is that it is often associated with a decrease in the overall adaptability of plants to unfavorable environmental factors, in particular to water stress [29]. Nevertheless, the instability of the negative correlation between the harvesting moisture and grain yield is noted, which allows for positive selection for both traits and the creation of hybrids with a quick moisture loss without a decrease in the yield [37].

For the northern zone of corn cultivation, the effectiveness of the selection for the ability to accelerate moisture loss in grain has not been determined, and the likelihood of manifesting the genetic potential of hybrids for this trait in the Urals requires an experimental assessment against the background of fluctuations in hydrothermal conditions, which are typical for the region.

In this study, it was established for the first time that in the south of Russia, under weather conditions that weakly limit the moisture loss in corn grain, its harvest moisture was determined by the moisture loss rate in the pre-harvest period, while in the Ural region, given a periodically unfavorable weather background, it was mainly due to the early maturity of the studied hybrids.

The work aimed to identify corn hybrids with an increased rate of moisture loss during grain ripening and to assess the manifestation of this trait under unfavorable hydrothermal conditions.

Materials and methods. The studies were conducted in 2016-2018 in two contrasting geographical points (the foothill zone of the North Caucasus, the experimental field of the All-Russian Research Institute of Corn, Pyatigorskiy settlement, Predgorny District, the Stavropol Territory and in the northern forest-steppe of the Southern Urals, Southern Ural GAU, village Miasskoye, Krasnoarmeysky District, the Chelyabinsk Province) on six ultra-early corn hybrids (*Zea mays* L.) Nur, Ross 130 MV, Obsky 140 SV, Kubansky 141 SV, Mashuk 150 MV, Uralskiy 150, and early ripening Bilyar 160. The field trials were arranged in triplicate according to randomized design with 28 m² plots. Sowing dates in the North Caucasus were decade 3 of April, in the Southern Urals decade 2 of May. The actual plant density with manual formation was 70,000/ha. The phases of germination, flowering of the ear, and physiological ripeness of the grain were registered, the onset of which was judged by the appearance of a dark layer at the base of the caryopsis [30, 31]. The moisture content of grain in the North Caucasus was estimated from July 25 to September 11, in the Southern Urals from August 15 to October 10.

The moisture content of the grain was determined by the gravimetric method. The samples with a mass of at least 70 g were dried at a temperature of

150 °C (drying oven SNOL 58/350, Elektrotechnika, AB, Lithuania) to constant weight (it was determined using an electronic weighing scale CAS MW-II, CAS Corporation, Republic of Korea). For the analysis, 10 average ear samples weighing from 1.2 to 1.8 kg were taken and completely threshed in 3 repetitions. When choosing a method, the authors were guided by the criterion of the minimum measurement error. The periodic grain sampling was conducted with an interval of 3-7 days, which provided from 8 to 16 control points.

The statistical hypotheses were tested by the methods of variance, correlation and regression analyses. The reliability of the differences between the mean for the variants was assessed by Fisher's test (F) and the least significant difference (LSD). The moisture loss rate in grain was calculated as the linear regression coefficient (b_i) of grain moisture at the i -th observation dates. The significance of the correlation and regression coefficients was assessed by the magnitude of the errors of these parameters (S_r , S_b) and Student's t -test. The standard deviation (S) was used to assess the intragroup variation in the values of harvest moisture. The level of significance of the critical values of statistical parameters is $p = 0.05$. For the statistical analysis, we used the Data Analysis add-in of the Microsoft Excel 2016 spreadsheet processor.

Results. During the years of research, the average monthly air temperature in the North Caucasus exceeded the same indicator in the Southern Urals in May by 3-8 °C, in July and June by 3-7 °C, and in August and September by 5-9 °C (Table 1). Monthly precipitation totals in both areas varied within significant limits, but their minimum was observed in the Caucasus region in August and September. This annually led to a decrease in the relative humidity of the air by the end of the vegetation period. In the Urals, a more uniform distribution of precipitation over months was observed, which, at a low temperature, caused a steady increase in air humidity during the period of grain ripening. The noted differences provided a more favorable background for plant development, accumulation of dry matter in grain, and a decrease in its moisture content in the pre-harvest period in the North Caucasus.

1. Weather conditions in the geographical areas of the study of corn (*Zea mays* L.) hybrids for moisture content in grain

Month	North Caucasus			Southern Urals		
	2016	2017	2018	2016	2017	2018
Average monthly air temperature, °C						
May	17.9	13.8	17.9	12.0	10.7	9.8
June	22.3	18.8	21.6	16.5	16.2	14.6
July	25.8	23.1	24.5	18.8	18.0	20.2
August	27.3	22.4	21.3	18.3	17.4	15.9
September	18.7	18.5	19.5	11.2	9.7	11.3
Monthly precipitation, mm						
May	93	218	80	16	60	40
June	134	61	79	78	113	60
July	46	56	65	40	91	37
August	0	25	39	56	26	95
September	9	8	20	38	16	19
Average monthly relative air humidity, %						
May	50.9	50.8	52.0	50.6	51.3	60.6
June	52.8	54.6	56.4	64.3	61.2	64.7
July	64.1	62.6	61.0	69.6	69.0	73.2
August	62.7	59.2	59.4	72.8	71.2	83.4
September	58.8	57.4	58.8	73.7	71.9	74.1

In the North Caucasus region, full corn seedlings were observed 8-16 days after sowing, depending on the rate of soil warming, while in the Southern Urals after 21-23 days. In 2016-2017, the further development of plants up to the flowering phase of the ear took place against a relatively favorable temperature background, thus the duration of the period from the germination to this phase in both

climatic zones was comparable (54–58 days). The exception was 2018, when, under a heat deficit under the conditions of the Urals, this period was 10 days longer on average. The early ripening hybrid Bilyar 160 was distinguished by the longest period from germination to flowering of the ear, the rest of the samples developed 3–4 days earlier and did not show significant differences in flowering dynamics within the ultra-early group. The calendar flowering dates in the northern research point occurred 18–40 days later than in the south of the country and fell on the third decade of July—early August (Table 2). In the conditions of the North Caucasus, flowering was observed in the third decade of June—early July. The grain reached physiological ripeness in the period from August 1 to 7, and the processes of maturation and filling of ultra-early hybrids took place under a favorable combination of air temperature and humidification. Consequently, the weather conditions in the south of the country did not limit the moisture loss rate in grain, which provided an objective assessment of the differences between hybrids according to the studied trait.

2. Dates of ear flowering and physiological ripeness in corn (*Zea mays* L.) hybrids assessed by the moisture loss rate in grain in two geographical zones (2016–2018)

Hybrid	Southern Urals			North Caucasus		
	2016	2017	2018	2016	2017	2018
Date of ear flowering						
Obsky 140 SV	20.07	28.07	07.08	27.06	03.07	30.06
Kubansky 141 SV	20.07	28.07	07.08	27.06	03.07	30.06
Nur	19.07	27.07	05.08	26.06	02.07	28.06
Mashuk 150 MV	20.07	28.07	06.08	27.06	01.07	28.06
Ross 130 MV	20.07	28.07	07.08	27.06	02.07	29.06
Uralsky 150	19.07	27.07	08.08	27.06	03.07	31.06
Bilyar 160	23.07	31.07	11.08	30.06	06.07	02.07
Date of physiological ripeness of grain						
Obsky 140 SV	12.09	13.08	09.10	02.08	01.08	02.08
Kubansky 141 SV	12.09	14.08	09.10	04.08	02.08	02.08
Nur	11.09	13.08	10.10	04.08	01.08	02.08
Mashuk 150 MV	12.09	14.08	10.10	03.08	01.08	02.08
Ross 130 MV	11.09	13.08	10.10	04.08	02.08	03.08
Uralskiy 150	12.09	14.08	11.10	04.08	03.08	03.08
Bilyar 160	15.09	17.08	—	07.08	05.08	06.08

Note. A dash means that the hybrid has not reached the corresponding development phase.

In breeding, methods for determining moisture content that do not violate the integrity of the caryopsis are preferable, for example, using contact digital moisture meters [31]. Without limitations in the volume of the examined material, when choosing a method, the authors were guided by the criterion of the minimum measurement error with the number of control points from 8 to 16. The methods of similar studies based on 2–4 observation sites [29, 31], according to our opinion, replace the stochastic nature of the dependence with a functional one or unreasonably underestimate the variance of the regression.

It should be noted that by the time of the onset of physiological ripeness of the grain under specific vegetation conditions, no significant differences were found between the hybrids. According to Fisher's test, the fluctuations in grain moisture were statistically proven only for geographical points, while there was a tendency to its decrease in the south. Thus, in the conditions of the Southern Urals, grain moisture in 2016 varied from 37.4 to 41.7%, in 2017 from 39.2 to 42.0%, in 2018 from 38.5 to 41.9%; in the North Caucasus from 33.9 to 36.4%, from 36.3 to 39.6%, and from 33.9 to 36.8%, respectively.

The influence of environmental conditions on the moisture content in the corn grain when it reaches physiological ripeness is noted in various climatic zones. Thus, against the arid background of Canada, by the onset of this phase, the moisture content of grain decreases to 30% [31], in the Central Black Earth

Region of Russia to 35-38% [33, 40], in the conditions of the Southern Urals only to 40-42% [41]. The obtained data are within the range of these fluctuations.

Based on the results of analysis of variance of the data in the North Caucasus in 2016, four groups of hybrids were identified according to their moisture loss (Table 3). The first group includes the only hybrid Bilyar 160, which is characterized by the maximum dynamics of moisture loss; the second one comprised Uralskiy 150, Mashuk 150 MV, and Kubansky 141 SV with a medium rate of moisture loss; the third one was Ross 130 MV and Obsky 140 SV with moderate ability; the fourth one was the Nur hybrid, which was characterized by the minimum moisture loss rate.

3. Characterization of corn (*Zea mays* L.) hybrids by the rate of moisture loss form grain (% per day) after reaching physiological ripeness (North Caucasus, $b_i \pm S_b$, 2016-2018)

Hybrid	Year			Averaged
	2016	2017	2018	
Nur	0.58±0.03	0.73±0.05	0.69±0.05	0.67±0.03
Ross 130 MV	0.64±0.06	0.64±0.11	0.61±0.05	0.63±0.04
Obsky 140 SV	0.66±0.07	0.64±0.06	0.64±0.04	0.65±0.04
Kubansky 141 SV	0.71±0.09	0.74±0.06	0.69±0.05	0.71±0.03
Mashuk 150 MV	0.71±0.07	0.76±0.04	0.67±0.05	0.71±0.03
Uralskiy 150	0.70±0.09	0.71±0.09	0.67±0.06	0.69±0.03
Bilyar 160	0.81±0.11	0.81±0.05	0.72±0.07	0.78±0.05
LSD ₀₅ (hybrids)	0.04	0.07	0.03	0.04
LSD ₀₅ (years)	—	—	—	$F_{\text{observed}} < F_{05}$
LSD ₀₅ (interaction)	—	—	—	$F_{\text{observed}} < F_{05}$

N o t e. The dashes mean that when analyzing the results for some years, the LSD values were not calculated.

A similar distribution of samples was observed in 2017 and 2018, except that the Nur hybrid was included in the second group, showing an average moisture-yielding capacity. A fairly stable manifestation of the trait was established: for most hybrids, the differences in the moisture-yielding rate by years were insignificant and were not statistically proven by Fisher's test ($F_{\text{observed}} = 2.87 < F_{05} = 3.23$). This fact, as well as the obviously favorable hydrothermal background, against which the final processes of grain ripening took place, allow concluding that the differences between hybrids in terms of the moisture loss rate are mainly due to their genetic characteristics and reflect the results of selection for this trait. Similar conclusions are made by Reid et al. [31] and Wang et al. [32]. In the studies, conducted in the Primorye Territory [26] and North China [28] using mid-season and mid-late hybrids, daily moisture loss values of more than 1% were achieved, which is significantly higher than the maximum value obtained in the authors' studies (0.81%). With a high probability, these discrepancies can be explained not only by the growing conditions but also by the use of hybrids of different maturity classes [34].

Fundamentally different conclusions follow from similar observations, carried out in the forest-steppe of the Chelyabinsk Region. Under the conditions of this region, two stages of pre-harvesting moisture dynamics were observed. As it was already noted [37], in the first of them, which lasts from the beginning of grain formation to physiological ripeness, the moisture decrease rate is due to the intensity of accumulating organic matter in the endosperm and depends mainly on the air temperature. At the second stage (from physiological to full ripeness), moisture loss is inversely related to the relative humidity of the air and the amount of precipitation [31, 37]. At the second stage, the influence of external conditions on the moisture-yielding rate is mediated by the morphological features of the hybrids [29].

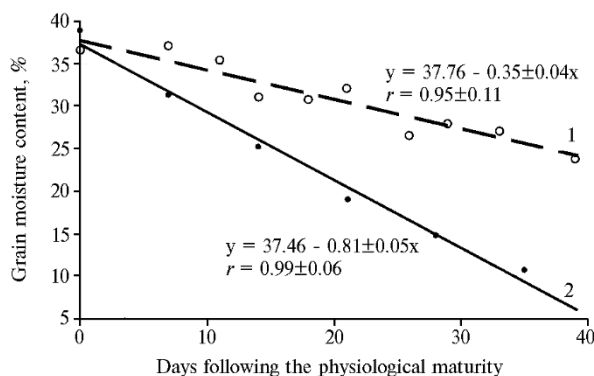


Fig. 1. Grain moisture in a corn (*Zea mays* L.) hybrid Bilyar 160 depending on the time interval after the physiological ripeness occurred in two geographical zones: 1 — Southern Urals, 2 — North Caucasus (2016).

(Fig. 1). This may be associated not only with a slowdown in the current moisture yield but also with the sorption of moisture by grain due to precipitation [39].

4. Characterization of corn (*Zea mays* L.) hybrids by the rate of moisture loss form grain (% per day) after reaching physiological ripeness (Southern Urals, $b_i \pm S_b$, 2016-2018)

Hybrid	Year			Averaged
	2016	2017	2018	
Nur	0.23±0.02	0.55±0.09	0.18±0.03	0.32±0.20
Ross 130 MV	0.23±0.02	0.53±0.12	0.19±0.02	0.32±0.19
Obsky 140 SV	0.21±0.03	0.53±0.14	0.18±0.03	0.31±0.19
Kubansky 141 SV	0.22±0.02	0.52±0.06	0.21±0.04	0.32±0.18
Mashuk 150 MV	0.24±0.02	0.58±0.10	0.17±0.02	0.33±0.21
Uralsky 150	0.24±0.03	0.58±0.06	0.19±0.04	0.34±0.21
Bilyar 160	0.35±0.03	0.72±0.07	No data	No data
LSD ₀₅ (hybrids)	0.06	0.07	$F_{\text{observed}} < F_{05}$	0.03
LSD ₀₅ (years)	—	—	—	0.02
LSD ₀₅ (interaction)	—	—	—	$F_{\text{observed}} < F_{05}$

Note. The dashes mean that when analyzing the results for some years, the LSD values were not calculated.

The distribution of hybrids according to the moisture-yielding rate was also different. By the method of two-way analysis of variance using Fisher's criterion, the reliability of the interaction was proved by the factors “geographic location” and “genotype”. That is, the differences between the hybrids in terms of moisture-yielding dynamics were due to the state of the external environment, which is indirectly confirmed by the data by Ghete et al. [27]. The main feature identified for the Ural region according to 2016 data was that only two groups of hybrids were reliably identified according to the target trait (Table 4). The first group with a high moisture loss rate (0.35%/day), as in the North Caucasus, was represented by one hybrid Bilyar 160. The rest of the samples were characterized by a relatively slow moisture loss (from 0.21 to 0.24%/day). The same distribution of hybrids was observed in 2017, despite the fact that the moisture-yielding rate was 2-2.5 times higher. In 2018, due to a significant shortage of heat during the vegetation period and late flowering, physiological ripeness was achieved by grain only by the end of the observation period, which did not allow revealing any peculiarities in the behavior of hybrids. For the hybrid Bilyar 160, the grain of which under these conditions did not reach physiological ripeness, the data on moisture loss in the last year of observations were not obtained.

The analysis of scientific publications confirms a significant variation in the moisture-yielding rate in corn grain due to the morphological features of the

As a consequence of the later flowering, the physiological ripeness of grain in the Ural region was reached in mid-late September (see Table 2), and a further decrease in moisture content was likely to occur against a background of low temperatures, high air humidity, and frequent precipitation. As a result, as established by the example of the hybrid Bilyar 160 in 2016, grain lost moisture more than 2 times slower than in the North Caucasus

hybrids. Thus, in the studies by Wang et al. [28], Reid et al. [31], and Voronina et al. [33], the differences between breeding samples for this trait were 0.22-0.24%/day. Ghete et al. [27] report fluctuations in moisture loss in inbred lines from 0.6 to 2.2%/day. It is noted that these results were obtained in traditional grain corn growing zones (to the south of 50° northern latitude). For more northern regions, no experimental data were found on the considered problem in the scientific literature.

The results of our research allow concluding that the ability of corn hybrids to accelerate moisture loss in grain is quite fully manifested against a favorable hydrothermal background, while against the background of excessive moisture in combination with a low temperature, the manifestation of this trait is suppressed. Therefore, in the northern zone of corn cultivation, one should periodically expect a leveling of the harvest moisture content of grain in hybrids of the same ripeness group.

The only exception can be made only by some genotypes, sharply differing in terms of the discussed trait. However, even for these samples, under conditions of a general heat deficit, genetic features are manifested irregularly. Thus, in the authors' study, the ability of the hybrid Bilyar 160 to quickly yield moisture was most fully manifested in the form of a minimum moisture level only in 2016 (Table 5). The next two years were characterized by a more or less pronounced delay in the development of all hybrids. Under these conditions, against the background of the shortened period from reaching physiological ripeness to the end of the vegetation period, the early maturation of the hybrids played a more significant role, which ensured the minimum grain moisture in the hybrid Uralskiy 150.

5. Grain moisture (%) at harvesting in corn (*Zea mays* L.) hybrids depending on vegetation conditions and a genotype (two geographical zones, $M \pm S$, 2016-2018)

Hybrid	North Caucasus			Southern Urals		
	2016	2017	2018	2016	2017	2018
Нур	19.6±0.8	17.9±2.4	14.6±1.3	26.9±0.6	34.4±1.9	41.3±2.6
Росс 130 MB	18.5±1.3	16.4±2.1	15.1±1.7	26.9±0.7	32.2±1.6	41.4±3.7
Обский 140 СВ	17.8±1.7	18.2±2.6	14.9±2.3	25.6±1.0	33.3±1.8	41.7±2.1
Кубанский 141 СВ	17.9±0.5	17.1±2.0	14.3±2.5	25.4±0.3	33.8±2.1	39.8±2.2
Машук 150 MB	17.6±0.9	17.8±3.2	14.8±2.3	26.3±0.5	34.4±1.6	41.9±3.4
Уральский 150	16.1±1.1	16.2±2.8	12.8±2.4	25.2±0.9	31.4±2.2	38.5±2.8
Билляр 160	15.5±1.6	17.5±1.6	14.4±2.1	23.7±0.9	34.9±1.2	43.5±4.3
HCP05	0.6	$F_{\text{факт.}} < F_{05}$	$F_{\text{факт.}} < F_{05}$	1.2	1.4	1.5

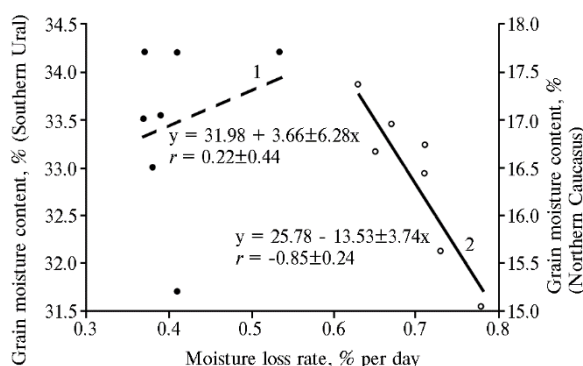


Fig. 2. Corn (*Zea mays* L.) grain moisture at harvesting vs. the rate of moisture loss after reaching physiological ripeness in two geographical zones: 1 — Southern Urals, 2 — North Caucasus (2016-2018).

An important consequence arising from the results of the conducted studies is the fundamentally different effect of the moisture loss rate on the harvest moisture content of grain in contrasting climatic conditions. As shown by the correlation and regression analysis, a close inverse dependence of the harvest moisture content of grain on the rate of moisture loss was observed only in the North Caucasus region (Fig. 2). These results confirm the conclusions by Wang et al. [28] for

North China, Sala et al. [29] for Argentina and Reid et al. [31] for Canada.

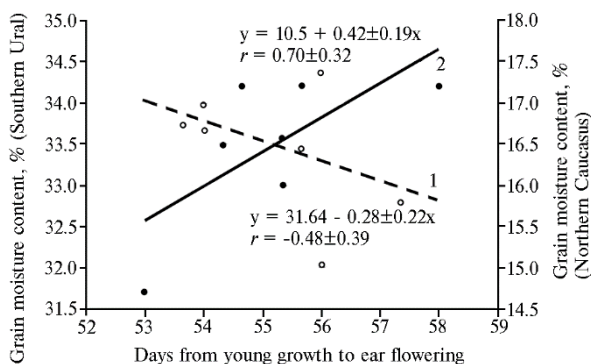


Fig. 3. Corn (*Zea mays* L.) grain moisture at harvesting vs. time period from seedlings to ear flowering in two geographical zones: 1 — Southern Urals, 2 — North Caucasus (2016–2018).

correlation coefficient ($t_r < t_{05}$).

Thus, the harvest moisture content of grain of corn hybrids is associated with two main features: early maturity and the ability to rapidly lose moisture at the final stages of ontogenesis. The contribution of each of these traits is determined by the conditions of plant vegetation and grain ripening. For the south of Russia, characterized by a favorable hydrothermal background in the pre-harvesting period, grain moisture is largely due to the genetic ability of the hybrid to accelerate moisture yield. Under these conditions, the rate of moisture loss in grain after reaching physiological ripeness varies for hybrids on average from 0.63 to 0.78% per day. On the contrary, in the Urals, with high relative humidity and low air temperature in the pre-harvest period, early flowering of the ear is of primary importance. Against this background, the moisture yield rate decreases to 0.21–0.35% per day under unfavorable hydrothermal conditions and to 0.52–0.72% per day under favorable conditions, and the differences between hybrids appear irregularly and are often leveled by the influence of the environment. Consequently, for the northern zone of corn sowing, the selection of corn for its ability to rapidly lose moisture in grain makes sense only in combination with selection for a short vegetation period, which should be considered as a priority trait when creating adapted hybrids. According to the results of the authors' research, an ultra-early hybrid Uralskiy 150 can be recommended for cultivation in the Southern Urals, which is characterized by early flowering in combination with a moderate loss of grain moisture in the pre-harvesting period. The high moisture-yielding ability, established for the early ripening hybrid Bilyar 160 in the North Caucasus, does not provide a consistently low harvest moisture content of grain in the Ural region.

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Under the conditions of the Southern Urals, this relationship was characterized as weak, and the main trait that depended on the moisture content of the grain during harvesting was the rapidity of the hybrids, defined as the duration of the period from germination to flowering of the ear (Fig. 3). In the North Caucasus, the dependence of humidity on the duration of the period “germination—ear flowering” was poorly expressed, with a statistically unproven significance of the

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ASSESSMENT OF THE RISKS OF CROSS-POLLINATION DURING CO-CULTIVATION OF MAIZE LINES IN THE SOUTH-EAST EUROPEAN RUSSIA

**Yu.S. GUSEV¹, O.V. GUTOROVA¹, E.M. MOISEEVA¹, V.V. FADEEV¹,
S.A. ZAYTSEV², D.P. VOLKOV², E.A. ZUK², I.V. VOLOKHINA¹, M.I. CHUMAKOV¹ ✉**

¹*Institute of Biochemistry and Physiology of Plants and Microorganisms RAS, 13, prosp. Entuziastov, Saratov, 410049 Russia, e-mail gusev_yu@ibppm.ru, olga.gutorova@mail.ru, em-moiseeva@mail.ru, vvf2593@gmail.com, volokhina_i@ibppm.ru, chumakovmi@gmail.com (corresponding author ✉);*

²*Russian Research, Design and Technology Institute for Sorghum and Maize, 4, 1-i Institutsii proezd, Saratov, 410050 Russia, e-mail zea_mays@mail.ru, genomix@mail.ru, e.a.zhuk@yandex.ru*

ORCID:

Gusev Yu.S. orcid.org/0000-0001-7379-484X

Gutorova O.V. orcid.org/0000-0002-6975-8675

Moiseeva E.M. orcid.org/0000-0001-9234-4000

Fadeev V.V. orcid.org/0000-0002-9725-3439

Zaytsev S.A. orcid.org/0000-0002-6829-1970

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Volkov D.P. orcid.org/0000-0001-8055-6516

Zuk E.A. orcid.org/0000-0001-6763-3724

Volokhina I.V. orcid.org/0000-0002-9088-481X

Chumakov M.I. orcid.org/0000-0002-6396-2851

Abstract

The new Russian Federal law (No. 358 of 03.07.2016) prohibits the commercial use of GM plants in agriculture, but allows, since 2018, for the first time in Russia their cultivation and testing for research purposes. Consequently, there is a need to assess and develop criteria for safe co-cultivation of non-GM and GM varieties, which are currently absent in Russia. In this paper, it was established for the first time that the 10-15 m distance is sufficient to prevent cross-pollination between maize lines with an acceptable presence of 0.9 % of the donor's genetic material, regardless of the recipient line, donor and recipient time flowering, and the wind direction in Saratov condition (South-West region of European part of Russia). The work aimed to assess the influence of the distance between pollen donor and recipient, wind direction, donor and recipient time flowering, and a buffer zone presence between them on the crossing frequency in mixed maize crops. The maize lines Korichnevyi marker (KM), GPL-1, Zarodyshevyi marker Saratovskii Purpurnyi (ZMS-P), Purpurnaya Saratovskaya (PS), as well as hybrids Purpurnyi (GP), Raduga and Tester 3 were grown (the experimental field of the Rossorgo, Saratov, South-West region of European Russia, 2018-2019). We planted the GP and ZMS-P lines as pollen donors in 2018 on a 3×80 m² area with planting density of 7-10 plants per 1 m². Between the pollen donor area, maize KM and GPL-1 lines were planted, and around them there were a 1290 m² area of yellow-colored grain recipients (Raduga and Tester 3 hybrids). In September, 5-12 ears from each pollen recipient were harvested. The cross-pollination frequency was calculated as the ratio of purple grains (GP pollination result) or yellow grains with a purple spot (ZMS-P pollination result) to the total grain number in recipient lines. In 2018 it was established that the maximum percentage (from 0.1 to 13.2 %) derived from cross-pollination with two pollen donors depends on different factors. At closer distances (1-4 m), the cross-pollination increased 4-fold for the earlier flowering recipient. The percentage of crosses for recipient Raduga decreased 3 times with a 10 m increase in the distance and 11 times at a 40 m distance from the donor plants. Experiments in 2018 indicate that the 10 m distance from the pollen donor guarantees the percentage of crosses not exceeding the 0.9 % GM threshold in food products accepted in the European Union and Russia. In 2019, we used PS inbred line as a pollen donor. The PS was planted on a 3×5 m plot with Sudanese grass (*Sorghum × drummondii*) Allegory cultivar as a buffer zone 3 m wide to the East and West and 15 m long to the South-West and North-East. Yellow-grain hybrid Raduga was planted around the buffer zone. The frequency of crosses was calculated as the ratio of the number of purple grains to the total number of Raduga grains per ear. In 2018, the frequency of crosses was also estimated

depending on the synchronism of flowering between pollen donors and recipients. The GPL-1 recipient with a 9-day difference from PG (pollen donor) in the beginning of flowering showed a 4 % lower pollination rate compared to a KM line with a flowering period closer to the PG pollen donor (1-day difference). Tall plants of PG donor of pollen prevented spreading pollen from a short ZMS-P donor to the Tester 3 and Raduga recipients in the direction of the wind rose. In 2019, no more than 0.9 % of purple grains were observed for the recipient Raduga when using a buffer zone of 15 m and more from the pollen donor in the wind rose direction. Based on the results of field experiments, the isolation distance from 15 m or more can be recommended to exclude cross-pollination of maize within the threshold of 0.9 % in the conditions of the South-East of the European part of Russia.

Keywords: GM plants, cross-pollination risks, maize, buffer zones

Maize (*Zea mays* L.) is one of the most important agricultural plants. Since 2010, the world's harvest of maize has grown from 800 to 1,122 million tons [1]. In the Russian Federation in 2019, according to the Ministry of Agriculture, the grain maize harvest amounted to 13.9 million tons, which exceeded the 1995 figure (1.7 million tons) by more than 8-fold (<http://mcx.ru/press-service/news/v-2019-godu-urozhaynost-zernovykh-v-rossii-vyroslo-na-4-7/>).

The photosynthesis of maize is of the C4 type, which implies a greater efficiency in absorbing carbon dioxide in conditions of good lighting and high temperatures. In addition, maize is drought-resistant, and climate warming, coupled with increasing droughts, will make it possible to expand the area of its cultivation in Russia. In Europe, an increase in the average annual temperature of 1 °C has already pushed the border of maize distribution by 200 km to the north [2].

The use of modern genomic technologies in crop production is one of the priority areas for the use of biotechnology in agriculture in the developed and developing countries [3]. The use of genetically modified (GM) plants for industrial cultivation began in 1996. Currently, almost a third of all cultivated maize is GM maize [4], which gives the largest yield of GM grain.

In the Russian Federation, until June 2018, there was a complete ban on the cultivation of GM crops. According to the Federal Law No. 358 in force since June 2018 (dated July 3, 2016), for the first time in Russia it is allowed to grow and test GM plants in the framework of scientific research. However, the parameters of safe cultivation of GM plants are not regulated and require studying. Each country-participant of the 1993 Convention on Biological Diversity, including Russia, must develop a strategy and program for the conservation and use of biological resources, taking into account their safe reproduction [5, 6]. Therefore, it is necessary to evaluate the criteria for the safe co-cultivation of non-transformed and GM varieties of plants, in particular, maize.

Maize is a monoecious wind-pollinated plant, which is a source of increased risk for the spread of pollen from GM varieties, crossing with which is undesirable in production crops. The world has not developed a unified approach to the legal regulation of the cultivation of GM crops. There are two opposite points of view on the problem of joint cultivation of GM and non-GM plants [7]. In the countries of the European Union and North and South America, regulation is based on fundamentally different risk assessment models for genetically modified organisms (GMOs). These approaches are called principle-based and product-based [8]. In the first case, the risk of genetic modification is recognized, in the second one, it is the risk associated with its result and object (GMO or derivative product) [9, 10]. In a number of countries (USA, Canada, Argentina, Brazil, and Spain), the cultivation of GM plants is permitted. Other states, in particular the countries of the European Union and Russia, have decided to ban the commercial cultivation of GM crops in their territory [11, 12].

The analysis of the world experience, accumulated over the past 20 years on the experimental assessment of the risks of cross-pollination in the joint

cultivation of GM and non-GM maize, is given in a number of reviews [13-15]. Buffer distances in different countries can range from 25 to 800 m, depending on legislative acts [16-18]. Moreover, the buffer distances measured in different regions of the world have very insignificant differences [19-21].

The distance between fields was identified as a key factor in spreading GM maize in non-transgenic crops [22]. As a consequence, the existing rules are mainly concerned with maintaining a fixed isolation distance between GM fields and the nearest non-GM fields [23]. However, other factors, such as the synchronicity of flowering of the donor and recipient of pollen, wind, relative sizes and shapes of fields, as well as the spatial distribution of different types of fields, can also affect the buffer distances [24, 25].

In Russia, the co-cultivation of GM and non-GM maize has not been systematically studied. In 2010, in the conditions of Krasnodar Territory, the first experimental assessment of the distribution of GM maize in non-transgenic crops was conducted [26]. The author states that cultivars of maize should be planted at least 200 m from transgenic varieties of maize, and barriers should be created in the direction of pollen flow. However, this work did not consider the question at what distance it is possible to grow GM and non-GM maize in order not to exceed the permissible content (0.9%) in Russia of GM product in the total grain yield. Since in Russia until June 2018 there was a complete ban on the cultivation of GM plants in an open environment, to study the distribution of GM maize pollen in field experiments that began in May 2018, the authors used a technology that simulates the distribution of GM pollen among production varieties under the conditions of free pollination. The essence of the technology is to assess the distribution of donor pollen based on the manifestation of a dominant trait (purple or white grain) in the progeny of yellow-colored grain lines of recipient plants [27-29].

In 2015, Russia developed an "Intra-industry standard for growing seed plots of maize, checking and preparing maize seeds for first-generation hybrids" (VOST 01.09.GK. Coordination Council for the development of maize seed production in Russia, 2015), which could serve as a guide for safe growing distances for maize. However, the standard lacks information about the experimental work that underlies these recommendations and does not take into account the possibility of the presence of acceptable 0.9% GMO products, which can significantly affect the safe distances for joint cultivation.

In this work, in the conditions of the Saratov Region (southeast of the European part of Russia), for the first time, it was found that a distance of 10-15 m was sufficient to prevent cross-pollination of maize lines with an acceptable presence of 0.9% of the donor's genetic material, regardless of the recipient line, the flowering time of the donor and the recipient, as well as wind direction.

The aim of the work was to assess the influence of the distance between the donor and recipient of pollen, the direction of the wind, the synchronism of the flowering of the donor and the recipient, and the presence of a buffer zone between them on the frequency of crossing of different maize lines in mixed crops.

Materials and methods. The experimental maize plants of the lines Korichnevyy marker (KM), GPL-1, Zarodyshevyy marker Saratovskii Purpurnyy (ZMS-P), Purpurnaya Saratovskaya (PS), as well as hybrids Purpurnyy (GP), of the selection of the Saratov State University named after N.G. Chernishevsky, as well as hybrids Raduga and Tester 3 of the selection of the Russian Research and Design Institute of Sorghum and Maize (RosNIISK "Rossorgo"), were grown in 2018-2019 on the experimental field of RosNIISK "Rossorgo" (Zonalny settlement, Saratov District, Saratov Region).

In 2018, the pollen donors were the Purpurnyy (GP) hybrid and the

Zarodyshevyi marker Saratovskii Purpurnyi (ZMS-P) line. GP was obtained by crossing the Purpurnaya Saratovskaya (PS) line and the Raduga hybrid and subsequently self-pollinated for several years (Gutorova, personal communication).

The planting density was 7-10 plants per 1 m². GP and ZMS-P were planted on an area of 3×80 m. Between the pollen donor areas, maize of the Korichnevyi marker (KM) and GPL-1 lines was planted, forming a buffer zone, and the areas with recipient lines were located around them (hybrids Raduga and Tester 3) with yellow caryopses, with a total area of 1290 m². In September 2018, 5-12 ears of each recipient line were taken, photographed in two projections, all caryopses were separated from the ear and analyzed. The frequency of cross-pollination was calculated on the ears of the recipient lines, calculating the ratio of the number of purple (the result of pollination with GP) or yellow grains with a purple spot (the result of pollination with the ZMS-P line) to the total number of grains in the recipient lines.

In the 2019 experiment, the inbred line Purpurnaya Saratovskaya, created on the basis of the American line, served as a pollen donor. The PS line was planted on an area of 3×5 m, around which a buffer zone was created with the sowing of Sudanese grass Allegory *Sorghum × drum-mondii* (Steud.) Millsp. & Chase, 3 m wide to the eastern and western directions and 15 m long to the southwest and northeast directions. Yellow grain maize of the Raduga hybrid was planted around the buffer zone. The calculation of the distances from the corners of the rectangular area of the donor to the corners of the rectangular plots of the recipient (near and far points) A5, A6, A10, A11 and F5, F6, F10, F11 was conducted by calculating the hypotenuses. The distances to the near and far points from the corresponding corner of the rectangular pollen donor site to the recipient plots A6, A11, F6, F11 were 6 and 12 m, to the A5, A12, F5, F12 plots 10 and 16 m. The frequency of crossings was calculated as the ratio of the number of purple grains to the total number of grains on the ears of the Raduga hybrid. In September 2019, the authors collected all the ears on each plot of the recipient line and counted the total number of ears on the plot and the total number of grains from the ears. The average number of grains per ear was calculated by dividing the total number of grains per plot by the number of ears.

The total number of harvested and analyzed grains in 2018 was 17,409 pieces, in 2019 201,284 pieces.

The statistical processing was carried out in Microsoft Excel 2010 using a statistics package. The arithmetic means (*AM*) and standard deviations (\pm SD) were calculated. The significance of differences was determined using Student's *t*-test ($p \leq 0.05$). In the 2018 experiment, each arithmetic mean was obtained on the basis of 5-12 repetitions. In 2019, for each distance, 5 repetitions (plots) were used, in each of which from 3 to 17 ears were analyzed.

Results. The layout of experimental maize crops on the experimental field of RosNIISK Rossorgo in both years of research is shown in Figure 1.

In 2018, the pollen donors were the Purpurnyi (GP) hybrid and the Zarodyshevyi marker Saratovskii Purpurnyi (ZMS-P) line. GP is tall-growing (2.0-2.5 m), has a dominant purple (anthocyanin) color of the stem, leaves, and grains. The genetically marked line of maize ZMS-P [30] has a purple coloration of vegetative parts of the plant, panicles, embryo, and endosperm; it is shorter (1.5 m) in comparison with GP and is mid-late ripening. The *R-g* gene provides the purple color of the aleurone layer of the endosperm and the embryo. When ZMS-P was crossed with yellow-grain recipient lines, hybrid caryopses had endosperm stained with a purple spot. In the 2019 experiment, the inbred line Purpurnaya Saratovskaya (PS), created on the basis of the American line Purple Tester [31] and the early-ripening line of the Saratov selection GPL-1, served as

a pollen donor. The mid-ripening tall-growing (1.8 m) line PS has a purple (anthocyanin) coloration of grain, leaves, and stem, determined by the dominant genes *A1*, *A2*, *B*, *Pl*, *Pr*, *R-g*, *C*. In particular, the *R-g* gene controls the purple coloration of grain aleurone [32].

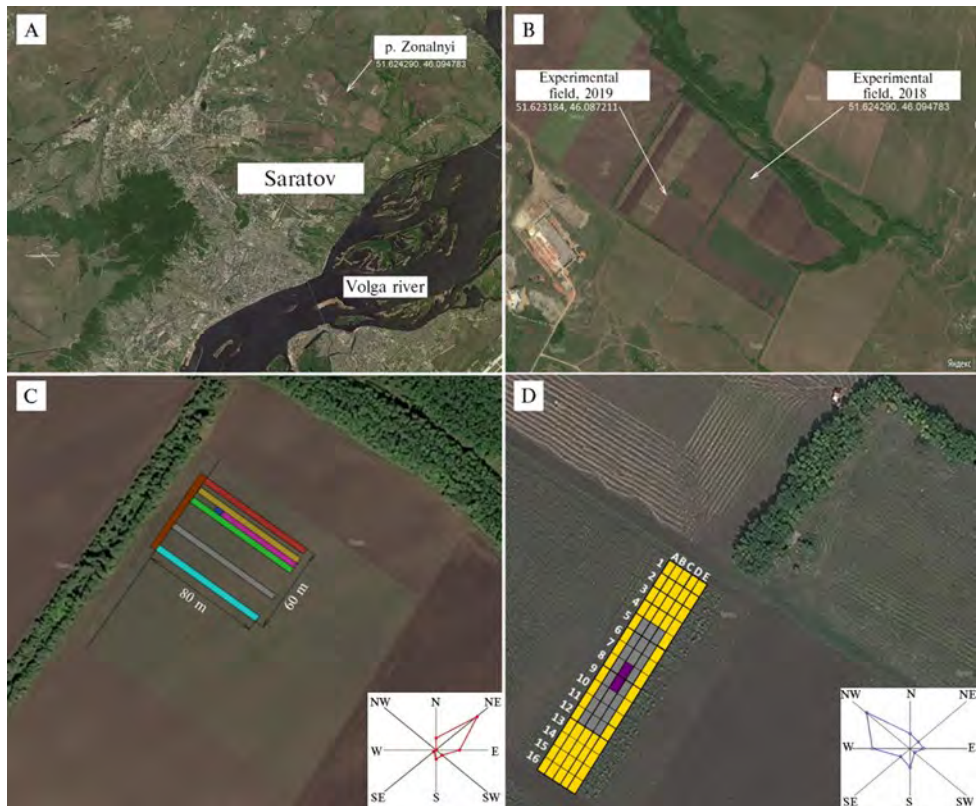


Fig. 1. The location of the experimental maize crops, where the risk of cross-pollination was assessed when growing genetically modified (GM) plants (<https://yandex.ru/maps/194/saratov>). The wind rose during the flowering period is given according to <http://weatherarchive.ru>. The orientation of the map (cardinal points) is indicated on the wind rose diagram.

A — the location of the experimental fields in 2018-2019 (Zonalny settlement) in relation to the city of Saratov and the Volga River.

B — general view of the experimental fields in 2018-2019.

C — the scheme of maize planting in the experimental field in 2018: green color corresponds to hybrid Purpurnyi (GP, pollen donor), yellow color to Zarodyshevyi marker Saratovskii Purpurnyi line (pollen donor); red color to hybrid Raduga (pollen recipient, 10 m from the GP to the east), turquoise color to hybrid Raduga (pollen recipient, 40 m from the GP to the west), brown color to hybrid Raduga (pollen recipient, 1-4 m from the GP to the north), gray color to hybrid Tester 3 (pollen recipient, 20 m from GP to the west), blue color to GPL-1 line, and pink color to Korichnevyi marker line.

D — planting scheme on the experimental field (2019): purple color corresponds to Purpurnaya Saratovskaya line (pollen donor, 30 m²), gray color to buffer zone with the sowing of Sudanese grass of the Allegory variety (330 m²), and yellow color to hybrid Raduga (pollen recipient).

In 2018, there was no rain during the maize flowering period, and the air humidity during the release of pollen from the anthers varied from 75% in the morning hours to 36% in the afternoon. The maximum wind speed during the flowering period was 5.7 m/s (July 31), the average wind speed was 3.1 m/s. The wind direction until August 1 was predominantly northeastern, from August 2, predominantly north and northwest (see Fig. 1, C). The temperature during the flowering period of maize in the morning hours ranged within 16-22 °C, in the daytime within 22-29 °C.

In 2019, it only rained on July 26 during the study period. The relative humidity in the flowering period during the release of pollen from the anthers fluctuated from 38 to 77%. The main wind direction was northwest, but in the period from 25 to 27 July, it was northeast (see Fig. 1, D). In the morning hours, the temperature ranged from 14–21 °C, the daytime temperature was 18–30 °C.

It is known that the distribution of pollen is influenced by temperature, air humidity, precipitation, viability, the total volume of pollen, wind flows, landscape profile, the configuration of the recipient field, and simultaneous flowering of paternal and maternal plants [12, 33]. However, there is no data on how the height of the pollen donor and the angle of arrangement of the leaves of the recipient affect the frequency of cross-pollination.

The viability of pollen, or the ability of pollen to germinate through the pistil filaments into the embryo sac, is an important prerequisite for cross-pollination. The duration of the viability of maize pollen (from 1 to 24 h) is influenced by humidity and temperature [34, 35]. As a rule, pollen spills out from the anthers in dry hot conditions, mainly from morning to noon [16, 36]. The flowering of maize pollen donor lines in the Saratov Region in 2018 continued from July 25 to August 5. Air temperature and humidity during this period were favorable for pollination.

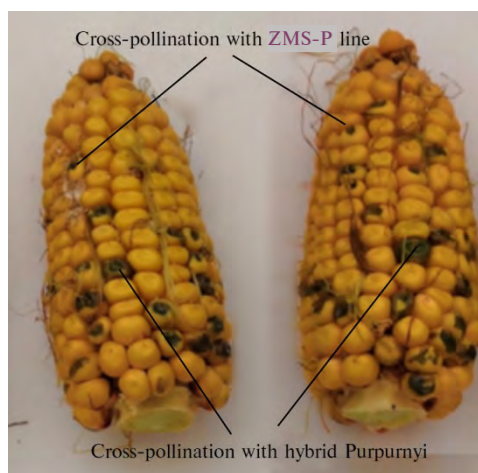


Fig. 2. The appearance of maize ears (*Zea mays* L.) obtained as a result of free crossing between pollen donors (hybrid Purpurnyi GP, Zarodyshevyi marker Saratovskii Purpurnyi ZMS-P) and the recipient line GPL-1 (experimental field of RosNIISK Rossorgo, Zonalny settlement, Saratov District, Saratov Province, 2018). The purple spots on yellow grains are the result of pollination with the ZMS-P line, the completely colored purple grain results from pollination by the hybrid Purpurnyi GP.

The distance to which the pollen will spread after being ejected from the anthers largely depends on the wind speed. During the period of experiments in 2018, the wind speed in the morning and afternoon was 2.4–5.7 m/s, and if the viability of the pollen was maintained for 1 hour, it could spread over 14 km. However, since maize pollen is rather large (average diameter 90 µm) and relatively heavy (0.25 µg), its sedimentation rate is high and, in real field conditions, pollen is spread relatively close [17, 39]. In 90–93% of all pollen from the donor line ZMS-P, its size was 120–140 µm and differed little from the size of pollen from other lines of diploid maize of the Saratov selection [37], that is, this trait probably did not affect the distribution of pollen.

In hot and dry conditions of the Saratov Region in 2018, the frequency of cross-pollination with pollen from donor lines ranged from 0.1 to 13.2% for differ-

ent recipient lines (Table 1). The maximum frequency of cross-pollination was observed for the lines KM and GPL-1 located in close proximity to pollen donors: 13.2% for KM when pollinated with Purpurnyi hybrid pollen and 10.5% for GPL-1 when pollinated with ZMS-P pollen. The appearance of maize ears obtained as a result of cross-pollination is shown in Fig. 2.

The frequency of crosses in the Raduga hybrid at a distance of 10 m was 3 times less (0.3%) ($p \leq 0.05$) than at a distance of 1–4 m when pollinated with GP pollen (0.9%) ($p \leq 0.05$), whereas when pollinated with ZMS-P pollen at the

same distance, the indicator was 0.05% ($p \leq 0.05$) and was 4 times less than at a distance of 1–4 m (0.2%) ($p \leq 0.05$). At a distance of 40 m from the GP, the crossing frequency decreased 11 times and amounted to 0.08% ($p \leq 0.05$), and we did not find plants pollinated with pollen from the ZMS-P line. Interestingly, in the Raduga hybrid, even at a close distance, the content of purple grains in the mass of yellow ones in the progeny practically did not exceed the threshold of 0.9% of the presence of GM products in food (harvest) accepted in the countries of the European Union and Russia.

In the work conducted in the conditions of the Krasnodar Territory [22], the question remained unclear, at what distance it is possible to grow GM and non-GM maize in order not to exceed the permissible content of GM product in Russia in the total grain yield. If it is taken into account that the total grain yield may contain 0.9% of the GM product, then the distance of 200 m, indicated by the author to prevent cross-pollination of maize, would be significantly reduced.

1. Frequency of purple grain occurrence in the progeny of yellow grain recipient lines of maize (*Zea mays* L.) with free pollination (AM \pm SD, experimental field of RosNIISK Rossorgo, Zonalny settlement, Saratov District, Saratov Province, 2018)

Recipient	A	B	C	D	E	F
KM	1–4 m, east	07/24–08/06	332 \pm 93.0	44.3 \pm 20.90	3.3 \pm 2.90	13.2/1.0
GPL-1	1–4 m, east	07/16–07/26	175 \pm 92.0	6.0 \pm 5.60	18.4 \pm 15.00	3.4/10.5
Raduga	1–4 m, north	07/23–08/01	539 \pm 206.0	5.0 \pm 3.20	1.3 \pm 1.50	0.9/0.2
Raduga	10 m, east	07/23–08/01	378 \pm 185.0	0.7 \pm 0.60	0.2 \pm 0.20	0.3/0.1
Raduga	40 m, west	07/23–08/01	506 \pm 213.0	0.4 \pm 0.40	0.0	0.1/0.0
Tester 3	20 m, west	07/24–08/01	590 \pm 70.0	1.0 \pm 0.80	0.0	0.2/0.0

Note. A — distance and direction from pollen donors; B — flowering period of recipient lines; C — the average number of all grains per 1 ear, pcs; D — the number of purple grains from the GP per 1 ear, pcs; E — the number of purple grains from ZMS-P per 1 ear, pcs; F — the proportion of purple grains by GP/ZMS-P donors, %. GP — hybrid Purpurnyi (donor), ZMS-P — Zarodyshevii marker Saratovskii Purpurnyi line (donor). The flowering period of the GP is from July 25 to August 5, ZMS-P from July 21 to August 7. The location of the experimental plots is shown in Figure 1.

In our experiments under the conditions of the Lower Volga region, the permissible proportion of crossings (0.9%) was observed for the tall-growing recipient Raduga even at a distance of 1–4 m from the donor (Table 1). The study conducted in 2000–2003 in 15 counties of England also showed a rapid decrease in the rate of cross-pollination in the first 20 m from the donor crop [38, 39]. In the experiments with maize in Mexico, the highest values of crosses were observed near the pollen source (12.9% at a distance of 1 m). The degree of crossing dropped sharply to 4.6, 2.7, 1.4, 1.0, 0.9, and 0.5% as the distance from the pollen source increased to 2, 4, 8, 12, 16, 20, and 25 m, respectively. At a distance of more than 20 m, the crossing frequency at all points was 0.9% ($p \leq 0.05$) and below [19].

In the dry summer of 2018, for the Tester 3 hybrid at a distance of 20 m to the west from the GP donor, the percentage of crosses was 0.2% ($p \leq 0.05$), which was significantly lower than the threshold value (see Table 1). It is interesting to note that approximately the same frequency of crossings (0.3%) ($p \leq 0.05$) occurred at a 10 m distance from the GP in the Raduga hybrid, but in the eastward direction, from where the winds mainly blew during flowering (see Table 1).

The effectiveness of the crossing depends on the simultaneous release of pollen from the anthers of the donor and the appearance of pistil filaments in the recipient [16, 40]. According to the literature, asynchronous flowering led to a decrease in the frequency of pollination in the recipient with a difference of 4–5 days by 25%, 6 days by 50% [34, 41]. The authors also observed a 4-fold decrease in the proportion of cross-pollination in the GPL-1 line (the difference

at the beginning of flowering is 9 days with GP) compared to the KM line with a closer (1 day difference) flowering period. At the same time, the frequency of cross-pollination in the recipient line GPL-1 (early flowering) and donor ZMS-P (mid-flowering) increased by 10 times compared with KM (recipient) and ZMS-P (donor) (see Table 1).

The height of pollen donor plants averaged 2.0-2.5 m for the Purpurnyi hybrid, 1.8 m for the PS line, and 1.5 m for ZMS-P. In 2018, the number of purple grains after pollination with GP in recipient lines was the maximum for the forms in close proximity and decreased with distancing. For the pollen donor ZMS-P, the percentage of crossings with recipient lines fell strongly in the north and north-east directions, and for the Raduga and Tester 3 recipient lines located in the west and southwest (see Fig. 1, C), cross-pollination from the ZMS-P line was not observed at all. Possibly, this is due to the fact that the tall-growing GP donor prevented the spread of pollen of the low-growing donor of the ZMS-P line in these directions. It can be noted that in ZMS-P, already at a distance of 1-4 m, the frequency of crossings with the tall-growing recipient Raduga was less than 0.9% ($p \leq 0.05$), and with the short-growing KM line slightly exceeded this value (see Table 1).

One of the possible factors limiting the flow of maize pollen is a buffer zone between the donor and the recipient in the form of an area not sown or sown with another plant. In 2019, the authors conducted an experiment to create a buffer zone with the sowing of the Sudanese grass Allegory, which is less tall than the donor. The maximum proportion (1.7-2.1%) ($p \leq 0.05$) of purple grains in the recipient (Raduga hybrid) pollinated by the PS donor was observed in the directions to the west (blocks 7A-12A) and east (blocks 7F-12F) with a buffer zone 3 m wide from the pollen donor (see Fig. 1, D, Table 2). For the south-western direction, with a buffer zone width of 15 m, the percentage of crosses (0.9%) ($p \leq 0.05$) did not exceed the permissible threshold (blocks 13A-13F, see Fig. 1, D).

2. The frequency of occurrence of purple grains in the progeny of the yellow grain maize (*Zea mays* L.) hybrid Raduga with free pollination by the donor of the line Purpurnaya Saratovskaya ($AM \pm SD$, experimental field of RosNIISK Rossorgo, Zonalny settlement, Saratov District, Saratov Province, 2019)

No. of row, plot	Direction and distance from the pollen donor, m	Number of ears, pcs.	Average number of yellow and purple grains per ear, pcs.	Proportion of purple grains, %
1A-1E	30-35 m, northeast	14	288.0 \pm 82.31/0.00	0.0
2A-2E	25-30 m, northeast	17	159.0 \pm 37.04/0.5 \pm 1.36	0.3
3A-3E	20-25 m, northeast	42	226.0 \pm 85.60/0.2 \pm 0.42	0.1
4A-4E	15-20 m, northeast	49	234.0 \pm 84.35/0.1 \pm 0.11	0.1
5A, 12A	10-16 m, west	26	419.0 \pm 29.63/1.2 \pm 0.62	0.3
6A, 11A	6-12 m, west	16	368.0 \pm 70.00/1.5 \pm 0.12	0.4
7A-10A	3-6 m, west	41	520.0 \pm 84.21/10.5 \pm 9.32	2.1
7E-10E	3-6 m, east	37	303.0 \pm 40.53/4.8 \pm 4.85	1.7
6E, 11E	6-12 m, east	28	303.0 \pm 7.02/0.9 \pm 1.13	0.3
5E, 12E	10-16 m, east	18	302.0 \pm 68.54/2.0 \pm 1.91	0.7
13A-13E	15-20 m, southwest	59	406.0 \pm 53.73/3.4 \pm 0.81	0.9
14A-14E	20-25 m, southwest	63	360.0 \pm 79.54/1.1 \pm 0.52	0.3
15A-15E	25-30 m, southwest	89	443.0 \pm 6.31/0.7 \pm 0.50	0.2

Note. The flowering period of the pollen donor and recipient is July 20-31. The location of the experimental plots is shown in Figure 1.

The maximum crossing frequency corresponded to the prevailing wind direction (see Fig. 1, D). With a buffer zone width of 3 m, the authors observed a significant excess of the permissible threshold of 0.9% ($p \leq 0.05$) for purple-colored caryopses in the harvest collected on plots 7A-10A and 7F-10F, which stood 0-3 m from the buffer zone (see Table 2). At distances farther from the donor (6-16 m), the proportion of crosses was within 0.3-0.7% ($p \leq 0.05$) in both directions. With a buffer zone width of 15 m at a distance of 15-20 m in

the southwestern direction from the donor, the Raduga hybrid had 0.9% ($p \leq 0.05$) purple grains in the ears, which even taking into account the coinciding wind direction did not exceed the permissible threshold (see Table 2).

It should be noted that the Raduga hybrid in the experiments of 2018-2019 showed a relatively low frequency of crosses (see Tables 1, 2). Recipient maize, especially tall-growing maize, itself serves as a barrier to the spread of donor pollen. Perhaps due to this, with a solid planting in 2018, the distribution of GP pollen was within the normal range already at a distance of 10 m from the donor, and in the presence of a buffer zone sown with a low-growing plant, in 2019 a threshold of 0.9% was recorded at a distance of 15 m. In the works of other researchers, it is also indicated that buffer zones with rupture plants, in contrast to barriers with tall-growing plants, do not lead to a decrease in safe distances [23]. Taking into account our findings, in the conditions of southeastern Russia, in order to eliminate the risks of cross-pollination of maize, it is possible to recommend a minimum isolation distance of 15 m in the presence of buffer (non-sown) zones.

Thus, under the conditions of the southeast of Russia, in order to exclude cross-pollination of maize above 0.9%, an isolating distance of 15 m or more can be recommended. In two independent experiments (2018 and 2019), it was found that at a distance of 10 and 15 m, respectively, from the pollen donor, the crossing frequency remained within the permissible norm, regardless of the recipient line, flowering time, and wind direction. At the same time, the synchronicity of flowering of donors and recipients of pollen had a significant effect on the frequency of crossings. In particular, under the conditions of 2018, in the recipient line GPL-1 (the difference at the beginning of flowering is 9 days with the donor hybrid Purpurnyi), the frequency of cross-pollination was 4 times less than in the Korichnevyy marker line with a period of flowering, which is closer to the donor. It was established that a tall-growing pollen donor, the Purpurnyi hybrid had an advantage in the spread of pollen as compared to the short-growing Zarodyshevyy marker Saratovskii Purpurnyi donor.

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Yield formation

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PHENOTYPIC VARIABILITY OF COMMON WHEAT (*Triticum aestivum* L.) BREEDING LINES ON YIELD COMPONENTS UNDER ENVIRONMENTAL CONDITIONS OF WESTERN SIBERIA AND TATARSTAN

A.I. STASYUK¹ ✉, I.N. LEONOVA¹, M.L. PONOMAREVA², N.Z. VASILOVA²,
V.P. SHAMANIN³, E.A. SALINA⁴

¹Federal Research Center Institute of Cytology and Genetics SB RAS, 10, pr. Akademika Lavrent'eva, Novosibirsk, 630090 Russia, e-mail stasyuk@bionet.nsc.ru (✉ corresponding author), leonova@bionet.nsc.ru;

²Tatar Research Institute of Agriculture — Subdivision of FRC Kazan Scientific Center RAS, 48, Orenburgsky trakt, Kazan, 420059 Russia, e-mail smponomarev@yandex.ru, nurania59@mail.ru;

³Stolypin Omsk State Agrarian University, 1, Institutskaya pl., Omsk, 644008 Russia, e-mail vp.shamanin@omgau.org;

⁴Kurchatov Genomics Center ICG SB RAS, 10, pr. Akademika Lavrent'eva, Novosibirsk, 630090 Russia, e-mail salina@bionet.nsc.ru

ORCID:

Stasyuk A.I. orcid.org/0000-0002-2931-6709

Vasilova N.Z. orcid.org/0000-0003-1135-486X

Leonova I.N. orcid.org/0000-0002-6516-0545

Shamanin V.P. orcid.org/0000-0003-4767-9957

Ponomareva M.L. orcid.org/0000-0002-1648-3938

Salina E.A. orcid.org/0000-0001-8590-847X

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Abstract

Spring wheat is one of the widely cultivated crops in the Russian Federation. Wheat breeding is aimed at creation of varieties characterized by high productivity and grain quality. When creating new varieties, the main attention is paid to ecological stability to environments, which negatively affects the agromomic traits and yield. Currently there is no enough information on the inheritance and manifestation of productivity traits in various agro-ecological conditions in advanced generations of the crosses between winter and spring wheat varieties. In this study, we analyzed the variability of the yield traits in wheat breeding lines (*Triticum aestivum* L.) of F₆₋₇ generations, obtained from hybridization of winter wheat varieties with spring donors of leaf rust resistance genes with the aim to identify promising genotypes as a source of valuable agromomic traits. Field trials were performed in 2018 in three regions, the Novosibirsk and Omsk region and the Republic of Tatarstan. The following traits were studied: tiller number per plant, the grain number per ear, the grain weight per ear, 1000 grain weight, and the grain weight per plant. Ecological plasticity of the lines was studied using indexes of the intensity and stability. Analysis of variance based on the results of field evaluation indicates a significant influence of the genotype, the environment and their interaction in the phenotypic manifestation of all the studied characters. The highest contribution of the genotype is shown for the grain number per ear (42.8 %) and the 1000 grain weight (57.0 %). A high contribution of environmental factors was found for tiller number (41.8 %) and grain weight per ear (40.3 %). The genotype × environment interaction had a significant effect on all traits, its contribution varied from 25.9 % (1000 grain weight) to 41.0 % (grain weight per ear), which indicates a significant response of genotypes to changing climatic factors. The results of field tests showed that there was a high variability of all characters in all three climatic zones, but the degree of variation differed. The averaged indicators of the studied traits were lower in the field conditions of the Omsk zone as compared to the Novosibirsk region and Tatarstan. Higher fluctuations were noted for the grain number per ear (13.0-69.0), the grain weight per ear (0.35-2.65 g), and the grain weight per plant (0.15-6.95) in Tatarstan's environments in comparison with other regions. The estimation of stability and intensity indices showed that 16 of 55 genotypes have intensive type, 35 were semi-intensive and 4 were extensive. The grouping of samples by the principal coordinate analysis method divided the genotypes into four main clusters according to the stability and intensity parameters. Molecular analysis for the presence of leaf rust resistance genes *Lr6Ai#2*, *LrAsp5*, and *LrT12* introduced from *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, *Aegilops speltoides*

Tausch and *Triticum timopheevii* Zhuk., respectively, showed that 10 out of 55 lines do not contain alien genetic material. The results of marker analysis for the presence of resistance genes did not correlate with the clustering of samples by intensity and stability types. This fact suggests that the presence of alien genomes does not influence on the stability of breeding lines. Based on the obtained results, genotypes with valuable characters were selected as sources of productivity.

Keywords: *Triticum aestivum*, common wheat, ecological plasticity, agronomic traits, yield, leaf rust

Soft wheat (*Triticum aestivum* L.) is of great importance throughout the world as a source of food for the population and fodder grain for farm animals. The Russian Federation currently occupies a leading position in the production of winter and spring wheat [1]. Over the past decade, there has been an increase in the sown area occupied by this crop in the country, an increase in the gross grain harvest, and an increase in productivity [2, 3].

The main producers of spring soft wheat in Russia are the Volga regions, the Ural and West Siberian regions, while up to 40% of the sown area of the crop falls on the Siberian region. However, despite the tendency to increase the yield of spring wheat, there is a significant variation from year to year. According to Silaeva and Barinova [3], the yield of spring wheat in 2007-2017 as a whole across the country varied from 9.5 to 18.5 c/ha. A significant fluctuation in yield is shown for both old and modern spring wheat varieties cultivated in Western Siberia [4-6].

The genetic basis of the variety is the main factor that determines the yield. In recent years, with the emergence of saturated molecular maps and the development of technologies for high-throughput geno- and phenotyping, significant progress has been made in the study of the genetic architecture of yield and its components in common wheat. Major and minor loci have been identified that contribute to the phenotypic manifestation of productive tillering, the number of grains and grain weight per spike, grain weight per plant, parameters of the root system, etc. [7-9]. Quantitative trait loci and candidate genes that determine yield under abiotic stress factors have been identified [10-13]. However, the implementation of the genetic potential of wheat largely depends on environmental factors. Therefore, at certain stages of obtaining new forms, it is necessary to study their phenotypic plasticity and adaptability in various soil and climatic conditions.

Winter wheat differs from spring wheat in higher productive tillering, grain weight per ear, and 1000 grain weight, which make the main contribution to the yield of varieties. However, in contrast to winter wheat, spring wheat is characterized by high indicators of grain and gluten quality and is more drought-resistant [14-16]. Since the middle of the 20th century, a strategy of combining the genetic pools of these crops has been used to obtain new spring and winter wheat varieties with high yield and disease resistance. Despite the fact that in most winter wheat varieties the combining ability when crossed with spring wheat is characterized as medium or weak, it has been shown that in some cases hybridization can give the desired genotypes [17, 18]. The general combining ability (GCA) of populations from crossing winter and spring samples was studied mainly at the F₁ stage to reveal the effect of heterosis and select the desired genotypes with higher indices for agronomically important traits [19, 20]. The GCA in the F₁-F₂ generations was assessed mainly on hybrid populations obtained on the basis of hybridization of either winter or spring varieties. The authors of these studies argue that the F₁ generation can be used for the initial stage of GCA assessment and donor selection. Thus, the study of the combining ability for the winter hardiness in 30 diallelic hybrids of the F₁ generation obtained from crossing 6 winter wheat varieties made it possible to identify genotypes characterized by high GCA effects and the participation of dominant alleles in an increase in the trait [21]. Based on the results

obtained, it was concluded that for the selection of unique genotypes with enhanced winter hardiness, it was necessary to test the hybrids of the F₄-F₆ generations. Studies for productivity traits performed using F₁-F₂ hybrids of spring and winter varieties indicate a significant contribution of genes with an additive type of action, which, according to the authors, makes it possible to start the selection of donors in early generations [22, 23]. As for the crosses of winter and spring wheat varieties, at present, there is not enough information on the manifestation and inheritance of productivity traits in advanced generations and in various agroecological conditions.

Earlier, the authors of this paper have created breeding lines based on hybridization of winter varieties and spring donors of resistance genes in order to expand the genetic diversity of common wheat for disease resistance genes [24, 25].

In the present study, these breeding lines were assessed according to the elements of the yield structure in different environmental conditions.

The aim of the work was to study the ecological plasticity of samples of advanced generations and to identify promising genotypes as sources of productivity traits for breeding.

Materials and methods. The studies were carried out on 55 lines of spring soft wheat of generations F₆₋₇, obtained from crossing the winter varieties Novosibirskaya 3 (N3), Novosibirskaya 40 (N40), Filatovka, and Biyskaya winter with spring donors of genes for resistance to leaf rust – the varieties Tulaykovskaya 10 (T10) and lines 5366-180 and 21-4, obtained on the varieties Saratovskaya 29 and Novosibirskaya 29, respectively. The variety T10 contains the *Lr6Ai#2* gene from *Thinopyrum intermedium* (Host) Barkworth & D.R. Dewey, line 5366-180 – the *LrTt2* gene from *Triticum timopheevii* Zhuk., Line 21-4 – the *LrAsp5* gene from *Aegilops speltoides* Tausch. [24, 26]. The lines were selected according to the earing time and productivity parameters in the F₃₋₅ generations and propagated to the F₆₋₇ generations.

DNA was isolated from the leaves of plants of the F₂ generation. A leaf fragment (2-3 cm) was placed in a 2 ml tube with Lysing Matrix Z (MP Biomedicals, USA) for grinding plant tissues, 700 µL of lysis buffer (1 ml 1 M Tris-HCl, 1 ml 5 M NaCl, 1 ml 0.5 M EDTA, 0.625 ml 20% SDS, and 0.04 g Na₂S₂O₅) was added, leaf fragment was grinded on a FastPrep-24 homogenizer (MP Biomedicals, USA) and incubated in a water bath at 60 °C for 30 min. Then, 700 µL of a mixture of chloroform: isoamyl alcohol (24:1) was added and centrifuged for 25 min at 12,000 rpm. The upper fraction was placed in a 2 ml tube, 1.4 ml of 96% chilled ethanol (–20 °C) was added and centrifuged for 15 min at 4500 rpm. Then, 700 µL of 70% ethanol was added to the sediment and centrifuged for 5 min at 4500 rpm. The supernatant was removed, the precipitate was air-dried and dissolved in 50 µL of TE buffer.

To determine genotypes containing the *Lr6Ai#2* gene from *Th. intermedium*, the authors used primers MF2 (5'-GATGTTCG-AGGAGCATTTTC-3'), MR1r2 (5'-GTGGTAGATTACTAGAGTTCAAGTG-3') and MR4 (5'-CGAATAGTTATACTAGGAGTAC-3') (Patent No. RU 2598275 C1, 2015). These primers are dominant, and two pairs, MF2/MR1r2 and MF2/MR4, must be used to identify plants homozygous and heterozygous for this gene. To identify introgressed fragments from *T. timopheevii* containing the *LrTt2* gene, the authors used the *Xbarc232* microsatellite marker (F – 5'-CGCATCCAACCATCCCCACCC-AACA-3', R – 5'-CGCAGTAGATCCACCACCCCGCCAGA-3') [27]. Translocations from *Ae. speltoides* containing the *LrAsp5* gene were identified using a pair of primers P1/P5 (P1 – 5'-TACCCCTGCTACCAAGTGC-3', P5 – 5'-GGCCAACCCTACACCCAAG-3') (Patent No. RU 2535985 C1, 2013).

Polymerase chain reaction (PCR) was carried out using a T-100 amplifier (Bio-Rad, USA) in a total volume of the reaction mixture of 20 µl containing DNA (50-100 ng), 10× buffer for Taq polymerase (650 mM Tris-HCl, pH 8.9; 160 mM (NH₄)₂SO₄, 25 mM MgCl₂, 0.01% Tween 20), 0.25 mM each dNTP, 1 ng each primer, 1 µl Taq polymerase (1 activity unit/µl), H₂O (to the final volume). The PCR conditions for gene detection have been described previously [24]. PCR products were separated by electrophoresis in 1.5% agarose gel containing ethidium bromide.

Phenotyping was carried out in the fields of the Novosibirsk and Omsk regions and the Republic of Tatarstan (Kazan) in 2018. The soil of the experimental plots in the Novosibirsk Region was leached chernozem, in the Omsk Province – meadow-chernozemic low-humus medium loamy soil, in Tatarstan – well-cultivated gray forest soil. The field trials were laid in 2 replicates by a systematic method on plots 1 m wide, 60 grains in a row.

The harvest was collected in sheaves, after which the number of productive stems, the number of grains in an ear, the weight of grain from the ear and from the plant, and the weight of 1000 grains were assessed. Structural analysis was performed for 20 plants of each sample.

The ecological plasticity of the lines was assessed on the basis of the trait "grain weight per plant" using the indices of the intensity index (I), stability index (SI), and reliability of stability index (R) according to the methodological guidelines [28]. The value of I, showing the reaction of the lines to the environments, was determined as the ratio of the difference in the grain weight per plant for its two extreme values for each line to the average weight for all lines on all environments: $I = (\bar{X}_{opt.} - \bar{X}_{lim.})/\bar{X} \times 100\%$, where \bar{X} is the average value of the grain weight per plant for all lines on all environments, $\bar{X}_{opt.}$ and $\bar{X}_{lim.}$ – average values of grain weight per plant for a sample against optimal and limited environments. The optimal environment was considered the region where the grain weight per plant for a particular line was the highest, the limited one was the region in which the grain weight per plant was the smallest. In addition, to assign a line to a specific type of intensity, a generalized intensity indicator for a hypothetical line (I) was calculated based on the average characteristics of all lines on tested backgrounds and the least significant difference (LSD) of partial averages for residual variance after two-way ANOVA [29]. The classification of lines according to the degree of responsiveness to the agricultural background was carried out according to the ratios: $I_{line} > I + LSD$ – intensive, $I - LSD \leq I_{line} \leq I + LSD$ – semi-intensive, $I_{line} < I - LSD$ – extensive. The stability index (SI), which characterizes the manifestation of homeostatic reactions of lines under different environmental conditions, was estimated as the ratio of the square of the average grain weight per plant in a sample against a specific environment to the value of the standard deviation of this indicator under certain conditions: $SI = \bar{X}^2/S$. The root-mean-square deviation was found by the formula $S = \sqrt{\sum X^2/n - (\sum X/n)^2}$, where X is the grain weight per plant in the line in each repetition against a certain environment (in the region), n is the number of repetitions in the conditions of this environment. The reliability index of the stability index (R), which characterizes the adaptive ability of the line, was determined by the formula: $R = (1 - SI_{opt.} - SI_{lim.})/SI \times 100\%$, where SI is the average value of the stability index in all experimental sites, $SI_{opt.}$ and $SI_{lim.}$ – indices of stability of lines against optimal and limited backgrounds. The environment, against which the calculated stability index was the highest, was taken as optimal, and the lowest, as limited.

Statistical processing of the results was carried out using MS Excel 2016 and STATISTICA v. 10.0 (StatSoft, Inc., USA). The mean (M), minimum (min), and maximum (max) values of the traits, median (Me) and standard error of the

mean (\pm SEM) were estimated. The contribution of the factors was calculated based on the mean square of the deviations (*MS*). The level of significance $p < 0.05$ was taken as a significant one. Principal coordinate analysis was performed using PAST v. 3.15 (30).

Results. The West Siberian region belongs to the territories with risky farming and includes various climatic zones, as evidenced by the meteorological data for the Novosibirsk and Omsk Regions in 2018. The weather conditions in the Novosibirsk Region during the growing season differed from the average long-term, the average temperature in May was significantly lower (6.9 °C), May and June were characterized by abundant precipitation, exceeding the norm by almost 3 times. In the Omsk Region, the average temperature did not differ from the average annual temperature, 259 mm of precipitation fell in the period of May–September, while their greatest amount fell on the second half of the growing season. In May, on the contrary, there was a deficit of precipitation in Tatarstan, in the rest of the months it fell unevenly; in June the average daily air temperature was below the norm, in the rest of the period – 2–3 °C above the norm.

The characteristics of 55 studied lines by the origin and the yield components during environmental tests are presented in Table 1 (see <http://www.agro-biology.ru>).

To assess the ecological plasticity, one of the main indicators of productivity was used — the grain weight per plant, which, in fact, serves as an indicator of plant productivity. Calculation of indicators of ecological plasticity made it possible to divide the studied lines according to intensity and adaptability (Table 2).

2. Parameters of ecological plasticity of 55 studied F₆₋₇ wheat lines from different cross combinations based on phenotyping tests in three regions of Russia (for each sample $n = 20$, 2-fold biological replication, 2018)

Line No.	I, %	R, %	SI			SI
			Novosibirsk	Omsk	Kazan	
Winter variety Biyskaya winter \times spring variety Tulaykovskaya 10						
1	130.27	–3.3	7.35	2.62	8.55	In/S
2	92.95	48.1	7.72	4.74	5.71	In/S
Winter variety Filatovka \times spring line 5366-180						
3	108.84	–2.5	5.09	2.32	8.20	In/S
4	71.00	51.3	5.59	6.48	8.38	P-In/S
Winter variety Filatovka \times spring variety Tulaykovskaya 10						
5	99.52	–52.7	14.15	7.02	15.78	In/US
6	22.78	84.4	6.36	5.46	5.52	P-In/S
7	89.56	54.6	7.73	9.31	10.34	In/S
8	231.49	–138.5	5.78	2.66	16.35	In/US
9	109.09	4.1	6.93	3.88	9.38	In/S
10	22.95	83.2	5.81	4.84	5.46	P-In/S
11	75.54	–24.2	9.36	2.23	4.32	P-In/US
12	97.12	31.1	8.21	4.30	4.26	In/S
13	91.31	6.5	7.73	2.36	4.54	In/S
14	41.58	30.5	4.03	4.71	8.02	P-In/S
15	44.98	72.3	6.86	6.10	5.27	P-In/S
16	60.83	11.7	7.05	1.98	4.93	P-In/S
17	84.00	–36.4	7.72	3.17	11.00	In/US
18	59.79	21.6	5.00	2.31	6.81	P-In/S
19	30.71	74.0	5.26	5.82	4.32	P-In/S
20	39.65	64.8	3.47	2.77	4.79	P-In/S
21	117.18	13.6	6.65	3.03	7.99	In/S
Winter variety Novosibirskaya 3 \times spring line 21-4						
22	33.05	52.6	4.68	2.64	5.36	P-In/S
23	72.26	30.9	5.58	3.76	7.73	P-In/S
24	34.73	75.0	4.10	3.95	5.38	P-In/S
25	31.93	85.4	5.14	4.77	4.30	P-In/S

	Winter variety Novosibirskaya 40 × spring line 21-4					
26	42.53	67.5	6.34	7.09	5.22	P-In/S
27	19.85	44.3	6.64	3.44	6.04	P-In/S
28	22.78	53.6	6.17	7.60	4.94	P-In/S
29	50.62	91.0	5.81	5.62	6.14	P-In/S
30	13.53	68.4	4.36	2.55	4.20	E/S
31	36.58	66.8	4.10	2.23	4.14	P-In/S
32	55.58	44.5	4.40	3.32	6.51	P-In/S
33	66.84	46.0	6.21	3.76	6.87	P-In/S
34	63.17	-27.9	10.36	3.54	3.01	P-In/US
35	54.90	39.1	7.20	3.70	4.58	P-In/S
36	82.37	48.8	7.99	6.19	9.13	In/S
37	28.07	85.9	3.45	4.18	3.37	P-In/S
38	52.26	17.6	9.28	4.55	4.72	P-In/S
39	38.26	54.2	5.16	2.53	4.30	P-In/S
40	16.54	96.4	4.60	4.40	4.42	E/S
41	18.26	5.1	9.03	3.58	3.90	E/S
42	36.76	76.2	5.55	4.19	4.43	P-In/S
43	42.93	-18.2	11.19	4.41	5.88	P-In/US
44	88.26	-46.2	12.28	3.89	7.14	In/US
45	81.10	23.5	7.45	3.06	5.54	In/S
46	42.57	85.0	4.39	4.05	4.91	P-In/S
47	46.41	53.6	6.97	4.31	5.97	P-In/S
48	92.70	28.8	8.24	5.54	4.15	In/S
49	33.38	32.7	9.54	5.87	5.68	P-In/S
50	66.14	-11.6	10.26	3.86	3.90	P-In/S
51	54.85	-16.0	11.38	4.84	4.72	P-In/US
52	96.35	37.2	4.49	5.19	8.09	In/S
53	15.06	57.7	5.31	2.88	4.72	E/S
54	64.79	-15.8	9.56	3.90	2.91	P-In/US
55	45.89	89.2	5.77	5.15	5.55	P-In/S

Note. I — intensity index, R — reliability of stability index, SI — stability index; In — intensive, P-In — semi-intensive, E — extensive; S — stable, US — unstable. For a description, see the Materials and methods section.

ANOVA, carried out on the basis of the results of field trials, showed a significant effect ($p < 0.001$) of the genotype, environmental conditions, and their interaction on the phenotypic manifestation of traits (Table 3). The contribution of the “genotype” factor varied depending on the trait, with the highest contribution noted for the grain number per spike (42.8%) and the 1000 grain weight (57.0%). The influence of genotypic factors on the manifestation of phenotypic variability of productive tillering and grain weight per plant was less significant compared to environmental factors (20.4 and 22.1%, respectively). The interaction genotype × environment was characterized by a high contribution to the phenotypic variability of all studied traits, which indicates a significant reaction of genotypes to soil and weather conditions.

A significant contribution of the “genotype × environment” factor has been described for the F₁ generation obtained from crossing winter and spring wheat varieties [19]. However, the authors found that the effect of heterosis depended not so much on the growing conditions of the hybrids, but on the genotype of the varieties used for hybridization. According to many studies, fluctuations in weather conditions in test regions, waterlogging at the beginning of the growing season, and low temperatures lead to significant variations in the yield and its components [31, 32]. The obtained results are consistent with the literature data, which indicate the unequal contribution of the genotype and the environments to the formation of the yield in common wheat [6, 33, 34]. The contribution of the genotype in the phenotypic variability of the yield is significantly less compared to environmental and agrotechnological factors, as evidenced by the results of most publications [35–37]. A number of works have shown that the realization of the genetic potential of varieties depends not so much on the influence of climatic factors, but on agro-technological measures and the use of intensive technologies that reduce the negative effects of the environments, which leads to the prevailing

influence of the genotype [38-40].

3. Analysis of variance based on the yield components in 55 studied wheat genotypes F6-7 from different cross combinations of winter wheats with spring wheats under environments in Novosibirsk, Omsk Regions, and the Republic of Tatarstan (for each sample $n = 20$, 2-fold biological replication, 2018)

Source of variation	Genotype	Region	Genotype \times region	Error
Trait, parameter				
Productive tillering:				
df	54	2	108	
MS	4.06	225.13	3.77	0.86
F	4.7*	260.6*	4.4*	
contribution, %	20.4	41.8	35.9	
The number of grains per ear:				
df	54	2	108	
MS	774	10451	325	80
F	9.7*	131.5*	4.1*	
contribution, %	42.8	21.4	35.9	
Grain weight per ear:				
df	54	2	108	
MS	1.22	23.11	0.72	0.16
F	7.6*	144.7*	4.5*	
contribution, %	34.6	24.4	41.0	
1000 grain weight:				
df	54	2	108	
MS	422	3514	94	28
F	15.3*	127.7*	3.4*	
contribution, %	57.0	17.6	25.4	
Grain weight per plant:				
df	54	2	108	
MS	9.33	458.70	7.94	1.43
F	6.5*	320.2*	5.5*	
contribution, %	22.1	40.3	37.6	

Note. df — the number of degrees of freedom, MS — the mean square; F — Fisher's test.

* The contribution is statistically significant at $p < 0.001$.

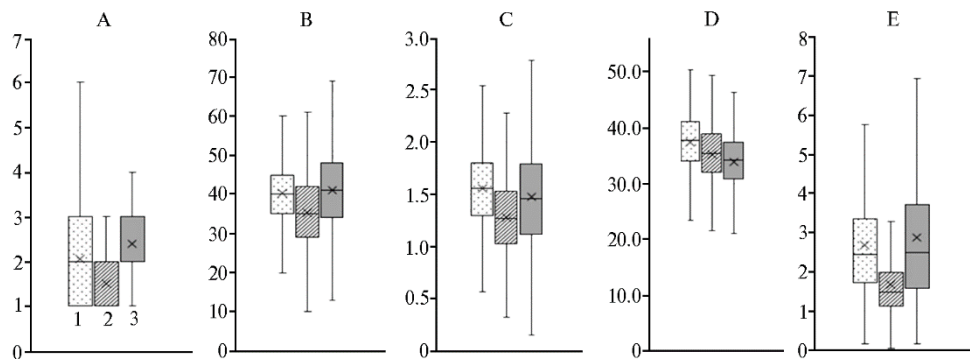


Fig. 1. Statistical analysis of the variability of the yield structure elements in 55 studied wheat lines F6-7 without taking into account their origin. The trials were conducted in 2018 in Novosibirsk Province (1), Omsk Province (2), and Tatarstan (3): A — productive tillering, psc.; B — the number of grains per ear, psc.; C — grain weight per ear, g; D — 1000 grain weight, g; E — grain weight per plant, g. Whiskers denote the minimum (min) and maximum (max) values of the trait, the horizontal line in the box is the median (*Me*), the average value of the feature is marked with “x”. The samples were obtained in combinations of crosses of winter varieties with spring forms. For each sample $n = 20$, 2-fold biological replication.

For a generalized assessment of the variability of yield traits in each of the three regions, we carried out statistical processing of field data for a total of 55 lines without taking into account their origin. As can be seen from Fig. 1, significant variability of all traits was observed when growing samples in all three climatic zones, but the degree of variation was different. For all signs, the obtained average value was lower in the field conditions of the Omsk Province. The most contrasting range of fluctuations was observed in the number of productive stems in the Novosibirsk Province (1-6 stems) compared to Omsk (1-2 stems) and Tatarstan

(1-4 stems). More significant fluctuations were observed for the ear grain number, ear grain weight, and grain weight per plant in Tatarstan as compared to other regions (see Fig. 1).

At present, various methods proposed by Eberhart and Russell, Tai, Udachin and Golovochenko, and others [28, 41, 42] are used to calculate the parameters of ecological plasticity. The most universal is the method of Eberhart and Russell [41], which is used by most researchers to assess the ecological plasticity and adaptability of cultivated varieties and at the final stages of breeding trials. However, the specified calculation method does not allow obtaining adequate and statistically reliable results in cases where the experimental samples are in the early hybrid stages or the number of field tests is limited. To assess the ecological plasticity of new breeding lines, the authors used the method of Udachin and Golovochenko [28], with the help of which it is possible to determine the tendency of plasticity formation at the initial breeding stages. The effectiveness of the method for obtaining preliminary information on the plasticity of varieties under conditions of a limited number of agricultural backgrounds and field seasons was confirmed in a number of works [6, 43, 44].

Based on the test results of 55 lines for each of the three climatic zones, stability indices (SI) were calculated, as well as intensity indices (I) and reliability of stability indices (R) (see Table 2). Intensity parameters allow determining the degree of responsiveness of genotypes to changes in plant cultivation conditions. The stability index shows how consistently a line is able to realize its potential in different environmental conditions. Lines with larger indices are more stable, that is, they are better adapted to changing conditions. The study found that 16 genotypes were included in the group of intensive type varieties, 35 in semi-intensive and 4 in extensive.

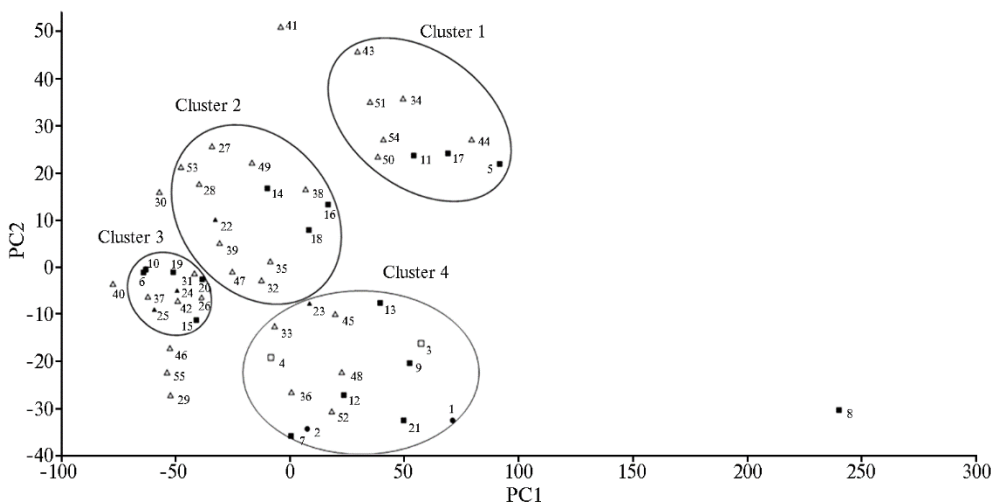


Fig. 2. Clustering by the method of principal coordinates of 55 studied wheat genotypes F6-7 from different cross combinations of winter wheats with spring wheats. Lines originating from different crossing combinations are indicated by the following symbols: ● — Biyskaya winter × Tulaykovskaya 10, □ — Filatovka × line 5366-180, ■ — Filatovka × Tulaykovskaya 10, ▲ — Novosibirskaya 3 × line 21-4, △ — Novosibirskaya 40 × line 21-4. The analysis was carried out according to the results of evaluating stability indices (SI), reliability of stability indices (R) and intensity indices (I), calculated for three test regions (for each sample $n = 20$, 2-fold biological replication, Novosibirsk Province, Omsk Province, and Tatarstan, 2018).

Principal coordinate analysis carried out on the basis of the parameters of ecological plasticity, additionally divided the studied breeding lines into four main clusters (Fig. 2). The first cluster includes 9 lines, 8 of which are characterized by

a low stability coefficient (R), which indicates an unstable line type. The exception was line No. 50, which belongs to the stable type. Among the genotypes of this group, there are samples of both intensive and semi-intensive types. Clusters 2 and 3 include, respectively, 13 and 11 samples of the stable type, with all lines, except for No. 53, being semi-intensive. A distinctive feature of cluster 3 is the higher stability indices of the stability index compared to the samples of cluster 2. Finally, cluster 4 unites 15 stable lines of predominantly intense type. Principal coordinate analysis also showed that clusters were formed from genotypes originating from different combinations of crosses. Seven lines (Nos. 8, 29, 30, 40, 41, 46, 55) were not assigned to any of the clusters, while line No. 8 significantly differed from all samples by a very low stability index ($SI = -138.54$) and high intensity factor ($I = 231.49$). Despite the fact that the combinations Biyskaya winter \times Tulaykovskaya 10 and Filatovka \times 5366-180 are represented by only two lines each, the results of evaluating these hybrid forms do not affect the grouping.

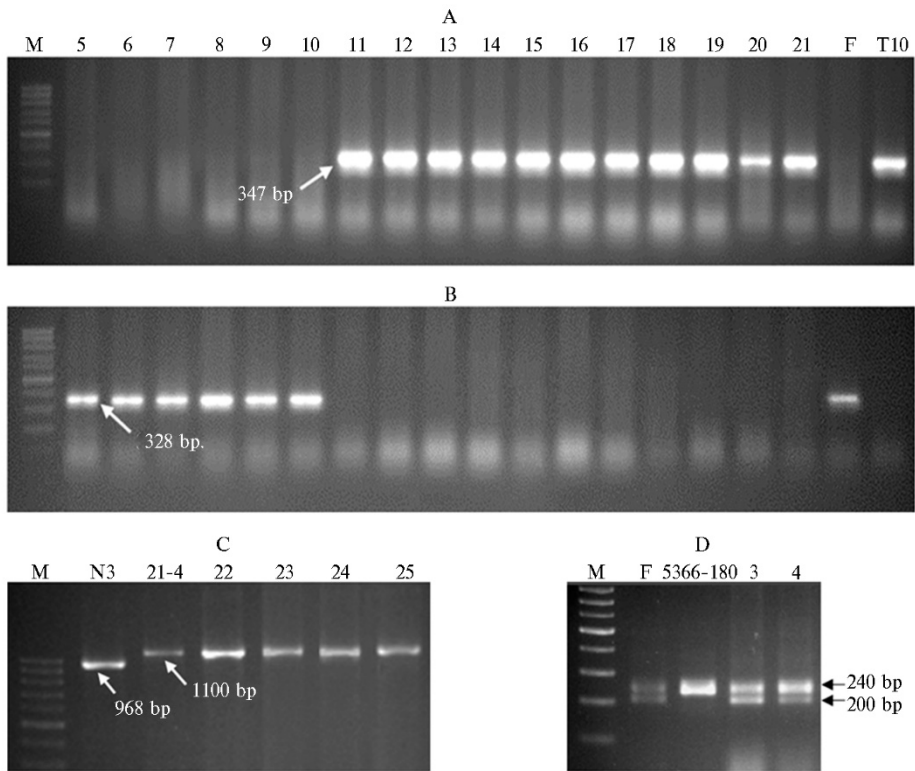


Fig. 3. An example of identification of genotypes containing genes for resistance to leaf rust among 55 lines F6-7 from different cross combinations of winter wheats with spring wheats. Primers MF2/MR1r2 (A) and MF2/MR4 (B) reveal the *Lr6Ai#2* gene, primers P1/P5 (C) reveal the *LrAsp5* gene, the Xbarc232 marker (D) reveal the *LrTt2* gene; 5-21 — the lines derived from crossing Filatovka \times Tulaykovskaya 10, F — variety Filatovka, T10 — variety Tulaykovskaya 10; 22-25 — the lines derived from crossing Novosibirskaya 3 \times line 21-4, N3 — variety Novosibirskaya 3, 21-4 — line 21-4; 3, 4 — the lines derived from crossing Filatovka \times line 5366-180. M — DNA 100 bp ladder (ZAO Biosan, Novosibirsk). Arrows point to diagnostic fragments 347 bp indicating the presence of translocations from *Thinopyrum intermedium*, and 328 bp indicating the absence of such translocations. The fragment 1100 bp shows the presence of translocations from *Aegilops speltoides*, and 968 bp — its absence. The fragment 240 bp indicates the presence of translocations from *Triticum timopheevii*, amplification of two fragments 200 and 240 bp indicates the absence of translocations.

Genotyping of the lines with *Lr6Ai#2*, *LrTt2*, and *LrAsp5* gene markers revealed the presence of amplification fragments with primers MF2/MR1r2 and MF2/MR4 in lines No. 11-21 (Filatovka \times Tulaykovskaya 10), suggesting that they

carry a *Th. intermedium* translocation (Fig. 3). We did not find alien translocations with resistance genes in lines No. 1 and No. 2 (Biyskaya winter × Tulaykovskaya 10), as well as in lines No. 5, 6, 7, 8, 9, and 10 (Filatovka × Tulaykovskaya 10). The presence of fragments of the *Ae. speltoides* genome was identified using primers P1/P5 in lines 22–55 obtained with the participation of Novosibirskaya 3 and Novosibirskaya 40 cultivars. PCR analysis of two lines participating in the study – no. 3 and no. 4 (Filatovka × line 5366-180) using the *Xbarc232* microsatellite marker showed the absence of introgression from *T. timopheevii* in these lines. The results of marker analysis for the presence of resistance genes did not correlate with the clustering of samples by the type of intensity and stability. The results obtained allow concluding that the presence of foreign genetic material in the genotypic environment of the recipient winter varieties does not affect the phenotypic manifestation of yield traits and the parameters of ecological plasticity.

So far, no unambiguous results have been obtained on the effect of alien translocations with resistance genes on the manifestation of productivity traits. It is known that foreign genetic material, which is part of extended introgressed fragments, can have both negative and positive effects on such traits as the number of grains per spike, the weight of grains per spike, and the 1000 grain weight [45, 46]. Similar effects are demonstrated by the example of wheat-rye translocations 1BL/1RS and 1AL/1RS, which are widely used in the world to create varieties with complex resistance to fungal diseases. Multidirectional effects on yield components during introgression of the genetic material of various species of wheat-grass *Thinopyrum* ssp., goat grass *Aegilops* ssp. and *T. timopheevii* in the common wheat genome are largely associated with the genotypic environment of the recipient cultivar, as evidenced by the literature data [47, 48].

Among the samples included in clusters 2 and 3, the authors selected 10 stable breeding lines of the semi-intensive type, distinguished by a higher grain weight per plant and a 1000 grain weight (Table 4). These lines will be involved in the next phase of testing to assess the prospects as sources of productivity.

4. Characteristics of stable breeding lines of semi-intensive type from different cross combinations of winter forms with spring ones, selected according to the results of field tests (Novosibirsk Province, Omsk Province, and Tatarstan, 2018)

Line No.	Yield structure component, $M \pm SEM$					Resistance genes		
	number, psc.		weight, g			<i>Lr6Ai#2</i>	<i>LrTi2</i>	<i>LrAsp5</i>
	productive tillering	grains per ear	grains per ear	grains per plant	1000 grains			
14	1.50±0.09	43.28±1.43	1.60±0.06	2.18±0.13	37.34±1.07	+	–	–
15	1.75±0.10	37.63±0.96	1.49±0.05	2.23±0.13	38.81±0.57	+	–	–
19	1.85±0.12	38.00±1.59	1.50±0.07	2.18±0.14	37.62±0.85	+	–	–
20	1.83±0.15	37.48±1.41	1.45±0.07	2.27±0.19	37.71±0.62	+	–	–
24	2.02±0.13	33.25±1.04	1.22±0.04	2.11±0.14	35.86±0.96	–	–	+
26	1.77±0.13	41.13±1.11	1.57±0.05	2.30±0.15	36.74±0.60	–	–	+
32	1.60±0.10	39.33±1.56	1.52±0.07	2.09±1.52	37.78±0.75	–	–	+
42	2.22±0.14	34.85±1.13	1.30±0.05	2.34±0.16	35.57±0.64	–	–	+
47	2.12±0.13	39.40±0.98	1.50±0.05	2.62±0.17	35.26±0.62	–	–	+
49	2.05±0.12	39.88±0.97	1.56±0.04	2.62±0.14	37.16±0.64	–	–	+

Thus, as a result of studying a set of common wheat breeding lines of F₆₋₇ generations in three ecological-geographical regions, it was found that the contribution of the genotype and environments to phenotypic variability depended on the trait under study. A high influence of genotype × environment interaction (25.9-41.0%) on all components of productivity was noted. The analysis of ecological plasticity showed that the lines were grouped into four main clusters according to the type of stability and intensity, while the clustering of samples was not affected by the origin of genotypes from crossing combinations and the

presence of fragments of alien material from *Thinopyrum intermedium* and *Aegilops speltoides*. Based on the assessment of ecological plasticity, stable genotypes of the semi-intensive type (Nos. 14, 15, 19, 20, 24, 26, 32, 42, 47, and 49) were selected for further testing with different cultivation technologies in the same region.

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PROSPECTS FOR PRECISION MANAGEMENT OF WHEAT PRODUCTIVITY IN THE CONDITIONS OF NORTHERN KAZAKHSTAN

B.R. IRMULATOV¹, K.K. ABDULLAEV¹, A.A. KOMAROV² ✉, V.V. YAKUSHEV²

¹Baraev Scientific-production Center for Grain Farming, p. Shortandy-1, Shortandinsky District, Akmola Region, 021601 Kazakhstan, e-mail irmulatov61@mail.ru, tsenter-zerna@mail.ru;

²Agrophysical Research Institute, 14, Grazhdanskii prosp., St. Petersburg, 195220 Russia, e-mail: zelenydar@mail.ru (✉ corresponding author), mail@agrophys.com

ORCID:

Irmulatov B.R. orcid.org/0000-0002-8155-7817

Komarov A.A. orcid.org/0000-0003-1430-0509

Abdullaev K.K. orcid.org/0000-0001-7760-4636

Yakushev V.V. orcid.org/0000-0001-8434-5580

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Abstract

More complete realization of the wheat genetic potential will ensure increasing its yield and quality in the conditions of the Republic of Kazakhstan. Innovative agrotechnological approaches, including a precision farming system (PFS) allow the problem to be addressed. Given the large area of Kazakhstan, the incorporation of precision farming systems needs assessment of the state of its vast territories using the Earth remote sensing (ERS) technology. In this paper, for the first time in the conditions of Northern Kazakhstan, the methods of precision management of spring wheat productivity were implemented. The aim of the work was to assess the prospects for precision farming in the conditions of Northern Kazakhstan and to develop an algorithm not only for passive monitoring of the state of crops, but also for active management of the spring wheat yield using ERS. The field trials were performed on dark chestnut soils in the seasons of 2019-2020 (a special precision farming landfill, Baraev Production Center of Grain Farming) with highly productive spring wheat (*Triticum vulgare* L.) varieties of local selection, the Shortandinskaya 95 and Astana. At the first stage, the main agrochemical indicators of the test sites were characterized using a ground-based mobile complex. Soil fertility parameters were assessed using survey grids with 1, 5, and 25 ha plot grids. The data were used to identify intra-field heterogeneity and calculate fertilizer doses for differentiated application. The second stage involved crop assessment using images from the Sentinel-2 group satellites and photographs from unmanned aerial vehicles (UAV). Plant development during ontogenesis was characterized by normalized difference vegetation index (NDVI), and the yields were finally evaluated using electronic yield maps. At the third stage, the data were processed using the Stat and Microsoft Excel 2010 software package and AGROS-1 software (version 2.09-2.11/1993-2009). The main fertilizers were used at sowing and surface applied. Non-root feeding was also performed. Polymer fertilizers Vitanoll-NP (1 l/ha) and Vitanoll-micro (1 l/ha) (Agromarket-24 LLC, Russia), a complex chelated fertilizer CHF (2 l/ha) (Agrophysical Research Institute, Russia), and a humic fertilizer Stimullife (0.3 l/ha) (Agrofizprodukt LLC, Russia) were used. It was found that only one agrochemical inhomogeneity of the field was partially leveled by the PFS-based differentiated introduction of fertilizers. Soil fertility, landscape profile and hydrological parameters (soil moisture availability) are shown to dominantly affect the spring wheat yield in the arid conditions of Kazakhstan. The changes in the agricultural landscape conditions due to the slope of the terrain, allows us to trace watercourses that characterize the productive moisture availability in a particular part of the field. On the basis of remote sensing data and coupled ground measurements used to precisely assess and record soil, landscape and soil moisture conditions, an algorithm has been developed to manage the yield formation process in plants. It is shown that the more precise the data analysis, the higher the effect of crop yield management. An increase in yield was approximately 10 % for a 5-ha plot grid and 2 times more for a 1-ha plot grid. The correlation revealed between the wheat yield and NDVI is strong and uneven as depends on the spatial direction, that is, the *r* values are 0.64-0.99 along the slope transect and 0.62-0.98 broadwise the field. A significant increase in yield was obtained both due to the differentiated application of

mineral fertilizers, by 9.5 % for the 5-ha grid and by 19.2 % the 1-ha grid, and due to timely non-root fertilizing, by 15–22.3 %. In general, our findings indicate the PFS prospects in the management of spring wheat productivity under the conditions of Northern Kazakhstan.

Keywords: spring wheat, Northern Kazakhstan, precision agriculture, remote sensing data, agro landscape, soil fertility, soil moisture

The Republic of Kazakhstan is one of the world's leading exporters of high-quality wheat. At the same time, the yield of soft wheat in Kazakhstan averages only 10–15 c/ha, which is due not only to natural and climatic factors but also to the imperfection of agricultural technologies. Increasing the yield and quality of wheat can be achieved by realizing its breeding potential [1, 2], which, among other things, provides resistance to pathogens [3]. It has been previously shown that, along with selection achievements, innovative agro-technological approaches, including the precision farming system (PFS), are of decisive importance [4, 5]. However, PFS technologies successfully implemented in the Russian Federation [4] and other countries [6, 7] require adaptation to the conditions of Northern Kazakhstan. Simple copying or replication of the PFS previously used in humid climates is unacceptable when it comes to the specific conditions of arid climates.

The introduction of precision farming in the conditions of Kazakhstan began relatively recently. Since 2018, LLP Baraev Production Center of Grain Farming has been developing a specialized program “Transfer and adaptation of technologies for precision farming in the production of crop products based on the principle of demonstration farms (landfills) in the Akmola Region”. Scientific research is focused on a specialized precision farming landfill with an area of 3000 hectares, where precision experiments have been carried out since 2019. One of the first elements of the introduction of precision farming in Kazakhstan (taking into account its length) is the assessment of the state of the cultivation zones of agricultural plants using modern technologies of Earth remote sensing (ERS) [8, 9]. On the basis of ERS, numerous tasks are currently being solved, including the predictive and actual assessment of the state and yield of spring crops in large areas [10, 11], which implies the assessment of the areas of not only cultivated plants but also the proportion of native species [12, 13].

To identify the ERS, it is required to be bound to the terrain conditions, as well as have a connection in time and space with ground-based studies, carried out taking into account the heterogeneity of the state of the soil cover, which can be realized only in precision studies. In addition, ERS provides typical monitoring observations aimed at assessing, analyzing, and only to some extent predicting the state of soils and plants [4, 14].

In this work, for the first time in the conditions of Northern Kazakhstan, methods of precision control of the productivity of spring wheat were implemented. It was shown that with a more detailed analysis of the data (the degree of precision on a grid of 1, 5, and 25 hectares), the resulting yield increased, which is associated with a more accurate differentiated use of fertilizers. It was revealed that in the arid conditions of Kazakhstan, soil-landscape-hydrological factors associated with the reserve and distribution of soil moisture were more important. Using ERS and the normalized difference vegetation index (NDVI), a close correlation was established between the remote sensing data and the yield map. In addition, the NDVI, determined using unmanned aerial vehicles, differed in information content and efficiency from satellite images. At the precision farming landfill, using ground and coupled ERS observations, it was possible to quickly identify zones of depression in plant development in the heterogeneous space of the field, which made it possible to timely carry out the necessary

correction of plant growth and development using foliar dressing.

The aim of the work is to assess the prospects for using the PFS in the conditions of Northern Kazakhstan and, on this basis, to propose an algorithm for the transition from passive monitoring measures to active control precision methods of increasing the productivity of spring wheat.

Materials and methods. The studies were carried out on dark chestnut soils typical for Northern Kazakhstan and southern carbonate chernozems in the conditions of the growing seasons of 2019-2020 (a specialized experimental land-fill of precision farming (LLP Baraev Production Center of Grain Farming). The main objects were highly productive varieties of spring wheat (*Triticum vulgare* L.) of local selection. The variety Shortandinskaya 95 (improved), a subvariety of Lutescens, was bred by the method of stepwise hybridization by means of mass selection from the hybrid population [(Pirotrix 28 × Justin) × Tselinnaya 21] × (Tselinnaya 60 × Lutescens 57/76). The variety is of a medium late type, the vegetation period is 95-100 days. The variety Astana, a subvariety of Lutescens, was bred by hybridization (line Lutescens I-2959 × Tselinnaya 90), medium-early ripening type, vegetation period 80-84 days.

The growth and development of plants were assessed both with the help of traditional ground-based observations and with the data of remote sensing of the Earth coupled with them.

Previously, at the precision farming landfill, the parameters of soil fertility were determined by means of a detailed agrochemical survey according to the generally accepted method, and in some variants – according to a 1-, 5- and 25-hectare grid. A precision farming technology with variable rationing of fertilization was used for the fields with a grid of 1 and 5 hectares, and the traditional technology for the fields with a grid of 25 hectares. Soil samples were taken with an automatic Wintex 3000 drill (Wintex Agro, Denmark) using a mobile complex with GPS referencing along a diagonal route for each cell of soil sampling. The data obtained were used to identify intra-field heterogeneity and calculate the doses of fertilizers for their differentiated application.

The state of plants was assessed using satellite images using the NDVI [8, 9]. Based on a detailed study of various vegetation indices, the most informative indicators with a spatial resolution of 30 m were previously identified [15]. Monitoring by satellites of the Sentinel-2 group was carried out using the LandViewer service (<https://eos.com>), which allows processing and analyzing images in real time. The authors also took into account the data of unmanned aerial vehicles (UAVs) [16] Geoscan 201 Agro (Geostoroiizyskaniya LLC, Russia) equipped with two cameras – an RGB camera and a modified infrared camera. The data from the first was used to create an orthomosaic, elevation maps, 3D models, and from the second, for NDVI maps.

Field coordinates on a 1-ha grid are 51°33'5.8"N (latitude), 71°02'30.7"E (longitude); on a 5-hectare grid 51°33'17.4"N, 71°02'08.7"E; on a 25-hectare grid 51°33'14.2"N, 71°03'25.1"E. For cluster sectors (along the field slope) at constant longitude 71°02'30.7"E, coordinates for sector 1 are 51°33'15.8"N, for sector 2 51°33'54.7"N, for sector 3 51°32'43.7"N. Spring wheat cv. Shortandinskaya 95 was cultivated in the 1- and 5-hectare fields with differentiated fertilization, wheat cv. Astana according to a standard technology. All fields were located within the same soil variety on dark chestnut soils.

The control techniques of precision farming were implemented through the differentiated application of basic fertilizers in two ways, i.e., with pre-sowing fertilization in the rows simultaneously with sowing seeds using the Bourgault complex (Bourgault Industries Ltd., Canada) and by the surface method using the Amazone ZA-M solid bulk fertilizer spreader (AMAZONEN-WERKE

H. Dreyer GmbH & Co. KG, Germany). Both of these complexes were equipped with control on-board electronics and GPS receivers, which made it possible to control fertilization with a specified accuracy.

Corrective control of the bioproduction process was carried out by foliar feeding. Top dressing was carried out with a solution at the rate of 50-100 l of tank mixture per 1 ha using equipment for applying liquid fertilizers (self-propelled sprayer John Deere m 4030, John Deere, USA). In the experiments, the research team used polymer fertilizers Vitanoll-NP (1 l/ha) and Vitanoll-micro (1 l/ha) (Agromarket-24 LLC, Russia) [25], complex chelated fertilizer CCF (2 l/ha) (FSBSI Agrophysical Research Institute, Russia) and humic fertilizer Stimulife (0.3 l/ha) (Agrofizprodukt LLC, Russia) [26]. Operational correction of productivity was carried out at the critical phases of ontogenesis which were assessed using ERS.

Statistical analysis was performed using the Stat and Microsoft Excel 2010 standard software packages and the AGROS-1 software (version 2.09-2.11/1993-2009). The reliability of the results of field experiments based on the analysis of variance, correlation and variation analysis was determined according to the method of the field experiment [17]. ERS data were assessed based on cluster analysis [18, 19] and time series analysis [20, 21], as well as NDVI dynamics for the growing season for each field [22-24]. Curves of NDVI dynamics by analysis time were smoothed in the dispersion mode. The data were presented graphically [21]. The software for precision farming FieldRoverII (Site-Specific Technology Development Group, Inc., USA) and SMS Advanced (Ag Leader Technology, Inc., USA), as well as own developments of Agro-Network Technologies (Kazakhstan) and API GIS (Russia), were used.

Results. A distinctive feature of the conducted studies is their precision nature, which for the first time made it possible to move from the assessment of the observed phenomena to the operational use of crop correction means.

The use of ERS in Kazakhstan is far from new. Earlier, the National Center for Space Research and Technologies introduced the AgroGIS information and analytical system, adapted to solve the problems of space monitoring of grain production in Kazakhstan [27]. Based on data from EOS MODIS (Moderate Resolution Imaging Spectroradiometer), Terekhov presented empirical relationships between productivity and various spectral characteristics of fields for non-cereal [28] and grain crops [29] over a number of seasons. Results have been obtained that confirm the close relationship between the vegetation indices and the yield of cultivated crops [27-29]. However, these and other monitoring studies in the conditions of Kazakhstan were carried out over large territories, therefore, they were rather statistically generalizing and predictive in nature and did not detail the data for each field separately in terms of their mosaicity in fertility and agricultural landscape features. At the same time, the intra-field diversity (heterogeneity) of each field has not yet been taken into account, which can be realized only with the help of precision farming technology.

Methods based mainly on geostatistics have been developed to identify intra-field heterogeneity; however, there are not many approaches for decoding ERS in an inhomogeneous field space. Based on many years of research by the Agrophysical Research Institute (API) on the introduction of precision farming technology, the authors have developed an algorithm for the transition from typical monitoring studies to new management techniques for wheat cultivation in the unfavorable arid conditions of Kazakhstan. So, to identify the boundaries of intra-field inhomogeneity, a new precision approach was proposed based on the conjugate processing of remote sensing and ground-based precision information

[4, 15], which was first implemented in the conditions of Northern Kazakhstan [30].

At the precision farming landfill, a detailed assessment of the heterogeneity of the state of the soil cover was carried out according to the main agrochemical indicators. A network of 159 elementary plots with an area of 1 hectare was formed, as well as a network of landfills of 5 and 25 hectares. According to the main agrochemical characteristics, the comparative heterogeneity of the massifs was revealed. So, in a 1-ha grid with an average N-NO₃ content of 15.2 mg/kg, the coefficient of variation (Cv) was 65%, for P₂O₅ (27.2 mg/kg) 27%, and for K₂O (733 mg/kg) 21%. An increase in the size of an elementary plot in determining the most variable indicator (the content of nitrate nitrogen in the soil) led to a decrease in the coefficient of variation. And with a decrease in the size of the elementary section, the inhomogeneity index for mobile phosphorus decreased.

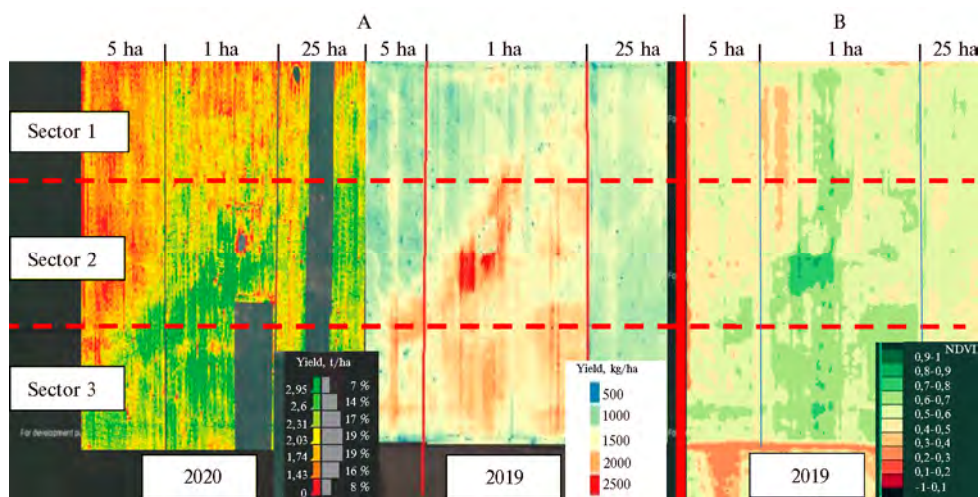


Fig. 1. Integration of terrestrial (yield maps of spring wheat *Triticum vulgare* L.) (A) and satellite remote sensing data (normalized vegetation index, NDVI) (B) with differentiation by assessment grids of 5, 1, and 25 hectares and clusters (sectors 1-3) in different years (LLP Baraev Production Center of Grain Farming, Kazakhstan).

Of particular practical interest were the data that serve as an information basis for subsequent calculations and analysis – an electronic yield map with a grid of 1, 5, and 25 hectares (Fig. 1). Each field had zones (designated as sectors, or clusters) with clearly lower and higher yields. These clusters were clearly manifested according to the data of the yield map for the growing season of 2019, as well as independently and unambiguously – in the conditions of 2020. Moreover, the cluster manifested itself as a separate zone of field heterogeneity, identified not only by the yield map, but also in the conjugate assessment with ERS.

The results obtained in the arid conditions of Kazakhstan in 2019 and confirmed in the conditions of the growing season of 2020 made it possible to reveal the dominant influence on the productivity of the crop rather than typical agrochemical parameters of soil fertility, but others related to the stock and distribution of soil moisture (soil-landscape-hydrological). Earlier [30], the authors, like other researchers [4-6], focused on the parameters of the agrochemical heterogeneity of the field and, using the differential application of mineral fertilizers, tried to eliminate nutritional deficiencies. Therefore, as a result of cluster and detailed analysis of the yield for each cell of the landfill for a 1 ha grid, it was not possible to reveal any significant interdependence with the parameters of soil fertility.

However, in the present experiment, a very close relationship was found between yield and NDVI. Along the long run of the field in the direction of the wheat sowing rows, the correlation coefficient between yield and NDVI along the transect was $r = 0.64-0.99$ (sample of 9 rows with a cell of 1 ha), across the field $r = 0.62-0.98$ (sample of 18 rows with a cell of 1 ha). However, based on the results obtained, as well as the data of other researchers [30, 31], the authors were unable to identify a factor that determines the increase in yield across clusters of a field that is heterogeneous in the direction from its northern part (sector 1) to its southern part (sector 3).

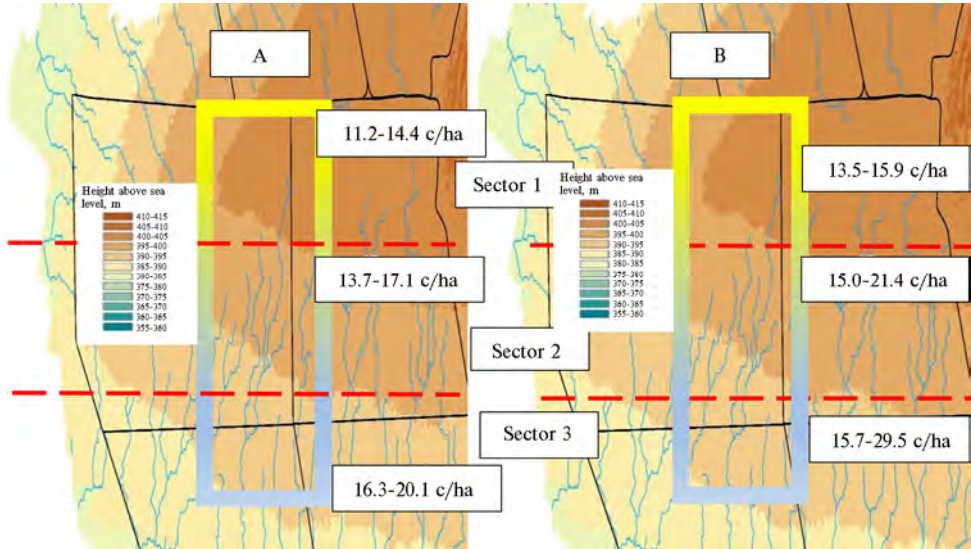


Fig. 2. The distribution of the yield of spring wheat *riticum vulgare* L. on the digital elevation model map in different years: A — 2019, Б — 2020. Sectors are marked with a coordinate reference at a constant longitude of 71°02'30.7"E with heights registration (h): for sector 1 51°33'15.8"N (h = 400-405 m), for sector 2 51°33'54.7"N (h = 390-395 m, for sector 3 51°32'43.7"N (h = 385-390 m) (LLP Baraev Production Center of Grain Farming, Kazakhstan). The rectangles in the center of images A and B illustrate an increase in moisture availability from the top of the figure to the bottom.

It was found that only one agrochemical heterogeneity of the experimental field was partially leveled by the differentiated introduction of nutrients. This heterogeneity was clearly manifested in the detailed accounting of yield data. Dividing the field according to the zones of heterogeneity of the array into three sectors (Fig. 2), it can be noted that the average yield for 2019 in the upper part of the field (sector 1) was 12.8 c/ha, in the center (sector 2) 15.4 c/ha, in the lower part 18.2 c/ha (sector 3). In 2020, this trend continued, but the average yield turned out to be slightly higher. It was found that with an increase in the degree of precision of studies (grids of 25, 5, and 1 ha), both the total yield and the differentiation of the yield along the slope increased. If in 2019, on a grid of 25 hectares, the average yield on an area of 82.9 ha was 14.6 c/ha, then in 2020, on an area of 559 ha, a yield of 18.1 c/ha was recorded; in a 5-ha grid, in 2019 and 2020, this indicator was 16.0 c/ha (area of 68.3 hectares) and 18.9 c/ha (65 hectares), respectively, in a 1 ha grid 17.4 c/ha (174.4 ha) and 22.6 c/ha (418 ha).

On the basis of coupled precision studies, the authors were able to identify the reason for the change in yield in different parts of the field, which was determined by the slope of the field and, accordingly, by different degrees of moisture supply. Here one can single out a factor associated with the stock and

distribution of soil moisture, due to the peculiarity of the terrain, the combination of soil and hydrological conditions of the agricultural landscape (that is, soil-landscape-hydrological conditions). This was realized with the help of remote sensing and the creation of a digital elevation model [31], for which mathematical and digital models of the earth's surface were used [32]. The more detailed the data analysis was, the higher the accuracy of estimating the yield increase was. Thus, the increase in yield when detailed on a 5-ha grid was slightly less than 10%, and with a 1 ha grid 2 times more compared to the traditional technology (on a 25-ha grid).

Based on the change in agrolandscape conditions determined by the slope of the terrain, it was possible to estimate the distribution of watercourses characterizing the supply of productive moisture in one or another part of the field (see Fig. 2).

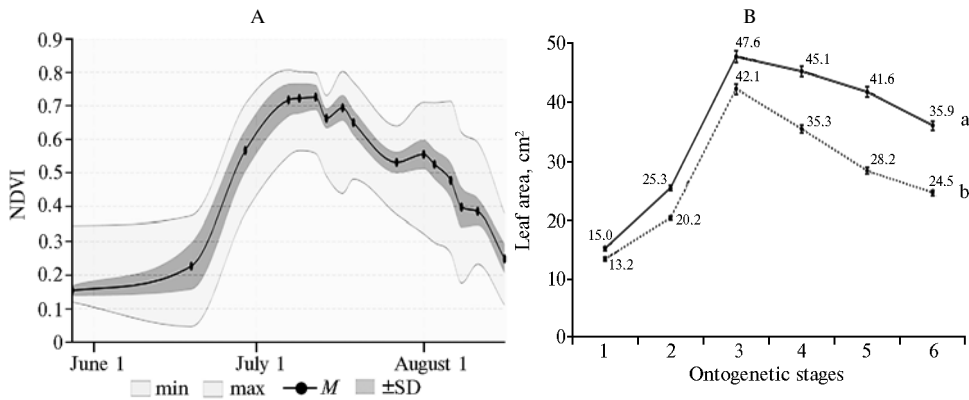


Fig. 3. Correspondence between the dynamics of the vegetation index by dates (A, Earth remote sensing data, LLP Baraev Production Center of Grain Farming, Kazakhstan, 2019) and change in the wheat leaf surface area (B) in ontogenesis under irrigation (a) and drought (b) [33]: 1 — tillering, 2 — stem elongation, 3 — earing, 4 — flowering, 5 — milk ripeness, 6 — wax ripeness.

When assessing the dynamics of plant development from May 26 to August 10, 2019, it was noted that the change in NDVI over time was uneven. A gradual increase in the vegetation index from 0.15 to 0.71-0.73 (July 6-9) characterized an increase in the vegetative mass and growth activation (Fig. 3). However, the vegetation index did not reach the expected values of 0.9-1.0, which indicated the insufficient completion of the physiological processes of accumulation of plastic substances in the plant biomass. The latter could be associated with arid conditions, high temperatures, and lack of moisture, which did not allow realizing the potential of cultivated varieties and agrotechnological solutions of precision farming [33].

A correlation (r) was found between the yield and ERS data according to the NDVI index: for sector 1 — 0.68, for sector 2 — 0.83, for sector 3 — 0.65. However, here the influence of not an agrochemical factor, but a hydrological factor, which determined the difference in yield across the estimated clusters, was already manifested.

Also in precision field experiments, it was found that when using foliar plant feeding at different phases of ontogenesis, wheat yield increased from 22.1 c/ha (high agricultural background) to 22.7-24.7 c/ha, or by 10-20%. A statistically significant ($LSD_{05} = 3.42$ c/ha) yield increase of 3.7 c/ha was noted for Vitanoll-NP fertilizer, of 4.1 c/ha for CCF, and 4.6 c/ha for Vitanoll-micro. This data was obtained in 2019, the first year of the research. However, in the conditions of the 2020 season, no statistically significant increases in yield due to

the use of foliar feeding were revealed. In the future, it is necessary to clarify the features and reasons for the ambiguity of these results.

Thus, as a result of the performed studies, an algorithm was proposed for the transition from passive monitoring activities using ERS to active control actions aimed at increasing the productivity and quality of spring wheat in the conditions of Northern Kazakhstan. This algorithm is based on the use of remote sensing data and associated ground precision measurements. It allows determining the specifics of differentiated fertilization, taking into account the response of plants to changes in the vegetation index NDVI, and controlling the physiological processes of growth and development of cultivated crops, which ultimately affects an increase in yield. So, with remote sensing using UAVs, it was possible to find out the features of the formation of the spring wheat crop in different zones of field heterogeneity. Such an indicative indicator as the vegetation index NDVI, determined using UAVs, was more informative than satellite images. With the help of remote sensing data, it was possible to assess the specificity of the dynamics of physiological processes during the growth and development of different varieties of wheat, to identify critical phases, and to determine the optimal timing for applying corrective actions. Significant increases were obtained when vegetating wheat plants were treated with Vitanoll-NP (1 l/ha) at germination and tillering (by 18%), CCF (1 l/ha) at tillering (by 19.9%), and Vitanoll-micro (1 l/ha) upon a combined application at tillering, earing, and milk ripeness of grain (by 22.3%). In addition, with the help of remote sensing, it is possible to quickly identify zones of depression in plant development in the heterogeneous space of the field and timely carry out the necessary fertilizing. On the basis of the chosen scheme of plant treatment according to the phases of ontogenesis using means of crop correction (foliar feeding), promising data were obtained. In general, precision control of wheat productivity in the conditions of Northern Kazakhstan is very promising and requires further comprehensive study.

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BIOLOGICAL FEATURES OF *Brassica rapa* L. VEGETABLE LEAFY CROPS WHEN GROWING IN AN INTENSIVE LIGHT CULTURE

A.M. ARTEMYEVA^{1, 2} ✉, N.G. SINYAVINA², G.G. PANOVA², Yu.V. CHESNOKOV²

¹Federal Research Center Vavilov All-Russian Institute of Plant Genetic Resources, 42-44, ul. Bol'shaya Morskaya, St. Petersburg, 190000 Russia, e-mail akme11@yandex.ru (✉ corresponding author);

²Agrophysical Research Institute, 14, Grazhdanskii prosp., St. Petersburg, 195220 Russia, e-mail sinad@inbox.ru, gpanova@agrophys.ru, yuv_chesnokov@agrophys.ru

ORCID:

Artemyeva A.M. orcid.org/0000-0002-6551-5203

Panova G.G. orcid.org/0000-0002-1132-9915

Sinyavina N.G. orcid.org/0000-0003-0378-7331

Chesnokov Yu.V. orcid.org/0000-0002-1134-0292

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Abstract

One of the main components of the successful production of plant products is the choice of crops with a high realization of their production potential under the formed conditions, including in the conditions of intensive light culture. The problem of compensating or eliminating the deficit of green crops is currently quite acute in Russia and in the world. The genetic diversity of the most widespread green *brassic* plants of the species *Brassica rapa* L. for use in protected ground facilities has developed in Southeast Asia and includes numerous morphotypes of Chinese cabbage, pakchoi, tatsoi, wutacai, mizuna, and leafy turnip. In Russia, leafy *B. rapa* vegetables are used to a limited extent, mainly as salad vegetables. The main areas of *B. rapa* crops breeding for artificial light culture include a high growth rate (the duration of vegetative period is not more than 28-35 days, depending on the biological characteristics of the crop), the yield is not less than 3-8 kg/m², depending on crops, a certain compact plant habit, high quality of leaves, valuable biochemical composition, resistance to early bolting, resistance to possible low light and high air temperature, suitability for growing in a thin layer of soil or hydroponics. The biological features of the diversity of *B. rapa* leafy crops as sources for breeding for intensive light culture conditions have not been sufficiently studied, first of all, this concerns little-known crops (tatsoi, wutacai, mizuna) and certain morphotypes within common crops (Chinese cabbage and pakchoi). In this study, for the first time, we studied the wide genetic diversity from the Russian worldwide *B. rapa* collection of the Russian Federation, stored in the VIR, and morphotypes within them (72 accessions), including genotypes contrasting in morphological characteristics, under different supply light intensity for the development of energy-saving agro-technologies in intensive light culture. For the first time, correction factors were determined for calculating the leaf surface area from easily measured linear dimensions for each studied crop and type of leaves, which will speed up the calculations of photosynthesis characteristics. A different degree of variation in the variability of the size and weight of plants of various crops, depending on the illumination, is shown, the highest in Chinese cabbage and leafy turnip. It was found that the intensity of the light regime is largely determined the increase in the productivity of plants of all studied crops, mostly of Chinese cabbage and pakchoi and leafy turnip. High-yielding accessions of Chinese cabbage, pakchoi and leafy turnip were identified (more than 6.3 kg/m² for 28 days of growing season). The studied accessions of wutacai and mizuna are low-yielding and have a longer growing season, but are of interest as vegetables for functional nutrition and for decorative purposes. Semi-headed types of Chinese cabbage (Shantung, Santo, Xiao), which are of primary interest for light culture, have been identified. We recommend Chinese cabbage accessions vr.k-1375, Bice (vr.k-1376), and pakchoi k-647 for thickened cultivation, and pakchoi Extra Dwarf Pak Choi (vr.k-1405) and tatsoi Xiao Ba Je Ta Cai (k-695) for growing with a small distance between the shelves of 25 cm. Accessions of Chinese cabbage Chokurei (k-177), Tokyo Bekana (k-395), Zao Shi No. 5 (vr.k-1120), and Fun jen F₁ (vr.k-1410) showed the highest salad qualities of the leaves. Under the conditions of a biopolygon, we managed to determine accessions with a stable manifestation of the traits, of early maturity and high productivity, practically independent of light intensity. These are accessions of Chinese cabbage Harumaki Shin Santousai (k-270), Tokyo Bekana (k-395), Xiaobaikou (k-74), pakchoi Kangre 605 (vr.k-1131), Gai Lang Jin Pin 25 F₁ (vr.k-1124). In addition, it was shown that the Chinese accession of Chinese cabbage Zao Shi No. 5 (vr.k-1120) and the Russian cultivar of the leafy turnip Selecta (vr.k-1371) have increased the activity

of the photosynthetic apparatus. The obtained data on the crops in general and the determined accessions of *B. rapa* are of interest for the practice of vegetable growing, in the development of elements of cultivar technology of *B. rapa* crops under conditions of light culture and as sources of valuable traits in breeding programs, including the development of city farming.

Keywords: *Brassica rapa* L., leafy crops, controlled-environment agriculture, artificial lighting, valuable traits, genetic sources

The main genetic diversity of the most common plants of the species *Brassica rapa* L. (turnip) for the protected ground has been formed in Southeast Asia: initially in China, then in Japan and Korea. It includes numerous morphotypes of pakchoi, Chinese cabbage, wutacai, tatsoi, mizuna, and leafy turnip. Chinese cabbage is the most widely cultivated on the globe, pakchoi is common in Central and Southern China, wutacai and tatsoi in Southern China, mizuna and leafy turnip in Japan [1].

In many industrially developed countries (Japan, USA, China, Singapore, South Korea, EU countries), fast-growing leafy *brassicac* crops with a valuable biochemical composition, in addition to traditional methods of cultivation (open field and greenhouses), have been grown along with lettuce (*Lactuca sativa* L.) in artificial lighting conditions of a new intensively developing type of plant production — vertical farms (plant factories) [2-7].

In Russia, *B. rapa* vegetables are traditionally used to a limited extent — mainly as salad vegetables. Chinese cabbage is grown in open field and protected ground, including industrial vegetable growing. Although the first Russian cultivar of Chinese cabbage Khibinskaya was created in 1962 at the Polar experimental station of VIR (Apatity, Murmansk Region) and was widely cultivated, later in greenhouses, Chinese cabbage gave way to lettuce. Pakchoi and mizuna in Russia are grown very limitedly in private vegetable growing (the first cultivars were included in the State Register of Breeding Achievements Allowed for Use in 2000 and 2002, respectively), leafy turnip and tatsoi are little known, wutacai is unknown (the State Register of Breeding Achievements Allowed for Use. Vol. 1. Plant varieties. Official publication. Moscow, 2020). It should be noted that all leafy *B. rapa* crops have a set of valuable features. They are early-maturing, productive, cold-resistant, and relatively easy in growing. According to the biochemical composition of these crops (especially rare), they are superior to lettuce (the content of vitamin C 40-80 mg/100 g, β -carotene 2-10 mg/100 g, chlorophylls 50-200 mg/100 g, a large number of organic acids, phenolic compounds, red-colored forms contain anthocyanins), which is due to the growing interest in such crops [8-11].

Eighty-four cultivars and hybrids of leafy *B. rapa* crops were included in the State Register of Breeding Achievements Allowed for Use (Vol. 1. Plant varieties. Moscow, 2020) in 2020. Among 57 samples of Chinese cabbage, 75% are F₁ hybrids, including 19 of Russian breeding, among 18 samples of pakchoi, 7 are hybrids, including 5 Russian hybrids, 5 samples of mizuna and 4 samples of leafy turnip are mainly represented by cultivars of Russian breeding. However, it should be noted that greenhouse farms in Russia so far provide only half of the required volume of green crops. At the same time, the State Register has no cultivars of leafy *B. rapa* crops created specifically for artificial lighting.

Intensive artificial lighting is increasingly used in the world and actively used in modern protected ground facilities, being an integral element of urbanized agricultural production, the development of which, according to experts, will steadily increase in the coming decades. It is due to its higher productivity and efficiency in the use of resources — space, soil, water, the number of crops, the volume of fertilizers, etc. (15-90 times higher compared to open field and 1.5-10

times – compared to modern greenhouse complexes of the protected ground) [2, 12, 13], as well as the gradual reduction (according to UN and FAO forecasts) of highly productive land due to their absorption by growing megacities, an expressed tendency of population concentration in cities, and the inability to ensure quantitative and qualitative demand for food through the traditional production chain and food supplies [14, 15].

One of the main components of the successful operation of urbanized plant production, especially in the case of energy-intensive production with the use of artificial lighting, is the choice of crops with a high realization of the productive potential in the conditions being formed. Studies of the influence of agrotechnical growing features in the protected ground and artificial lighting, including the level of irradiation and the spectral composition of light, on the productivity and biometric indicators of various vegetable crops, are conducted in Russia [16–19] and abroad [5, 7, 20–23], but many issues related to ensuring sustainable profitability of production have not been resolved yet.

Systematic fundamental long-term research work on physical modeling and the study of the influence of life-support factors of cultivated plants on their production process, the quality of formed plant products under regulated conditions is carried out in Russia only in several research institutes, including the Agrophysical Research Institute (ARI), where, based on a systematic approach, developments are carried out to optimize methods for obtaining consistently high crops of high-quality plant products in fully formed conditions of light, air, root environment, and depending on agrotechnological factors [24]. Prototypes of mobile and stationary plant factories with original technologies developed and tested at the Institute's biopolygon showed high efficiency and productivity [25].

Biological features of the diversity of leafy *B. rapa* crops of the worldwide VIR collection in the conditions of artificial lighting have not been sufficiently studied. The criteria for the selection of genotypes for growing in conditions of intensive artificial lighting, which is necessary for the breeding of forms for innovative urbanized agricultural production – city farming, have not been developed yet. First of all, it concerns little-known crops in Russia (wutacai, tatsoi, mizuna) and different morphotypes within common crops (pakchoi and Chinese cabbage).

This paper is the first to assess the wide genetic diversity of the Russian collection of leafy crops and the morphotypes within them in the species *Brassica rapa* L. by morphological and phenological characteristics associated with productivity, at different light availability in artificial lighting culture. The degree of variation in the size and mass of different types of plants depending on the illumination is estimated. For each of the studied crops (pakchoi, Chinese cabbage, wutacai, tatsoi, mizuna, and leafy turnip), the sources for breeding for yield, early maturity, salad qualities, and resistance to low illumination conditions have been identified. Correction factors have been established for calculating the leaf surface area for each crop and leaf type, which will speed up the calculations of photosynthetic characteristics.

The work objective was to study the growth reactions of the genetic diversity of leafy *brassic* crops in different illumination conditions when using low-volume technologies for growing plants in intensive artificial lighting.

Materials and methods. The sample of leafy crops of the species *Brassica rapa* L. included 72 samples of different origin (VIR collection, Federal Research Center, Vavilov All-Russian Institute of Plant Genetic Resources), including 36 samples of Chinese cabbage, 23 samples of pakchoi, 2 samples of wutacai, 3 samples of mizuna, 3 samples of tatsoi, and 5 samples of leafy turnip.

The plants were grown under the conditions of a regulated agroecosystem

(ARI development) in an original long-tier vegetation light installation [25] equipped with DNaZ-400 lamps (OOO Reflux, Russia) with a short day (12-hour photoperiod) and two illumination modes, 15-20 klx (high) and 10-15 klx (low). The spectral composition of the radiation of DNaZ-400 lamps is close to sunlight with a predominance of the long-wave range (<http://www.reflux.ru>). The distance from the lamp to the soil surface was 55 cm, from the lamp to the top of the plant 25-50 cm, depending on the age of the plants and the height of the rosette of the studied samples. Daytime ambient temperature was 24 ± 2 °C, night 20 ± 2 °C.

As a substrate, we used terrestrial peat with mineral additives [26], the thickness of the root layer was 3-4 cm. Watering was carried out daily, three times a week, instead of tap water, root fertilizing with 0.5 normal Knop's solution was used. The crops were thinned twice, at 2 weeks of age (the cultivation scheme was 10×8 cm, for some samples of pakchoi, wutacai, tatsoi, and leafy turnip – 10×5 cm, two samples of pakchoi were also grown according to the scheme of 10×10 cm). The replication in each variant was 12-20 plants.

Samples of pakchoi and tatsoi were also grown in the Pushkin laboratories of VIR in a glazed greenhouse in the early spring period (sowing on March 1) and in the open field (sowing on July 1). The growing scheme is the same under all conditions.

For biometric evaluation and statistical data processing, 5 plants per option were analyzed. Harvesting was carried out on the 28th day after sowing with sprouted seeds. During harvesting, the main biometric indicators were evaluated: the habitus and plant weight, the number of leaves, the size of the leaf lamina, and petiole [27].

The coefficient for determining the area of different types of leaves was calculated after processing photos of plants with smoothed leaves in Adobe Photoshop (Adobe, Inc., USA). The area of one leaf was determined as the result of multiplication of the length and width of the leaf lamina and the coefficient calculated for each sample, the area of the leaf surface of the plant — as the result of multiplication of the obtained value and the number of leaves.

Statistical data processing was performed by the method of variance analysis using the program STATISTICA v. 12. 0 (StatSoft Inc., USA). The mean value of each feature (M), the standard error of the mean (\pm SEM), the LSD at the 5% significance level, the coefficients of variation of the feature values, and the correlation coefficients of the studied features have been determined. The significance of the differences between the options has been determined by the indicator of the least significant difference.

Results. In the VIR worldwide collection, there are more than 1050 samples of all morphotypes of vegetable leaf crops of the *B. rapa* species, received since 1924, including 470 samples of Chinese cabbage, 123 samples of pakchoi, 7 samples of wutacai, 23 samples of tatsoi, 21 samples of mizuna, and 58 samples of leafy turnip. The uniqueness of this collection reaches 50%. When selecting from existing cultivars and creating special ones that allow getting 10-12 crops per year per unit area, the profitability of their cultivation can be very high, despite the cost of lighting in traditional protected ground facilities and intensive artificial lighting [2, 19, 24].

For growing in artificial lighting, representatives of *B. rapa* species must have a high growth rate (the duration of one turn is no more than 28-35 days, depending on the biological characteristics of the crop), a yield of at least 3-8 kg/m², a certain habitus of the plant (preferably an erect leaf rosette up to 30 cm high, a lower high is desirable), high leaf quality (no hairiness or weak hairiness, delicate consistency, good taste, valuable biochemical composition), resistance to early bolting, resistance to possible low illumination and high air temperature,

suitable for growing in a thin layer of soil substrate or on hydroponics.

In long-day leafy *brassic* crops used in Russia mainly for salads, the morphological and physiological features of the production process, including the time of transition to flowering, largely depend on the duration of the light period, illumination, spectral composition of light, adaptive reactions to growing in unfavorable light conditions, including in artificial lighting at a relatively high temperature [28].

We selected 72 samples of leafy crops of the species *B. rapa*, including landraces and samples of modern breeding, of various origins, mainly from countries where the consumption of this species is developed and the selection of its varieties is carried out (Table 1). Previously, the morphological description of the samples of the collection was carried out when growing in the field and the winter glazed greenhouse of the Pushkin laboratories of VIR (St. Petersburg) [29] and selected samples that are presumably close to the model of the desired variety for artificial lighting in terms of a set of characteristics. The number of samples of each crop in the selected option depended on the existing natural diversity of the culture morphotypes.

1. Origin and belonging to the ecological and geographical cultivar type of samples of East Asian leafy crops of *Brassica rapa* L. from the VIR collection selected for study (biopolygon of the Agrophysical Research Institute, St. Petersburg, 2019)

Catalog No.	Name	Varietytype/subtype	Origin
Chinese cabbage <i>Brassica rapa</i> L. subsp. <i>pekinensis</i> (Lour.) Hanelt			
k-74	Xiaobaikou	Xiao	China
k-112	Round Shantung	Shantung	Japan
k-123	Xiaobaikou	Xiao	China
k-126	Kairyochitose	Matsushima	Japan
k-177	Chokurei	Shantung	Japan
k-185	Mestnyi	Xiao	China
k-201	Hsiao Pai kou pai tsai	Kasin	China
k-227	Pyacha	Xiao	Kazakhstan
k-230	Digeson	Xiao	Korea
k-236	Untitled	Chosen	Mongolia
k-252	Untitled	Santo	Japan
k-270	Harumaki Shin Santousai	Santo	Japan
k-294	Xinfeng 2	Shantung	China
k-306	Zelenaya Malenkaya 1	Kasin	Korea
k-307	Osennyaya 3	Kasin	Korea
k-309	Chammad-baechu	Chosen	Korea
k-311	Pyongyang 2	Kasin	Korea
k-329	Maruba Santo	Santo	Japan
k-359	Nagaoka F1 Azuma	Shantung	Japan
k-387	Untitled	Xiao	China
k-395	Tokyo Bekana	Santo	Japan
k-406	Shantung Tropical Round (F1)	Shantung	Japan
k-410	Shirokuki Santousai	Santo	Japan
k-440	Hakata Chirimen	Chirimen	Japan
k-457	Osennyaya 2	Chosen	Korea
k-497	No. 55	Kasin	China
k-578	Teplichnaya 56	Kasin	China
k-629	Yoko F1	Datsinkou	The Netherlands
k-645	Beijingxiaoza 56	Kasin	China
vr.k-965	Kikunishiki F1	Kaga	Japan
vr.k-1120	Zao Shi No. 5	Shantung	China
vr.k-1375	Untitled	Chosen	Kyrgyzstan
vr.k-1376	Bitse	Santo × Hatou	Kyrgyzstan
vr.k-1400	Chirimen Hakusai	Chirimen	USA
vr.k-1410	Funjen F1	Santo	USA
vr.k-1557	MiniRaioh F1 50	Matsushima	Japan
Pakchoi <i>Brassicarapa</i> L. subsp. <i>chinensis</i> (L.) Hanelt			
k-116	Taisai	Taisai	Japan
k-470	F1 Mei Qing Choi	Syusman	The Netherlands
k-529	Zelenaya 9	Syusman	China
k-538	Green Boy	Piorbai	China
k-558	Joi Choi	Joy Choi	China
k-583	94 No. 6	Chinese × rosellate	China

k-584	Xia L V No. 2 Pakchoi	Syusman	China
k-585	Lingguan No. 1 PC	Syusman	China
k-647	Untitled	Taisai	Tajikistan
vr.k-932	Pack Choi F ₁	Piorbai	Japan
vr.k-1107	Heiyekuishantian	Chinese × rosellate	China
vr.k-1124	Gai Lang Jin Pin 25 F ₁	Syusman	China
vr.k-1128	Hanging	Syusman	China
vr.k-1130	Wuyemanyoucai	Syusman	China
vr.k-1131	Kangre 605	Syusman	China
vr.k-1251	Untitled	Syusman	China
vr.k-1343	Shatu	Syusman	China
vr.k-1403	Chinese Pack Choi	Piorbai	USA
vr.k-1404	Dwarf Pack Choi	Joy Choi	USA
vr.k-1405	Extradwarf Pack Choi	Joy Choi	USA
vr.k-1406	Petitestar F ₁	Babysyusman	USA
vr.k-1408	Redchoi F ₁	Syusman × Piorbai	USA
vr.k-1521	Untitled	Leychoy	Kyrgyzstan
T a t s o i <i>Brassica rapa</i> L. subsp. <i>narinosa</i> (Bailey) Hanelt			
k-695	Xiao Ba Je Ta Cai	Tatsai	China
vr.k-1398	Yukina Savoy	Chrysanthemum	USA
vr.k-1409	Redtatsoi F ₁	Tatsai	USA
W u t a c a i <i>Brassica rapa</i> L. subsp. <i>chinensis</i> (L.) Hanelt var. <i>pupuraria</i> Kitam			
vr.k-1357	Untitled	Untitled	The Netherlands
vr.k-1358	Untitled	Untitled	Unknown
M i z u n a <i>Brassica rapa</i> L. subsp. <i>nipposinica</i> (Bailey) Hanelt			
k-463	Shirojuki Sensujo Kyo Mizuna Nakate	Mizuna	Japan
k-506	Sensuji Kio Mizuna	Mizuna	Japan
bp. k-1360	Untitled	Mizunamagenta	The Netherlands
L e a f y t u r n i p <i>Brassica rapa</i> L. subsp. <i>rapifera</i> Hook. var. <i>komatsuna</i> M a k i n o			
k-335	Hiroshimana	Hiroshimana	Japan
k-598	Dapungrudatoubaixinie	Chinese × leafy turnip	China
vr.k-1371	Selecta	Komatsuna	Russia
vr.k-1372	Biryuzs	Komatsuna	Russia
vr.k-1418	Komatsuna	Komatsuna	USA

The set of Chinese cabbage studied in this work included mainly early-maturing samples of semihead Shantung type (including the Santo subtype with exceptional salad qualities), Xiao, open-topped head Kasin and Chosen, as well as some samples of common head Chifu type (the Matsushima subtype from Japan), Kaga, Dacinkou, a hybrid between the Santo and Hetou types, as well as the leafy type Chirimen, bearing the features of a leafy turnip (the presence of a short petiole and a thickened root). The samples are salad, with a compact leaf rosette, a large leaf lamina (often length is more than 40 cm), a dense consistency, without hairiness or with weak soft hairiness. The rosette leaves are directed mainly obliquely upright [30].

The study included all cultivar types of pakchoi, except for the relatively late-maturing Yutsai. Cultivars of the Taisai type have a closed rosette of upright leaves, with a very long (more than 25 cm) white or pale green petiole. The Piorbai type with a semi-spreading rosette and relatively numerous leaves on petioles of medium length (15-20 cm) includes productive samples, often resistant to alternariosis and peronosporosis, with a dense, tender consistency of a dark green leaf lamina and a light green petiole.

The most popular samples of pakchoi belong to the Syusman type. This is a typical pakchoi, with a compact leaf rosette of the “vase” type, often the plants are resistant to thickening. Petioles are relatively short (8-13 cm), very wide (5-7 cm), thick, light green. Special attention should be paid to the samples of mini-pak-choi (baby-pak-choi), very early-maturing (25-28 days), with a small compact rosette (15-18 cm).

The Leychoi type is represented by stable hybrids between samples of the Yutsai and Syusman types, tatsoi, and leafy turnip. They are characterized by thick bright white petioles of shiny dark green convex leaves, relatively high productivity (5-7 kg/m²), resistance to bolting. In samples of a similar type of Joychoi, the leaf

rosette is small (18-20 cm), spreading and semi-spreading, the leaves are weekly uprisen, the consistency of the leaves is very dense, crisp.

Tatsoi samples are characterized by a compact leaf rosette with obliquely upright (*Chrysanthemum*) and slightly raised numerous indistinctly lyre-shaped leaves (Tatsai, flat cabbage, black Chinese cabbage); petioles are short and of medium length (6-13 cm), narrow, green, leaf lamina of deep dark green color, shiny, convex, plicate-rugate. Tatsoi includes cultivars with a leaf rosette of small and very small sizes (12-18 cm), decorative, similar to a flower. The samples are low-yielding but have a very valuable biochemical composition, 35-60 mg/100 g ascorbic acid, 125-230 mg/100 g chlorophylls, and 4-7 mg/100 g β -carotene.

Samples of Japanese cabbage of the Mizuna type with strongly dissected leaves are decorative, with a semi-spreading rosette, resistant to bolting. Wutacai samples have long petioles (20-25 cm), upright and obliquely upright, purple; they are characterized by a high content of biologically active substances with average productivity.

Leafy turnip leaves are large, upright, entire and lyre; petioles are long (15-25 cm) and of medium length, light green. The samples are productive, resistant to bolting, and used after cooking.

Thus, the formed set of samples of *B. rapa* leafy crops had a potential diversity of qualitative characteristics and adaptive responses to environmental conditions, including light and temperature.

The main limit factor when growing *brassicas* crops in winter and early spring periods in greenhouses is insufficient illumination. It is shown [31] that at low illumination, plant adaptations manifest themselves in the enhanced development of the light-harvesting complex due to an increase in the assimilation surface and the number of photosynthetic pigments, with more economical use of assimilates for growth with a decrease in the specific surface density (SSD) of leaves, although an increase in SSD was observed in tuberous rooted mustard plants with strongly dissected leaves. For example, Tohoku Hakusai turnip plants were distinguished by increased shade tolerance compared to a sample of k-18 rooted mustard [31]. In low illumination conditions, turnip plants had an increased leaf area and an increased content of photosynthetic pigments [31].

The length of the light period (12-hour day) and high illumination in biopolygon conditions prevent the transition of long-day plants of the species to the reproductive phase of ontogenesis. Most crops of the *B. rapa* species are cold-resistant, in the centers of diversity formation they are often grown in the autumn, winter, and early spring periods, usually in fertile soil with a large space (especially Chinese cabbage). To identify samples with a stable display of valuable traits associated with productivity, we studied the morphophysiological features of the genetic diversity of the selected samples under light installation conditions at temperatures above the biological requirements of crops, in a thin layer of soil substrate, and at different luminous power. In addition, we took into account the fact that in the absence of separate rooms for each crop, the temperature regime is determined by the requirements of crops with a narrow reaction rate, grown together with greengrocers (in this study tomato and cucumber).

The leaves are the main photosynthetic and food organ of green *brassicas* crops. The important features that characterize the activity of the photosynthetic apparatus of plants include the leaf surface area. The area of the leaves in the study of the photosynthetic apparatus is determined in modern conditions using a photoplanimeter or a digital image analysis system SIMAGIS[®] MesoPlant[™] (Smart Imaging Technologies Co., USA). At the same time, methods for calculating the

leaf area from easily measured linear dimensions also retain value. The leaf area of some vegetable crops can be calculated using the regression equations according to Konyaev [32, 33]. Young entire leaves of Chinese cabbage and leafy turnip are most similar in shape to the leaves of white cabbage young plants, and the lyrate-sected leaves of mizuna are most similar to the leaves of radish, for which Konyaev proposed calculation formulas. Nikolenko and Kotov proposed a method for calculating the area of a strawberry leaf, Kiseleva for the area of a pear leaf [34, 35]. In the available literature, we did not find the leaf area calculations for pakchoi, wutacai, tatsoi, and crops with a similar leaf shape. In addition, the type and shape of the leaf lamina of samples of the same crop, primarily pakchoi and Chinese cabbage, which include a wide diversity of morphotypes, differ significantly. The leaves of the adult Chinese cabbage plant are mostly entire sitting, whereas the first true leaves in most samples are entire with a petiole of different length. The calculated coefficients obtained by the authors for determining the leaf area of the studied images differ (Table 2). For calculations based on the photo, the leaf was inscribed in a rectangle, the length and width of which correspond to the largest length and width of the plant leaf (excluding the petiole). The correction factor for the analyzed leaf was the quotient of dividing the leaf area in pixels by the rectangle area. The average for 5 leaves was taken as the average correction factor for the studied plant, then the coefficients for each sample were calculated as the average of five plants in the sample. By grouping the samples by leaf type, the authors found the average values for each leaf type.

2. Correction factors obtained for calculating the leaf surface area of samples of East Asian *brassic*s crops from the VIR collection ($n = 5$, biopolygon of the Agrophysical Research Institute, St. Petersburg, 2019)

Leaf type	Leaf lamina shape	Coefficients		
		min	max	mean
	C h i n e s e c a b b a g e <i>Brassica rapa</i> L. subsp. <i>pekinensis</i> (Lour.) Hanelt			
Entire	Broad-lanceolate	0.567	0.802	0.671±0.055
	Elongate inversely egg-shaped	0.607	0.628	0.618±0.012
	Inversely egg-shaped	0.646	0.672	0.660±0.027
	Oval	0.684	0.698	0.690±0.011
	Wide-oval	0.717	0.748	0.729±0.031
	Rounded	0.778	0.802	0.790±0.035
	P a k c h o i <i>Brassica rapa</i> L. subsp. <i>chinensis</i> (L.) Hanelt			
Lyre-shaped	Truncated-elliptical/oval	0.552	0.821	0.651±0.073
Unclear lyre-shaped	Truncated-elliptical/oval	0.633	0.645	0.640±0.018
Entire	Egg-shapedt	0.660	0.682	0.672±0.230
	Oval	0.690	0.719	0.703±0.238
	Rounded	0.733	0.794	0.765±0.042
	Wide-round	0.805	0.821	0.818±0.016
	T a t s o i <i>Brassica rapa</i> L. subsp. <i>narinosa</i> (Bailey) Hanelt			
Unclear lyre-shaped	Wide-oval	0.645	0.655	0.649±0.016
	M i z u n a <i>Brassica rapa</i> L. subsp. <i>nipposinica</i> (Bailey) Hanelt			
Lyre-shaped	Unpaired pinnatis sected	0.443	0.464	0.451±0.011
	W u t a c a i <i>Brassica rapa</i> L. subsp. <i>chinensis</i> (L.) Hanelt var. <i>pupuraria</i> Kitam			
Entire	Oval	0.663	0.721	0.692±0.041
	L e a f y t u r n i p <i>Brassica rapa</i> L. subsp. <i>rapifera</i> Hook. var. <i>komatsuna</i> Makino			
Entire	Wide-egg shaped/wide-oval	0.791	0.814	0.801±0.028

It was reported [31] that reduced illumination caused the formation of a larger leaf surface area in turnips and mustard. In our studies, the size of the leaf rosette, the number of leaves, and the linear dimensions of the leaf lamina of *brassic*s crops, depending on the illumination, differed on average by 9-25% (Table 3, Fig. 1) and were in all cases larger in high illumination conditions. This conclusion is consistent with the results of the study of cutting lettuce *Lactuca sativa* L. [35-37], tatsoi [38], and wutacai [39] at different levels of irradiation.

3. Morphological characteristics and yield in samples of East Asian *brassic*s crops from the VIR collection in the intensive artificial lighting at different levels of illumination ($n = 5$, $M \pm \text{SEM}$; biopolygon of the Agrophysical Research Institute, St. Petersburg, 2019)

Illumination level	Rosette dimensions, cm		Number of leaves, pcs.	Leaf lamina dimensions, cm		Area of one leaf lamina/leaf surface of the plant, cm ²	Plant weight, g	Yield, kg/m ²
	height	diameter		length	width			
C h i n e s e c a b b a g e <i>Brassica rapa</i> subsp. <i>pekinensis</i> <i>Brassica rapa</i> L. subsp. <i>pekinensis</i> (Lour.) Hanelt (36 samples)								
High (15-20 klx)	26.60±0.83	26.50±0.71	8.60±0.24	25.70±0.80	11.90±0.37	204.91/1762.23	50.40±3.49	6.30±0.32
Cv, %	19.56	16.65	17.44	19.37	19.61		43.26	
Low (10-15 klx)	23.80±0.65	24.20±0.56	8.20±0.21	22.90±0.67	10.40±0.30	159.57/1308.47	33.90±2.25	4.24±0.23
Cv, %	16.49	13.97	15.55	17.55	17.44		39.79	
P a k c h o i <i>Brassica rapa</i> L. subsp. <i>chinensis</i> (L.) Hanelt (23 samples)								
High (15-20 klx)	23.30±0.94	19.80±1.14	8.30±0.29	11.20±0.58	8.80±0.40	73.92/613.54	41.50±4.01	5.19±0.37-8.30±0.57 ^a
Cv, %	19.78	28.26	17.08	25.26	22.21		48.33	
Low (10-15 klx)	19.90±1.13	15.80±1.04	7.90±0.33	9.20±0.60	7.30±0.44	50.37/397.92	30.10±3.59	3.76±0.31-6.02±0.48 ^a
Cv, %	27.32	31.68	20.42	31.29	29.11		57.38	
T a t s o i <i>Brassica rapa</i> L. subsp. <i>narinosa</i> (Bailey) Hanelt (3 samples)								
High (15-20 klx)	22.00±1.55	20.70±2.02	15.40±6.21	9.40±1.33	7.20±0.88	43.99/677.45	33.60±3.30	4.20±0.28-6.72±0.49 ^a
Cv, %	15.76	21.81	69.73	31.71	27.34		21.96	
Low (10-15 klx)	17.90±1.16	17.50±2.9	13.60±2.91	7.50±1.15	6.50±0.94	31.69/430.98	25.90±8.27	3.24±0.49-5.18±0.78 ^a
Cv, %	11.21	28.68	47.86	26.36	25.14		55.36	
M i z u n a <i>Brassica rapa</i> L. subsp. <i>nipposinica</i> (Bailey) Hanelt (3 samples)								
High (15-20 klx)	23.60±1.91	19.10±0.93	13.70±4.12	10.70±0.45	7.10±0.29	34.19/468.40	22.70±1.97	2.84±0.22
Cv, %	14.00	8.45	52.03	7.30	7.06		15.00	
Low (10-15 klx)	20.80±0.20	17.20±1.25	12.10±3.23	9.80±0.91	7.10±0.74	31.31/378.85	17.80±1.39	2.23
Cv, %	1.67	12.58	46.16	16.09	18.20		13.49	
W u t a c a i <i>Brassica rapa</i> L. subsp. <i>chinensis</i> (L.) Hanelt var. <i>pupuraria</i> Kitam (2 samples)								
High (15-20 klx)	23.10±1.30	22.40±3.00	5.70±0.30	10.30±1.45	8.50±1.25	60.58/345.31	19.50±0.50	2.44±0.06-3.90±0.08 ^a
Cv, %	7.96	18.94	7.44	20.01	20.92		3.59	
Low (10-15 klx)	21.50±1.10	19.00±0.40	5.30±0.10	8.60±0.25	7.40±0.60	44.04/233.41	14.60±3.93	1.83±0.27-2.92±0.44 ^a
Cv, %	7.24	2.98	2.67	4.14	11.47		37.99	
L e a f y t u r n i p <i>Brassica rapa</i> L. subsp. <i>rapifera</i> Hook. var. <i>komatsuna</i> Makino (5 samples)								
High (15-20 klx)	29.00±3.16	29.90±5.19	8.30±0.78	24.70±3.65	12.60±1.54	248.98/2066.53	63.80±14.40	7.98±1.11-12.76±1.91 ^a
Cv, %	24.41	34.80	21.03	33.07	27.35		50.47	
Low (10-15 klx)	24.40±2.47	27.20±3.16	7.60±0.64	20.30±2.93	10.50±1.21	170.52/1295.95	33.70±7.37	4.21±0.63-6.74±0.01 ^a
Cv, %	22.64	26.01	18.82	32.28	25.84		48.88	
Averaged	23.00±0.62	21.60±1.07	9.60±0.80	14.20±1.77	8.80±0.50	96.20±19.23/831.60±149.65	32.30±3.04	
LSD ₀₅	1.85	3.20	2.39	5.32	1.49	57.69/448.96	9.13	
N o t e. The values (^a) are given for a group of samples grown according to the 5×10 cm seeding scheme.								

N o t e. The values (^a) are given for a group of samples grown according to the 5×10 cm seeding scheme.

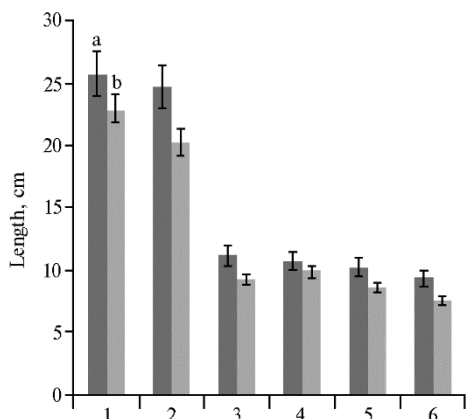


Fig. 1. Leaf lamina length in samples of East Asian *brassic* crops from the VIR collection in the intensive artificial lighting at high illumination (a, 15-20 klx) and low illumination (b, 10-15 klx): 1 — *Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt, 2 — *Brassica rapa* L. subsp. *rapifera* Hook. var. *komatsuna* Makino, 3 — *Brassica rapa* L. subsp. *chinensis* (L.) Hanelt, 4 — *Brassica rapa* L. subsp. *nipposinica* (Bailey) Hanelt, 5 — *Brassica rapa* L. subsp. *chinensis* (L.) Hanelt var. *pupuraria* Kitam, 6 — *Brassica rapa* L. subsp. *narinosa* (Bailey) Hanelt ($n = 5$, $M \pm SEM$; biopolygon of the Agrophysical Research Institute, St. Petersburg, 2019).

weight, and the yield of pakchoi, Chinese cabbage, and leafy turnip, the leaf surface area of the leafy turnip.

In all likelihood, it is due to the plant nutrition area, which is close to optimal under these growing conditions, allowing plants to avoid mutual shading, and the presence of a relatively large number of shade-tolerant genotypes, as well as the optimal regime of mineral nutrition. At the same time, the leaf surface area of one plant between the crops differed depending on the illumination by 24-59%, while the smallest difference between the leaf surface area with different illumination was noted in mizuna, the largest — in leafy turnip, pakchoi and tatsoi.

The coefficients of variation of the morphological characteristics of each crop, regardless of the illumination, were average and did not exceed 20% in the samples of Chinese cabbage and wutacai, as well as in tatsoi and mizuna (in the latter two crops — except for a highly variable number of leaves), that is, the growth strength (size) of the plants studied samples of *brassic* crops were close. The samples of pakchoi and leafy turnip differed significantly with coefficients of variation up to 32% in pakchoi, up to 35% in leafy turnip.

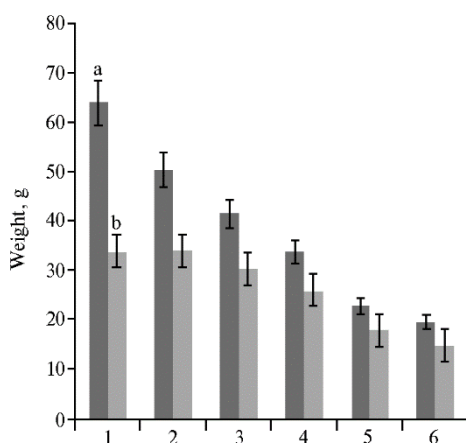


Fig. 2. Plant weight in samples of East Asian *brassic* crops from the VIR collection in the intensive artificial lighting at high illumination (a, 15-20 klx) and low illumination (b, 10-15 klx): 1 — *Brassica rapa* L. subsp. *rapifera* Hook. var. *komatsuna* Makino, 2 — *Brassica rapa* L. subsp. *pekinensis* (Lour.) Hanelt, 3 — *Brassica rapa* L. subsp. *chinensis* (L.) Hanelt, 4 — *Brassica rapa* L. subsp. *narinosa* (Bailey) Hanelt, 5 — *Brassica rapa* L. subsp. *nipposinica* (Bailey) Hanelt, 6 — *Brassica rapa* L. subsp. *chinensis* (L.) Hanelt var. *pupuraria* Kitam ($n = 5$, $M \pm SEM$; biopolygon of the Agrophysical Research Institute, St. Petersburg, 2019).

The differences in plant weight and, accordingly, yield depending on the illumination was significant (more than LSD_{05} 9.13 g) and especially significant in leafy turnip and Chinese cabbage, to a lesser extent in pakchoi; the excess plant weights of wutacai, tatsoi, and mizuna at a high level of illumination were unreliable (see Table 3, Fig. 2). The variability in plant weight was very strong between the samples of pakchoi and Chinese cabbage, and leafy turnip (coefficients of the trait variation were 40-57%).

A relatively low yield (more than 2 times lower than the yield of Chinese cabbage and leafy turnip) was noted in mizuna and wutacai, but the decorative and valuable biochemical composition, especially the high content of chlorophylls, carotenes, and anthocyanin [7] make them also promising for growing in artificial lighting.

All the studied quantitative characteristics (plant size and weight) correlate to a high degree (correlation coefficients $r = 0.72-0.98$; $p \leq 0.05$), including the average leaf surface area and plant weight of the studied set of samples that are strongly correlated ($r = 0.88$; $p \leq 0.05$). The number of leaves is associated with a weak to medium-strength inverse correlation dependence (r from -0.11 to -0.38 ; $p \leq 0.05$) with all indicators of the size of the rosette and leaves and does not correlate with the plant weight.

Among the studied samples of *brassic* crops, the authors identified sources for breeding for early maturity, productivity, and resistance to early bolting and marginal blight. Thus, 44% of the samples of Chinese cabbage from the studied set had a plant weight higher than the average (yield higher than 6.3 kg/m²) in high illumination conditions; in particular, 9 samples of mainly salad types of Shantung, Santo, Xiao had a yield of more than 7.5 kg/m², that is, 22-109% higher than the average for the crop. All these samples formed a high yield in 28 days, that is, they showed early maturity. The highest plant weight among the samples of Chinese cabbage was observed in the line isolated from the Japanese hybrid of the semi-headed Shantung type Shantung Tropical Round F₁ (k-406) (105.40±17.50 g) and in local Chinese samples of the Xiao type k-185 (97.00±22.25 g), and k-123 (73.90±6.31 g). However, with a decrease in illumination, their productivity fell by 46-125%, which indicates their high demands on the illumination level (Table 4).

4. Variability of productivity indicators in perspective samples of East Asian *brassic* crops from the VIR collection in the intensive artificial lighting depending on the illumination level ($n = 5$, $M \pm \text{SEM}$; biopolygon of the Agrophysical Research Institute, St. Petersburg, 2019)

Catalog No.	Sample name	Leaf surface area, cm ²		Plant weight, g		Yield, kg/m ²	
		high	low	high	low	high	low
Chinese cabbage <i>Brassica rapa</i> subsp. <i>pekinensis</i> <i>Brassica rapa</i> L. subsp. <i>pekinensis</i> (Lour.) Hanelt							
k-406	Shantung Tropical Round (F ₁)	3106.55	1460.67	105.40±17.50	46.80±3.71	13.18±1.80	5.85±0.45
k-185	Mestnyi	3191.37	2682.94	97.00±22.25	55.50±8.72	12.13±1.98	6.94±0.70
k-270	Harumaki Shin Santousai	3143.61	3473.24	86.40±6.97	71.70±5.98	10.80±0.65	8.96±0.54
k-629	Yoko F ₁	2368.28	1952.48	82.40±7.23	68.20±3.48	10.31±0.72	8.52±0.34
vr.k-1120	Zao Shi No. 5	1273.59	934.61	77.40±7.50	58.60±1.85	9.68±0.78	7.32±0.22
k-123	Xiao bai kou	1908.81	1444.41	73.90±6.31	50.50±2.90	9.24±0.74	6.32±0.25
vr.k-1410	Funjen F ₁	2191.75	1446.71	71.50±5.46	47.70±1.27	8.93±0.63	5.97±0.16
vr.k-1376	Bitse	2050.61	1602.77	68.60±8.00	48.50±2.50	8.57±0.86	6.06±0.24
k-395	Tokyo Bekana	2006.45	1557.81	65.90±16.80	62.80±10.33	8.24±1.49	7.85±1.01
k-74	Xiao bai kou	1938.98	1636.79	65.70±4.11	56.30±5.80	8.21±0.37	7.04±0.56
k-457	Osennyaya 2	2158.34	2081.80	59.50±6.08	53.40±4.58	7.44±0.51	6.68±0.46
P a k c h o i <i>Brassica rapa</i> L. subsp. <i>chinensis</i> (L.) Hanelt							
k-116	Taisai	1087.59	901.55	82.80±17.10	65.10±12.15	12.85±2.31	8.14±1.38
vr.k-1128	Hanging	899.90	742.28	77.20±13.25	57.90±7.91	9.65±1.54	7.24±0.80
vr.k-1131	Kangre 605	836.71	828.79	67.20±8.52	64.70±3.82	8.40±0.92	8.09±0.48
vr.k-1124	Gai Lang Jin Pin 25	675.73	576.68	54.80±6.30	55.10±7.51	6.85±0.62	6.89±0.88
vr.k-932	Pack Choi	606.21	494.74	50.50±7.88	42.90±4.25	6.31±0.82	5.36±0.43
vr.k-1405	Extra dwarf PC	415.81	324.56	44.70±5.94	42.30±6.39	8.95±0.90	8.46±1.02
T a t s o i <i>Brassica rapa</i> L. subsp. <i>narinosa</i> (Bailey) Hanelt							
k-695	Xiao Ba Je Ta Cai	613.86	544.99	40.10±4.38	39.70±5.17	8.01±0.64	7.94±0.80
vr.k-1398	Yukina Savoy	721.59	539.01	32.30±2.37	26.10±3.30	6.45±0.38	5.23±0.53
L e a f y t u r n i p <i>Brassica rapa</i> L. subsp. <i>rapifera</i> Hook. var. <i>komatsuna</i> Makino							
vr.k-1418	Komatsuna	3720.04	2966.65	102.20±11.10	61.50±17.27	12.78±1.28	7.69±1.69
k-335	Hiroshimana	3307.82	1084.51	90.30±9.50	30.00±4.18	11.29±0.91	3.75±0.43
vr.k-1372	Biryuza	2533.19	1755.17	75.00±4.16	52.70±2.15	9.38±0.37	6.59±0.20
vr.k-1371	Selecta	1263.09	896.37	68.70±7.88	46.40±2.94	8.59±0.86	5.80±0.35

In addition, the old Japanese cultivars of type Santo Harumaki Shin Santousai (k-270) (86.40 ± 6.97 g) and Tokyo Bekana (k-395) (65.90 ± 16.80 g), and the old Chinese sample of the type Xiao Xiao bai kou (k-74) (65.70 ± 4.11 g) were distinguished by a high plant weight, while the morphological parameters of plants, including their weight, decreased by no more than 5-20% with reduced illumination, and the highest stability was achieved by the Tokyo Bekana sample which showed traits at different illumination conditions (the differences between the plant weight indicators at high and low illumination conditions are 3 times lower than LSD_{05}).

The remaining samples of Chinese cabbage were characterized by a yield of less than 6.3 kg/m^2 and a reduced growth rate. Despite the resistance of most of them to low illumination, they are not recommended for intensive artificial lighting, although they can be included in breeding programs as sources of adaptability, such as the Korean cultivars with high leaf quality traits Osenyaya 2 (k-457) of the Chosen cultivar type.

Three samples of pakchoi in high illumination had a plant weight of more than 60 g: this is an old Japanese sample with the long-petiole Taisai type (k-116) (82.80 ± 17.10 g) and modern Chinese cultivars of the Syusman type with a closed compact rosette Hanging (vr.k-1128), and Kangre 605 (vr.k-1131) (77.20 ± 13.25 and 67.20 ± 8.52 g, respectively), while for the first two cultivars, the trait values under high illumination level were 27-33% higher, and of the last one were almost independent of the illumination level.

The representatives of the Piorbai, Syusman, Joychoy, and Leychoy cultivar types — four modern pakchoi cultivars from China, Japan, and the United States had a plant weight of 50-60 g, and four other samples from China, the United States, and Kyrgyzstan 40-50 g. The samples of pakchoi high-yielding Gai Lang Jin Pin 25 F₁ (vr.k-1124) and Pack Choi F₁ (vr.k-932) with an average yield practically did not react to the change in illumination. Samples of pakchoi with a yield below 5 kg/m^2 are not recommended for growing in artificial lighting culture.

Among the studied samples with consistently high productivity, there were samples with a super-compact rosette, i.e., pakchoi Extra Dwarf Pack Choi (vr.k-1405) ($8.46\text{--}8.95 \text{ kg/m}^2$) and tatsoi of Xiao Ba Je Ta Cai (k-695) ($7.94\text{--}8.01 \text{ kg/m}^2$), suitable for thickened cultivation with a planting scheme of 10×5 cm. The samples are squat, they can be grown at a distance between the shelves no more than 30 cm, if modern LED lamps with high efficiency are used as light sources, which emit significantly less heat energy than the DNaZ-400 lamp. For thickened cultivation, a sample of tatsoi Yukina Savoy (vr.k-1398) (yield $5.20\text{--}6.40 \text{ kg/m}^2$) with relatively stable productivity and a very valuable biochemical composition is also of interest.

The studied samples of mizuna for 28 days could not form a plant weight higher than 25 g, while in two cultivars, the excess plant weight under high light was 36-66%; in the mizuna cultivar with red-purple leaves (vr.k-1360), the productivity parameters were the lowest ($2.05\text{--}3.80 \text{ kg/m}^2$, depending on the illumination level). With thickened cultivation, the yield of mizuna in high illumination was $3.80\text{--}5.10 \text{ kg/m}^2$, it was the highest ($5.10 \pm 0.48 \text{ kg/m}^2$) in the Japanese cultivar Shirojuki Sensujo Kyo Mizuna Nakate (k-463).

Samples of wutacai from the Netherlands also require longer cultivation: for 28 days, the plant weight was 19-20 g only, and in the sample vr.k-1357, it practically did not depend on illumination.

Four samples of leafy turnip Komatsuna and Hiroshimana types showed good productivity. Under high illumination conditions, the plant weight in the

Komatsuna sample (vr.k-1418, USA) was 102.20 ± 11.10 g, in the Hiroshimana sample (k-335, Japan) 90.30 ± 9.50 , in the Russian cultivars Selekt (vr.k-1371) and Biryuza (vr.k-1372) 68.70 - 75.00 g, that is, the yield reached 8.59 - 12.78 kg/m². However, in low illumination conditions, the productivity of all leafy turnip samples fell by 43-81%.

Some of the studied samples formed a high plant weight with a small leaf surface area, for example, the Chinese sample of Chinese cabbage Zao Shi No. 5 (vr.k-1120), the Russian cultivar of leafy turnip Selekt (vr.k-1371), which indicates the intensive work of their photosynthetic apparatus.

The *brassic* samples were mostly resistant to early bolting, although up to 10% of the plants entered to bolting of three samples of Chinese cabbage, two samples of pakchoi, and one sample of leafy turnip (all samples of South Chinese and Japanese origin).

In Chinese cabbage of the Kasin type (headed with an open-top) and three samples of the Shantung and Xiao types, the onset of the marginal blight was observed – a physiological disorder caused by calcium deficiency (observed mainly in headed Chinese cabbage, which develops rapidly at elevated temperature and humidity).

5. Variability of plant size and weight in samples of pakchoi and tatsoi *Brassica rapa* L. from the VIR collection under different growing conditions ($n = 5$, $M \pm SEM$; biopolygon of the Agrophysical Research Institute, St. Petersburg, winter glazed greenhouse and experimental open field of Pushkin laboratories VIR, Leningrad Province, 2019)

Growing conditions	Dimensions, cm				Plant weight, g	Yield, kg/m ²
	leaf rosette		leaf lamina			
	diameter	height	length	width		
S a m p l e Gai Lang Jin Pin 25						
Biopolygon:						
high illumination (15-20 klx)	24,40±0,92	16,20±0,8	11,10±0,55	8,20±0,49	54,76±5,88	5,48±0,49
low illumination (10-15 klx)	22,00±1,89	15,00±0,95	10,40±0,51	7,60±0,51	55,28±6,83	5,53±0,61
Greenhouse	24,30±1,38	15,30±0,91	10,10±0,74	7,30±0,62	52,20±6,43	5,22±0,57
Field	22,10±1,47	14,80±0,83	9,70±0,73	7,00±0,65	50,60±6,21	5,06±0,53
Cv, %	3,22	3,18	5,15	4,35	3,82	3,91
S a m p l e Kangre 605						
Biopolygon:						
high illumination (15-20 klx)	26,20±1,74	20,40±1,12	12,60±0,74	9,60±0,58	65,22±4,78	6,52±0,38
low illumination (10-15 klx)	25,80±0,86	22,00±1,86	13,80±0,97	10,20±0,38	66,88±7,94	6,69±0,72
Greenhouse	24,30±1,28	21,40±1,33	13,00±1,12	9,20±0,35	61,40±5,15	6,14±0,46
Field	23,70±0,92	20,60±1,75	12,50±0,87	9,10±0,43	60,20±4,83	6,02±0,42
Cv, %	5,28	4,47	3,90	3,82	4,95	5,04
S a m p l e Extra dwarf Pack Choi						
Biopolygon:						
high illumination (15-20 klx)	18,60±0,74	10,00±0,54	7,30±0,58	8,90±1,32	42,30±6,39	8,46±1,02
low illumination (10-15 klx)	18,40±0,86	9,00±0,32	6,50±0,71	8,30±0,70	44,70±5,94	8,95±0,78
Greenhouse	17,40±0,92	10,80±0,45	7,20±0,47	7,80±0,82	41,10±5,11	8,22±0,83
Field	17,00±1,14	9,10±0,28	6,60±0,52	7,40±0,57	40,80±5,43	8,16±1,06
Cv, %	5,31	4,82	6,45	5,38	6,07	6,24
S a m p l e Xiao Ba Je Ta Cai						
Biopolygon:						
high illumination (15-20 klx)	20,80±1,31	13,40±0,59	6,40±0,69	5,90±0,29	39,70±5,17	7,94±0,80
low illumination (10-15 klx)	19,40±0,94	13,60±0,51	5,80±0,20	5,20±0,30	40,10±4,38	8,01±0,64
Greenhouse	20,20±0,85	12,30±0,62	5,70±0,44	5,70±0,47	36,50±4,12	7,32±0,66
Field	20,90±0,12	13,36±0,93	6,40±0,54	5,40±0,38	35,60±3,61	7,11±0,57
Cv, %	3,25	4,14	5,26	3,38	5,66	5,79

The highest salad quality of leaves was recorded in samples of Chinese cabbage Chokurei (k-177), Tokyo Bekana (k-395), Zao Shi No. 5 (k-1120), and Fun jen F₁ (k-1410). In terms of quality, the samples of vr.k-1375 and Bitse (vr.k-1376) from Kyrgyzstan with an erect rosette of upright leaves are also of interest, as well as a sample of pakchoi k-647 with a green petiole from Tajikistan.

In the studied set, among the samples of pakchoi and tatsoi we identified

highly stable, preserving morphological and phenological parameters in contrasting conditions of open field, greenhouses, and agricultural biopolygon, with very low variability of traits, which is mainly determined by the genotype. These are some modern cultivars, including, most likely, those carrying dwarfism *dwf* genes [40] (Table 5). The selected forms, in our opinion, should be included in the breeding process.

Thus, as a result of the research, it was found that the increase in plant productivity of all leafy crops of the *Brassica rapa* species was largely determined, as a rule, by the light flux intensity. For the conditions of intensive artificial lighting, the most high-yielding (more than 6.3 kg/m²) samples of cabbage and leafy turnip, as well as some samples of pakchoi, were selected. The studied samples of wutacai and mizuna are low-yielding and have a longer growing period, but are of interest as vegetables for functional nutrition and decorative purposes. The Chinese cabbage types (Shantung, Santo, Xiao), which are of primary interest for artificial lighting, have been identified. Under biopolygon conditions, we found perspective samples with a complex of valuable traits for various directions of breeding. Thus, the samples of Chinese cabbage Harumaki Shin Santousai (k-270), Tokyo Bekana (k-395), Xiaobaikou (k-74), pakchoi Kangre 605 (vr.k-1131), Gai Lang Jin Pin 25 F₁ (vr.k-1124) showed stable early maturity and high productivity almost independently of the level of illumination. It is shown that the Chinese sample of Chinese cabbage Zao Shi No. 5 (vr.k-1120) and the Russian cultivar of leafy turnip Selecta (vr.k-1371) have the increased photosynthesis intensity. Samples of Chinese cabbage vr.k-1375, Bitse (vr.k-1376), pakchoi k-647 are recommended for thickening conditions, pakchoi Extra Dwarf Pack Choi (vr.k-1405) and tatsoi Xiao Ba Je Ta Cai (k-695) for thickened cultivation at a small distance between shelves. Samples of Chinese cabbage with very high salad quality of leaves were identified — Chokurei (k-177), Tokyo Bekana (k-395), Zao Shi No. 5 (k-1120), Fun jen F₁ (vr.k-1410). The obtained data reveal some features of the reaction of different genotypes of *brassic*as crops of the *B. rapa* species to changes in the illumination level and increased temperature. The morphophysiological diversity of the studied forms will make it possible to manage the production process in vegetable growing more effectively, and the identified samples can be used as sources of valuable traits in the selection of specialized varieties for city farming.

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***Miscanthus sacchariflorus* IN SIBERIA — BIOLOGICAL YIELD PARAMETERS AND DYNAMICS OF BIOFILIC ELEMENTS**

S.Yu. KAPUSTYANCHIK¹ ✉, A.A. DANILOVA², I.E. LIKHENKO¹

¹*Siberian Research Institute of Plant Cultivation and Breeding — Branch of Institute of Cytology and Genetics, SB RAS, 21, ul. C-100, Krasnoobsk, Novosibirsk Province, Russia 630501, e-mail kapustyanchik@bionet.nsc.ru (✉ corresponding author), lihenko@mail.ru;*

²*Siberian Federal Scientific Center of Agro-Bio Technologies RAS, ul. Tsentralnaya, Krasnoobsk, Novosibirsk Province, 633501 Russia, e-mail Danilova7alb@yandex.ru*

ORCID:

Kapustyanchik S.Yu. orcid.org/0000-0002-2954-0620 Likhenko I.E. orcid.org/0000-0002-0305-1036

Danilova A.A. orcid.org/0000-0002-2212-3074

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Abstract

Throughout the world, bioenergy crops are grown to replace fossil resources and reduce greenhouse gas emission. *Miscanthus* is one of the bioenergetic plants that is widely cultivated in countries with optimal hydrothermal conditions. There is little research on the specifics of *Miscanthus* spp. cultivation in the continental climate. The aim of the study was a preliminary assessment of the possibility of *Miscanthus* cultivation in Siberia. The production process of *Miscanthus sacchariflorus* cv. Soranovsky (The State Register of Breeding Achievements, patent No. 6931 dated 06/06/2013) was assessed under conditions of the Central Forest-Steppe of Novosibirsk Ob (Novosibirsk Province, 54°53'13.5"N, 82°59'36.7"E, agro-gray soil, the experimental base of the Siberian Research Institute of Plant Cultivation and Breeding). During the formation of *Miscanthus* long-term stands (1-4 years), the aboveground biomass averaged 13 t/ha, the belowground biomass 17 t/ha. These figures are close to the average for this species in the world. The total N, P and K removal with biomass of stems and leaves was 51, 6, and 49 kg/ha, and with stems it was only 23, 3, and 26 kg/ha, respectively. Accumulation of N, P and K in the belowground biomass was 130, 10, and 126 kg/ha, respectively. Therefore, our experiment did not show any depletion of biophilic elements in soil during a four-year growth of miscanthus. Rough estimates have shown the presence of objective prerequisites for the atmospheric carbon sequestration in the fractions of the soil organic matter. During the first year of *Miscanthus* vegetation, at least 300 kg/ha of carbon accumulated in the mobile fractions of the soil organic matter; an increase in the C/N ratio in the belowground biomass of *Miscanthus* up to 74 vs. 20 in the initial soil was accompanied by a significant decrease in the mineralization rate of newly incoming plant residues. The parameters of the production process of *Miscanthus sacchariflorus* cv. Soranovsky on agro-gray soil in the Central Forest-Steppe of Novosibirsk Ob region correspond to the growth characteristics of *M. sacchariflorus* cultivated in other regions of the world on different soil types. We conclude that cultivation of *M. sacchariflorus* in Siberia is ecologically and commercially reasonable.

Keywords: bioenergy crops, *Miscanthus sacchariflorus*, cv. Soranovsky, aboveground biomass, belowground biomass, soil, nutrient removal, carbon sequestration

The production of renewable sources of fuels and raw materials is becoming one of the conditions for the survival of humankind. The cultivation of bioenergetic plants is recognized as one of the most important ways to solve this problem. Among such plants, representatives of the genus *Miscanthus* spp. of the family Poaceae are considered to be among the most promising in the world. This is due to both the valuable chemical properties of biomass and the characteristic high

growth rates and colossal biological productivity of this plant, as well as the conditions of a moderate climate. *Miscanthus* biomass is used as a renewable source of fuel for the production of lignocellulosic biomass, construction and composite materials (substitutes for wood and plastics) [1, 2]. Chlorophyll is also obtained and paper is produced from biomass [3].

The genus *Miscanthus* includes more than 20 species, distributed from tropical and South Africa to East and Southeast Asia. In Russia, in the Far East, there are 3 species: sugar-flowered miscanthus (*Miscanthus sacchariflorus*), reddening miscanthus (*M. purpurascens*), and Chinese miscanthus (*M. sinensis*) [4].

In the world (in Norway, Germany, Denmark, Great Britain, USA, Canada, and other regions), a fairly large experience has been accumulated in cultivating energy crops, including several species of the genus *Miscanthus*. At present, the main areas of their planting are located mainly in countries with an average annual positive air temperature above 5-10 °C and total annual precipitation of at least 600 mm (North America, Europe). The most widely cultivated species is *M. giganteus*, which stands out for its particularly high productivity in the above-mentioned climatic zones. Low frost resistance of this species [5-7] reduces its prospects for growing in the conditions of the Russian Federation.

In Siberia, miscanthus is quite famous as an ornamental plant. The problem of growing crops for biomass production in Siberia remains practically unexplored. It became necessary to select local varieties of miscanthus capable of maintaining high productivity under the limiting factors of the continental climate. Based on a collection of wild miscanthus species selected in the Far East of Russia, the cultivar Soranovsky was bred [8], which, by phenotyping and DNA analysis, was assigned to the species *M. sacchariflorus* [9]. The plants of this species are long-rhizome, have a height of up to 2.0-2.5 m, the stem is straight, rigid, with a pubescent leafy sheath around it. The leaves are narrow, linear-lanceolate, up to 60 cm long. The abaxial surface of the leaf plate is glabrous and non-pubescent, the adaxial surface is pubescent with a whitish, noticeable middle and solid edge. The inflorescence is in the form of a fan-shaped panicle of pale purple color at the beginning of flowering and white-gray at its completion, up to 25 cm long. The axis of the inflorescence is short, with long racemes.

M. sacchariflorus of the cultivar Soranovsky, in comparison with *M. giganteus*, is characterized by a higher frost and drought resistance, which makes it possible to recognize its potential for cultivation in the conditions of Siberia [10]. A distinctive feature of the variety is the formation of very long rhizomes that quickly colonize the free space. As a result, in a short period of time, a continuous flat plantation is formed, which successfully competes with weed vegetation. As it is known, the plantation of *M. giganteus*, due to its short rhizomes, slowly colonizes the space and represents overgrown hummocks with empty areas occupied by weed vegetation [10].

A large-scale experiment aimed at comparing the productivity of 15 genotypes of *M. giganteus*, *M. sinensis* and *M. sacchariflorus* in different climatic zones (Great Britain, Germany, the Netherlands, Ukraine, Turkey, Russia — Moscow Province) showed that *M. sacchariflorus*, in comparison with *M. giganteus* and *M. sinensis*, had more pronounced dependence of the biomass value on climatic conditions [11]. In this regard, the substantiation of the possibility of cultivating *M. sacchariflorus* in continental conditions requires special studies.

In addition, to regulate the production process of miscanthus, in particular, to answer the question regarding the doses and types of fertilizers, the

data on the turnover of biophilic elements in the soil-plant system are required. As it is known, the relatively low alienation of biophilic elements with the yield of miscanthus is due to the fact that when the biomass dries up, a significant part of the elements returns to the belowground part of plants (reutilization). As it is noted [12-14], the amount of reutilization depends on climatic conditions, the type of miscanthus, as well as the timing of harvesting.

The value of energetic plants is not limited to producing a renewable source of fuel and raw materials. In recent years, the interest in these crops, especially miscanthus, has been increasing due to the problem of binding the greenhouse gas CO₂. It is assumed that the colossal productivity of miscanthus biomass will contribute to the sequestration of atmospheric CO₂ in stable fractions of soil organic matter (SOM) [15-19].

In this work, the authors have shown for the first time that a new culture for Russia *Miscanthus sacchariflorus* of the cultivar Soranovsky can be grown in the conditions of the arid and cold climate of Western Siberia. Miscanthus with an average yield of 10±1.5 t/ha of absolutely dry matter for the first four years of plantation formation did not deplete the soil with elements of mineral nutrition of plants, contributed to the atmospheric carbon sequestration in the fractions of SOM (1±0.15 t C/ha per year in mortmass, 150±30 kg C/ha per year in mobile fractions).

The aim of the study was to assess the possibility of growing *M. sacchariflorus* of the cultivar Soranovsky in the continental conditions of Siberia based on the analysis of plant productivity, dynamics of carbon in the agroecosystem, and determination of the need for mineral fertilizers.

Materials and methods. The experiments were conducted in the conditions of the Central Forest-Steppe of Novosibirsk Ob (Novosibirsk region, 54°53'13.5"N, 82°59'36.7"E). A miscanthus plantation with an area of 0.3 hectares was laid in the spring of 2015 at the scientific and experimental base of the Siberian Research Institute of Plant Cultivation and Breeding – a branch of the Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences. The rhizomes of *M. sacchariflorus* cv. Soranovsky (originator – Institute of Cytology and Genetics, Siberian Branch of the Russian Academy of Sciences) were used as planting material [9]. The observations continued during 2015-2018.

According to the data by AMS Ogurtsovo, during the growing seasons in 2015, 2017, and 2018, the precipitations were 322, 316, and 297 mm, respectively, the sum of temperatures above 0 °C was 2396, 2340, and 2199 °C, in 2016 231 mm and 2478 °C with vs. long-term values of 263 mm and 2248 °C. Consequently, 2015, 2017, and 2018 were characterized as moderately humid, and 2016 was relatively dry; in all years the hydrothermal coefficient was the same (HTC = 1).

Before the establishment of the miscanthus plantation, a soil-agrochemical survey of the experimental site was performed. The soil was agro-gray typical. The depth of the humus horizon was 30-35 cm, the content of C_{org.} was 1.57-1.76% (according to Tyurin), N_{tot.} 0.13% (according to Kjeldahl), P₂O₅ 30-40 mg/100 g (according to Chirikov), K₂O in the extract 10-13 mg/100 g (according to Chirikov), hydrolytic acidity 0.5-1.5 mg-eq/100 g of soil (according to Kappen in the modification of CINAO), and the content of exchangeable Ca and Mg was about 70% of the cation exchange capacity. The granulometric composition was medium loamy.

To study the accumulation of plant biomass, plots of 50×50 cm were used. The aboveground plant biomass (AB) was sampled in four-fold repetition annually during the growing season according to the phases of miscanthus development. The biomass was counted separately for plant organs – stems, leaves, and

inflorescences (panicles). The belowground biomass (BB) was taken into account by the method of monoliths (height 25 cm, cross-sectional area $10 \times 10 \text{ cm}^2$) annually in 5-fold repetition at the onset of the phase of decline of AB. Mortmass and rhizomes were separated by decantation of the soil with water on a sieve with a mesh diameter of 0.25 mm. The biomass was dried to an absolutely dry state. The NPK content in the aboveground plant biomass was determined annually in the phases of germination, flowering, and decline. After the desiccation of the biomass to an absolutely dry state, its wet ashing was conducted according to the Ginzburg method, the total nitrogen content was determined according to Kjeldahl, phosphorus according to Truog, potassium photometrically (atomic absorption spectrometer with flame atomization AAS Kvant 2A MT, OOO KORTEK, Russia) [20]. The analytical replication was 3-fold.

Based on the actual content of elements in the biomass and the total value of the latter, the authors calculated the removal of nitrogen, phosphorus, and potassium during the period of maximum development of the AB by the end of August—early September (flowering phase) and when it dried up by the end of September—early October. The difference between these values was used to assess the amounts of elements returned to the soil with dry plant residues.

To determine the content of NPK and organic carbon in BB on plots of $50 \times 50 \text{ cm}$, the rhizomes were taken in 5-fold biological repetition and each sample was analyzed in 2-fold repetition. The NPK content in the rhizomes was measured in the autumn of 2016. The analysis procedure was the same as for determining NPK in AB. The accumulation of elements in the biomass and their removal with the harvest were calculated from the value of the biomass and the accumulation of the corresponding element in it. The content of organic carbon (C_{org}) and total nitrogen (N_{tot}) in BB was determined on an elemental CHNS analyzer Vario EL III (Elementar Analysensysteme GmbH, Germany) according to the attached manufacturer's instructions.

Soil sampling to assess the NPK content in 0–20 and 20–40 cm layers was performed in 3-fold repetition on the $50 \times 50 \text{ cm}$ plots annually at the beginning (germination phase) and at the end (senescence phase) of miscanthus vegetation. The analytical repetition was 3-fold. The agrochemical analysis of the soil was performed by the standard methods: phosphorus was determined in two extracts according to Chirikov and to Karpinsky-Zamyatina (GOST 26204-91, Moscow, 1992; DSTU 4727: 2007, <http://www.chemicalnow.ru/chemie-6387.html>), nitrate nitrogen ionometrically (GOST 26951-86, Moscow, 1986), potassium in Maslova's extract (AAS Kvant 2A MT, OOO KORTEK, Russia) (GOST 26210-91, M., 1992), the content of organic carbon (C_{org}) according to Tyurin [21].

The indicators characterizing the carbon metabolism in the soil were assessed by the absorption method according to the production of C-CO_2 under laboratory conditions [22]. A sample of soil (200 g of air-dry mass) was placed in plastic vessels with a volume of 1 L with a sealed lid. The incubation was conducted at 25°C and humidity 60% of the field water capacity. The emitted CO_2 was captured with 0.1 n. NaOH. The remainder of the alkali was determined by titration using a 916 Ti-Touch titrator (Metrohm AG, Switzerland).

To determine the amount of carbon in the mobile fraction of SOM (laboratory experiment 1) after the first 2 years of vegetation, the authors simplified a bit the known technique [23]. The experiment involved soil samples from under miscanthus and from under pure fallow. The amount of carbon in the latter was taken as the initial one, since a miscanthus plantation was made on this soil. The experiment was repeated 3 times. The incubation was conducted until the signifi-

cant differences in the rate of soil respiration between the variants of the experiment disappeared, which was considered to indicate the complete decomposition of the stock of mobile SOM accumulated in the soil under the miscanthus. Further, the value of mobile SOM was calculated from the difference in the total release of C-CO₂ from the soil from under the miscanthus and fallow.

To study the direction of carbon metabolism in the soil under miscanthus (laboratory experiment 2), ground wheat straw was added to soil samples (from under fallow and miscanthus) at a dose of 600 mg C/kg of soil. The response of the respiratory activity of the soil was assessed as an increase in the production of C-CO₂ in the variant with the introduction of wheat straw (%) compared to the control (soil samples in which no straw was added). The experiment was repeated 3 times.

The experimental data were statistically processed using the MS Excel program. The results are presented as means (*M*) with standard errors of means (\pm SEM) or confidence intervals at a significance level of $p = 0.05 (t_{0,05} \times \text{SEM})$. The reliability of the differences was assessed by the results of calculating the paired Student's *t*-test. With the actual value of the criterion below the level accepted in the experiment (0.05), the differences between the options were considered significant.

Results. As a result of the research, it was required to answer the following questions: how does the productivity of *M. sacchariflorus* of the cultivar Soranovsky compare with the development parameters of this and other miscanthus species in different regions of the world; is the soil depleted in terms of the main biophilic elements during the cultivation of miscanthus and is there a need for fertilization; what is the direction of the dynamics of carbon in the agroecosystem of *M. sacchariflorus* of the cultivar Soranovsky in the first years of plantation formation.

The phases of miscanthus development, in which the plant biomass was taken into account, are presented in Table 1.

1. Dates of the onset of phenological phases in *Miscanthus sacchariflorus* plants of cv. Soranovsky by the years of observation (Novosibirsk Province, 54°53'13,5"N, 82°59'36,7"E)

Phase	2015	2016	2017	2018
Planting	05/14	—	—	—
Germination	06/08	05/24	05/24	05/29
Internode growth	06/21	06/02	06/06	06/07
Belowground tillering by means of rhizomes	07/14	—	—	—
Stem elongation	08/25	07/14	07/15	07/17
Flowering	09/18	08/23	08/31	09/04
Decline	10/14	09/30	10/06	10/04
Duration of the vegetation period, days	129	130	136	129

Note. Dashes in the table indicate the absence of data.

2. Biomass dynamics (absolutely dry matter, t/ha) in *Miscanthus sacchariflorus* plants of cv. Soranovsky during the formation of the plantation ($n = 12$, $M \pm \text{SEM}$; Novosibirsk Province, 54°53'13,5"N, 82°59'36,7"E)

Year (planting age)	Aboveground								Belowground
	seedlings	flowering			drying				
	leaves and stem	leaves	stem	panicle	leaves	stem	panicle	total	
2015 (1 year)	0.009±0.001	0.30±0.04	0.5±0.1	0.04±0.01	0.3±0.1	0.5±0.1	0.05±0.01	0.8±0.1	4.5±0.7
2016 (2 years)	1.0±0.1	6.30±2.00	7.1±2.2	0.40±0.10	6.0±0.9	6.3±0.7	0.30±0.10	12.6±1.5	9.6±1.8
2017 (3 years)	2.7±0.7	7.80±1.00	8.1±0.8	0.10±0.09	7.4±1.2	8.1±1.2	0.30±0.07	15.9±0.6	13.7±0.8
2018 (4 years)	2.3±0.6	5.60±1.30	6.6±1.5	0.05±0.02	4.5±1.2	7.4±1.5	0.20±0.04	12.1±1.2	17.0±1.1

Table 2 shows the dynamics of accumulating the AB and BB of miscanthus over the years of the observation.

In the year of planting (2015), miscanthus formed a well-developed bush.

The stock of BB in the first year of the growing season exceeded the stock of AB by more than 5 times. In the second year, the stock of AB exceeded the indicator of the first year by almost 16 times; the stock of BB increased by about 2 times and did not reliably differ from the stock of AB. In the third and fourth years of the growing season, the value of AB stabilized, while BB continued to increase. For 4 years, the yield of AB averaged 10 t/ha, BB 11 t/ha.

Under the conditions of the authors' experiment, during the vegetation period, the content of nitrogen and potassium in the biomass changed the most significantly (the indicator decreased from seedlings to desiccation by 9 and 6 times, respectively) (Table 3).

3. Content of biophilic elements in plant biomass of *Miscanthus sacchariflorus* cv. Soranovsky ($n = 12$, $M \pm \text{SEM}$; Novosibirsk Province, 54°53'13,5"N, 82°59'36,7"E, 2015-2018)

Phase	N			P			K		
	leaves	stem	panicle	leaves	stem	panicle	leaves	stem	panicle
Seedlings		2.99±0.31			0.34±0.03			2.43±0.35	
Flowering	1.08±0.20	0.26±0.04	1.37±0.25	0.19±0.01	0.11±0.02	0.28±0.05	1.38±0.11	0.61±0.17	1.21±0.25
Drying	0.46±0.06	0.24±0.09	0.93±0.29	0.12±0.04	0.08±0.02	0.14±0.05	0.48±0.03	0.26±0.02	0.51±0.19

One of the advantages of miscanthus over other energetic plants is the low removal of biophilic elements from the soil, since when the plants desiccate, an intensive outflow of substances from AB to BB occurs (reutilization). In the year of planting (2015), the biomass was not removed from the field due to its small amount (0.8 t/ha), therefore, for comparison, the following vegetation years of the plant (2016-2018) were taken. In the authors' experiment, the final value of the removal of elements depended on the harvesting technology (Table 4). If only the stems were harvested, the nitrogen removal was 23.3, phosphorus 2.8, and potassium 25.9 kg/ha. The amount of reutilization was, respectively, 74, 69, and 76% of the content of the element in the flowering phase. When harvesting with leaves, the alienation of biophilic elements increased by about 2 times, and the amount of reutilization decreased to 43, 34, and 54%, respectively.

4. Removal and return (kg/ha) of biophilic elements into the soil by *Miscanthus sacchariflorus* cv. Soranovsky plants ($n = 12$, $M \pm \text{SEM}$; Novosibirsk Province, 54°53'13,5"N, 82°59'36,7"E, 2016-2018)

Plant organ	N	P	K
Flowering			
Leaves	65.1±7.3	5.2±0.8	67.4±8.9
Stem	21.1±0.6	3.6±0.2	37.5±2.8
Panicle	2.4±0.6	0.2±0.1	1.8±0.6
Sum (removal)	88.7	8.9	106.7
Drying			
Leaves	27.3±4.8	3.1±0.3	23.3±3.3
Stem	22.1±1.8	2.7±0.1	25.5±1.5
Panicle	1.2±0.6	0.1±0.1	0.4±0.1
Sum (total removal)	50.6	5.9	49.2
Removal without leaves	23.3	2.8	25.9
Return to soil including leaves and stems	38.1	3.0	57.5
Return to soil excluding leaves (stem harvesting)	65.4	6.1	80.8

To assess the balance of biophilic elements in the soil-plant system, the stock of elements in BB was estimated as the closest source of nutrients. The stock of N, P, K in BB was, respectively, 131, 10, 126 kg/ha, that is, 1.6-2.6 times higher than the amount alienated with the harvest.

Thus, for 3 years of miscanthus vegetation, the maximum removal of N, P, and K with the aboveground plant biomass was, respectively, 150, 18, and 150 kg/ha. It was interesting to observe how this affected the agrochemical properties

of the soil. According to the generally accepted scale [24], the content of phosphorus and potassium in the studied soil is assessed as high. After 4 years of vegetation of miscanthus, the content of phosphorus in the Chirikov extract did not change in both the upper and lower soil horizons (Fig. 1, A). The content of water-soluble forms of the element (Karpinsky extract) at the end of 2018 in the upper soil layer was significantly lower in comparison with the initial value of the indicator, which reflects a decrease in the reserves of readily available phosphorus in the soil during the growth of miscanthus (see Fig. 1, B). It should also be noted that the phosphorus content in the soil remained at the “high” gradation level. The supply of potassium to the soil after 4 years of miscanthus growth did not change (see Fig. 1, C).

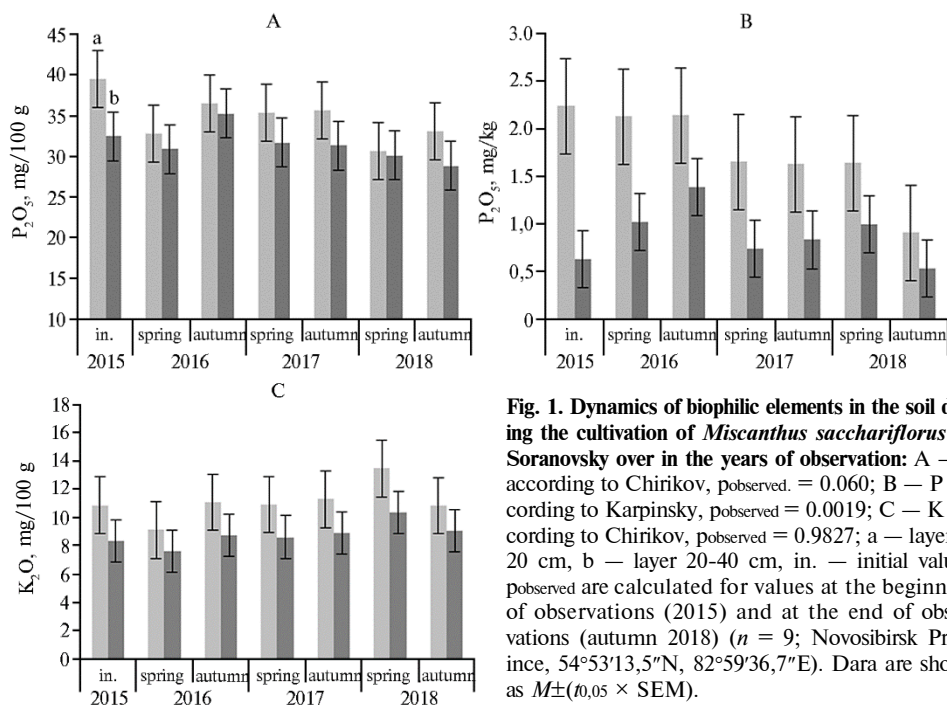


Fig. 1. Dynamics of biophilic elements in the soil during the cultivation of *Miscanthus sacchariflorus* cv. Soranovsky over in the years of observation: A — P according to Chirikov, $p_{\text{observed}} = 0.060$; B — P according to Karpinsky, $p_{\text{observed}} = 0.0019$; C — K according to Chirikov, $p_{\text{observed}} = 0.9827$; a — layer 0-20 cm, b — layer 20-40 cm, in. — initial values; p_{observed} are calculated for values at the beginning of observations (2015) and at the end of observations (autumn 2018) ($n = 9$; Novosibirsk Province, 54°53'13,5"N, 82°59'36,7"E). Data are shown as $M \pm (t_{0,05} \times \text{SEM})$.

It is rather difficult to obtain such information on nitrogen in the soil due to the specificity of the cycle of this element in the agroecosystem. Determining nitrate nitrogen showed quite an expected result: during the vegetation period of miscanthus in 2015-2017, only trace amounts of this compound were present in the soil; in 2018, the authors did not find nitrate nitrogen. A low content of nitrate nitrogen is typical for perennial crops of any cereal perennial grasses [20]; therefore, an insignificant content of nitrate nitrogen in the experiment is not evidence of a deficiency of this element for plants. Thus, for 4 years of the vegetation of miscanthus in the studied agro-gray soil, no significant decrease in the content of the main biophilic elements was found.

The interest in the data on the dynamics of carbon in crops (plantings) of energy crops is associated with the problem of greenhouse gas CO₂ sequestration in SOM fractions. After 4 years of vegetation of miscanthus, the content of C_{org.} in the 0-20 cm soil layer was $1.97 \pm 0.16\%$ ($M \pm \text{SEM}$), and in the initial soil $1.76 \pm 0.16\%$, that is, a reliable accumulation of C_{org.} in the soil was not established. As it is known, the content of total carbon in soil (C_{org.}) is a very stable indicator and it is rather difficult to note its change in short-term experiments

[23]. Nevertheless, rough estimates are possible using the available standard methods, in particular, based on the dynamics of carbon accumulation in BB. Table 5 presents the data on the content and reserves of carbon and nitrogen in mortmass and rhizomes of miscanthus in autumn 2016. As the initial values of the studied parameters, we took the values for one-year fallow, since the miscanthus plantation was formed after the one-year fallowing of a wheat field. The sample in the experiment did not differ significantly in terms of the carbon content in BB (see Table 5). The decrease in the nitrogen content in the mortmass during the cultivation of miscanthus in comparison with the initial soil indicates a less deep processing of organic material in the soil under the miscanthus. The nitrogen content in the rhizomes of miscanthus was significantly lower than in mortmass, so the C/N ratio increased sharply.

5. Content and accumulation of nitrogen and carbon in belowground biomass and in the soil under the fallow and under *Miscanthus sacchariflorus* cv. Soranovsky plants ($n = 10$, $M \pm \text{SEM}$; Novosibirsk Province, 54°53'13,5"N, 82°59'36,7"E, autumn 2016)

Sample	Content, %		C/N	Accumulation, kg/ha	
	N	C		N	C
Fallow (mortmass)	2.12±0.24	43.39±1.01	25±2.3	40.3±3.9	760±80
Miscanthus (mortmass)	0.79±0.04	44.61±0.21	66±3.3	41±5.3	2750±300
Miscanthus (rhizomes)	0.48±0.05	44.61±0.65	96±13.3	72±7.3	4760±670

For two years of miscanthus growth, the carbon stock in mortmass increased 3.6 times compared to the initial value (fallow). As a result, the total carbon stock in the BB of miscanthus was 7.5 t/ha, that is, it increased by about 10 times in comparison with the indicator established before the planting. The increase in the total carbon stock in the BB occurred mainly due to the rhizomes where the C/N ratio reached 100.

Thus, the carbon sequestration in the BB in the first 2 years of the vegetation period of miscanthus was 1 t C/ha per year in mortmass and 2 t C/ha per year in rhizomes. These data give a general idea of the dynamics of SOM without taking into account the changes occurring in the fractions of organic matter of the soil itself. A fairly simple method is known for assessing the amount of the mobile fraction of SOM, which is most accessible for microbial decomposition. The soil is incubated until the release of C-CO₂ ceases to increase [23]. However, it is clear that this procedure is quite long-term. In a laboratory experiment, the authors tried to estimate the amount of the most accessible part of SOM accumulated by miscanthus during the first 2 years of vegetation. For this, the soil from under fallow and from under miscanthus was incubated under optimal conditions of heat and humidity. After 6 weeks, the rate of CO₂ production in the samples of both soils reached the same value (Fig. 2).

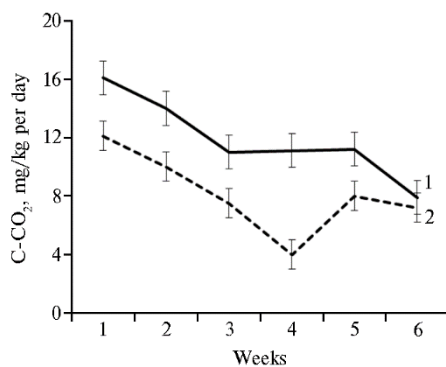


Fig. 2. Comparative rate of C-CO₂ production by soil under *Miscanthus sacchariflorus* cv. Soranovsky (1) and one-year fallow land on which the plantation was formed (a basal level) [2]; $p_{\text{observed}} = 0.004$ for weeks 1-5, $p_{\text{observed}} = 0.980$ for week 6 (lab test, $n = 3$; Novosibirsk Province, 54°53'13,5"N, 82°59'36,7"E, 2016). Data are shown as $M \pm (0.05 \times \text{SEM})$.

For 6 weeks, the total release of C-CO₂ from the soil under miscanthus was 1039±25 kg/ha, under the fallow 750±15 kg/ha, that is, the C accumulation of the mobile part of organic matter under miscanthus was almost 300 kg/ha more than under the fallow. It follows

from this that under the plantings of *M. sacchariflorus* of the cultivar Soranovsky for two vegetation periods (2015–2016), about 300 kg C/ha of mobile SOM was accumulated in the soil in comparison with its initial stock in the soil of the annual fallow land, on which the plantation was formed.

The content of SOM is due to the resulting rate between the rate of entry of organic residues into the soil and their mineralization to the final products. The changes in the components of the balance of these events are small and, as a consequence, the intensity of SOM accumulation is difficult to estimate. However, with the help of laboratory measurements, it is possible to determine the tendencies of this process by assessing the respiratory response of the soil to the introduction of plant residues and thus to measure the ratio of removed and retained OM in the soil. The response of the respiratory activity of the soil to the input of new organic matter serves as one of the criteria for the intensity of carbon metabolism in the soil: the lower the response, the lower the rate of mineralization, that is, the dynamics are directed towards the accumulation of carbon in the SOM.

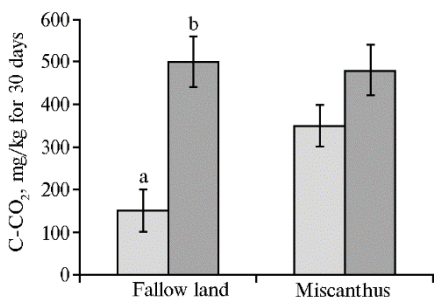


Fig. 3. C-CO₂ production by the soil under one-year fallow on which the plantation was formed (a basal level) and under *Miscanthus sacchariflorus* cv. Soranovsky in control (a) and with wheat straw incorporation (b); $p_{\text{observed}} < 0.001$ for the fallow, $p_{\text{observed}} = 0.038$ for miscanthus (lab test, $n = 3$; Novosibirsk Province, 54°53'13,5"N, 82°59'36,7"E, 2016). Data are shown as $M \pm (t_{0,05} \times \text{SEM})$.

Fig. 3 shows the results of such an experiment. Of incorporated C (600 mg/kg as straw) from the fallow soil, approximately 350–400 mg C-CO₂/kg were released, while for the soil under miscanthus, the differences between the variants (control and experiment) were minimal. Respiratory response for fallow soil was 250%, for the soil under miscanthus 40%. From the obtained data, it follows that the microbial decomposition of the new organic matter in the soil during the cultivation of miscanthus was much weaker than against the background of fallow. The inhibition of mineralization processes in the variant with miscanthus is obviously associated with the accumulation of plant residues with a high C/N ratio.

6. Yield (absolutely dry matter, t/ha) of different species of *Miscanthus* for the first 3 years of vegetation calculated according to literature data [25, 26]

Harvesting period	Value	<i>M. sinensis</i>	<i>M. sacchariflorus</i>	<i>M. giganteus</i>
Autumn	Average	12	15	26
	Min-max	4.6–22	1.4–35.0	13.8–37.8
Winter	Average	9	10	19
	Min-max	3.1–17.6	0.4–22.4	9.2–26.4

Thus, the presented work summarizes the results of the first stage of studying the possibility of growing miscanthus for biomass in Siberia. Here, the data were obtained in the harshest climatic conditions (arid cold climate) compared to the published results worldwide. Nevertheless, the average yield of *M. sacchariflorus* of the cultivar Soranovsky, established in the work (10–13 t/ha) (see Table 2), is within the values typical for this species (Table 6). When analyzing Table 6, attention is drawn to the very wide range of fluctuations in the yield of miscanthus in the world. This is due to the fact that the authors of the reviews [25, 26] tried to collect the maximum amount of factual data obtained under a variety of conditions (namely, climatic, soil, and agrotechnical ones). At the same time, the

range of yield fluctuations in the *M. sacchariflorus* species was wider than in the other two species. This once again confirms the conclusion of other authors [11] regarding a more pronounced dependence of the yield of *M. sacchariflorus* on soil and climatic conditions.

7. Removal of biophilic elements with biomass of *Miscanthus* in different regions of the world (according to literature data)

Country, region	Average annual values		Species	Yield, t/ha	Removal, kg/ha			References
	T, °C	precipitation, mm			N	P	K	
USA:								[13, 29]
State of New Jersey	11.2	1211	<i>M. giganteus</i>	9.5	27.1	4.3	32.6	
State of Kentucky	12.8	1166	<i>M. giganteus</i>	19.0	112.9	14.4	74.5	
State of Illinois	11.1	1043	<i>M. giganteus</i>	15.6	54.2	3.4	39.6	
State of Nebraska	9.7	704	<i>M. giganteus</i>	27.7	116	7.9	165	
Spain	13.9	100.3	<i>M. giganteus</i>	17.6	46.4	3.9	23.0	[14]
France	11.5	557	<i>M. giganteus</i>	16.9				[16]
France (autumn/winter)	17.8	390	<i>M. giganteus</i>	22/15.5	101/29	19/7	139/45	[27]
Russia:								[11]
Moscow	4.5	620	<i>M. sinensis</i>	7.8				
			<i>M. giganteus</i>	5.7				
			<i>M. sacchariflorus</i>	4.2				
Western Siberia	1.7	459	<i>M. sacchariflorus</i>	13/7	50/23	6/3	49/26	Current research

N o t e. Slashes indicate the values for harvesting the entire biomass and only the stems. The gaps indicate the absence of data.

The comparison of the data we obtained on the removal of biophilic elements is rather difficult to perform both geographically and by the species of miscanthus. For Russia, we did not find such information; moreover, in other countries, this information concerns mainly the species *M. giganteus*. As it can be seen from Table 7, nitrogen and potassium are most intensively removed with the biomass of miscanthus. The total removal depends on the yield of the crop and the timing of harvesting. As the literature data show [27, 28], during winter harvesting, in comparison with autumn harvesting, the removal of elements can decrease three times. The calculation of the removal of elements per unit of yield is presented in Table 8. The indicator varies greatly from different sources. In our experiment, the specific removal was rather low and approached the indicators [28] obtained during winter harvesting.

8. Removal of biophilic elements with biomass of *Miscanthus* (kg/t) in different regions (calculations per unit of the yield mass are based on the literature data of Table 7)

Country, region	Timing/harvesting technology	N	P	K
USA, State of New Jersey	Winter	2.9	0.5	3.4
USA, State of Kentucky	Autumn	5.9	0.8	3.9
USA, State of Illinois	Autumn	3.5	0.2	2.5
USA, State of Nebraska	Winter	4.2	0.3	6.0
Spain	Winter	2.6	0.2	1.3
France	Autumn	4.6	0.9	6.3
	Winter	1.9	0.5	2.9
Russia, Western Siberia (current research)	Harvesting with leaves	3.0	0.4	2.9
	Harvesting the stems	1.4	0.2	1.5

Our data on the yield and removal of elements with biomass are, on the whole, close to those obtained in the world for other soils and climatic conditions.

As to the dynamics of biophilic elements in the soil-plant system, according to the literature data [13, 30], the most deficient element in perennial plantings of miscanthus is phosphorus, which, despite its relatively low removal, is poorly reutilized. The same tendency is observed in the authors' experiment, although after 4 years of cultivation of miscanthus, a decrease was noted in the content of only the most mobile fraction of this element in the soil. The analysis of the ratio of the reserves of elements in BB and their removal shows that already in

the first years of the vegetation period, a sufficient supply of nitrogen, phosphorus, and potassium was formed to successfully replace the removal of elements with the biomass of miscanthus. The same pattern was revealed by the studies in other regions of the world. In particular, Dohleman et al. [12] in the USA (the State of Illinois, 40°03'21.3"N, 88°12'3.4"W) showed that for 5-7 years of vegetation, the yield of *M. giganteus* (about 40 t/ha) did not decrease even without fertilization. The authors attribute this to the dynamic release of elements, in particular nitrogen, from rhizomes and, in general, from BB. Thus, the nitrogen reserve in rhizomes, which in April was 264 kg/ha, decreased to 145 kg/ha by June, and then, due to reutilization from AB, increased by December to 373 kg/ha. From the presented data, it follows that 45% of nitrogen contained in the rhizomes was actively absorbed by the growing biomass. In our experiment, with nitrogen removal equal to 50 kg/ha (including leaves) or 23 kg/ha (when harvesting only stems), the reserve of the element in the rhizomes was 130 kg/ha, that is, the removal compensation was sufficient.

According to our findings, over 4 years of development of *M. sacchariflorus* plants of the cultivar Soranovsky, no signs of soil depletion by biophilic elements were found. Moreover, during this period, a significant supply of nitrogen and carbon was formed in BB.

The possibility of the carbon sequestration of greenhouse gas CO₂ in the SOM fractions during the growth of miscanthus as a whole is beyond doubt. In particular, a quantitative forecast has been developed for the UK, according to which, upon obtaining stable yields of miscanthus of 12 t/ha per year, by 2090 it is possible to stabilize the positive dynamics of SOM [18]. Such expectations are mainly based on the colossal productivity of the AB and BB of miscanthus. The value of carbon sequestration in BB in the authors' experiment (approximately 1 t C/ha in biomass, 2 t C/ha in rhizomes per year) is quite close to the literature data. Thus, according to the generalized data by Robertson et al. [17], under 3-4-year-old *M. giganteus* plantings, the average annual carbon accumulation in the roots was about 860 kg C/ha, in rhizomes 2660 kg C/ha. The transfer of this carbon to SOM is a dynamic process that depends on the rate of biomass mineralization to end products, which is determined by specific soil-climatic and agro-technical conditions. We have experimentally shown that, under miscanthus, the intensity of this process decreased sharply already in the first 2 years of the vegetation due to a significant increase in the C/N ratio, which may be a prerequisite for the accumulation of organic matter in the soil. As it is known, the imbalance between biophilic elements is the most effective natural mechanism that prevents the rapid decomposition of biomass on the planet to end products [31]. In our experiment, we did not find a significant increase in the total carbon content in the soil. According to the publications, even after 20 years of *M. giganteus* vegetation, the expected increase in the content of SOM may not be observed [17]. One of the probable reasons is the gradual replacement of the already existing SOM with a new one. Thus, it was reported [15] that for 6 years in 10-year-old *M. giganteus* plantings, the annual replenishment of SOM with C₄ plant carbon was 780 kg/ha, and 68% of detritus carbon in the 0-10 cm soil layer belonged to miscanthus. Moreover, the share of miscanthus carbon in the fraction resistant to NaOCl oxidation was 15%. The authors emphasize that the replenishment of stable fractions of SOM can occur in a relatively short period of time. Researchers continue to study the reasons why the accumulation of SOM under miscanthus often does not match the amount of incoming plant residues. In particular, with the help of the ¹³C isotope, it was shown that the residues of C₄ plants in the soil were mineralized to final products faster in comparison with those of C₃

plants [19]. Thus, the problem of air carbon sequestration in SOM fractions is rather complicated and requires additional study under specific soil and climatic conditions.

Thus, for *Miscanthus sacchariflorus* of the cultivar Soranovsky, the possibility of its cultivation in the arid and cold climate of Western Siberia was assessed. It was found that on the agro-gray soil of the central forest-steppe of the Ob region during the formation of perennial plantings (1-4 years), the AB value of miscanthus reached 13 t/ha, and the average yield for 4 years was 10 t/ha. These indicators are generally comparable to the productivity observed in *M. sacchariflorus* in the world under more favorable climatic conditions. The ratio of the removal of the main biophilic elements with the yield of AB, as well as a sufficient reserve of the latter in BB, allows concluding that there is no depletion of the soil with elements of mineral nutrition of plants during the cultivation of miscanthus. This conclusion is confirmed by the stability of the agrochemical properties of the studied soil over the observation period. Rough estimates of the components of the carbon balance (C accumulation in mortmass of 1 t/ha, in the mobile SOM fraction of 150 kg/ha per year) in the agroecosystem showed the objective prerequisites for atmospheric carbon sequestration in fractions of SOM. Thus, the cultivation of *M. sacchariflorus* for biomass in Siberia is quite reasonable ecologically, agrotechnically, and economically.

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STIMULATING EFFECTS OF PRE-SOWING SEED TREATMENT WITH METAL NANOPARTICLES ON WINTER WHEAT GROWTH AND DEVELOPMENT

T.A. YURINA¹, G.V. DROBIN¹, O.A. BOGOSLOVSKAYA² ✉, I.P. OLKHOVSKAYA²,
N.N. GLUSHCHENKO²

¹Novokubansk Branch of Russian Research Institute of Information and Feasibility Study on Engineering Support of Agrobusiness, 15, ul. Krasnaya, Novokubansk, Krasnodar Territory, 352243 Russia, e-mail agrolaboratoriya@mail.ru, director@kubnitiim.ru;

²Semenov Federal Research Center for Chemical Physics RAS, the Talrose Institute for Energy Problems of Chemical Physics, 38/2 Leninsky pr., Moscow, 119334 Russia, e-mail obogo@mail.ru (✉ corresponding author), nnglu@mail.ru, iolkhv@gmail.com

ORCID:

Yurina T.A. orcid.org/0000-0002-6158-5714

Olkhovskaya I.P. orcid.org/0000-0003-2322-7868

Drobin G.V. orcid.org/0000-0002-6673-1776

Glushchenko N.N. orcid.org/0000-0002-0227-9282

Bogoslovskaya O.A. orcid.org/0000-0003-1632-4010

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Abstract

The use of nanotechnologies in agriculture is an advanced course enabling to reduce the dependence of crop tonnage and quality on external factors. A special section is represented by studies of pre-sowing treatment of seeds with metal nanoparticles (NPs). In this work, it is shown for the first time that pre-sowing treatment of seeds by metal nanoparticles with specific physico-chemical parameters affects morphometric indices of the of winter wheat growth at all stages of its development as well as the plant resistance to pathogens, grain quality, the degree of its damage from fusarium and elemental composition of the soil after harvesting. Effects depended on the type of metal used. Our aims were to study (1) effects of pre-sowing seed treatment with iron, zinc, and copper NPs on the growth parameters and grain quality of winter wheat, and (2) whether this treatment affects the soil after harvesting. Iron, zinc, and copper NPs were obtained by the method of high temperature condensation at the Migen-3 apparatus (Institute for Energy Problems of Chemical Physics RAS, Russia). The shape and size of NPs were evaluated by JSM-7401F scanning electron microscope (JEOL Ltd., Japan). X-ray phase analysis was carried out using an ADP-1 X-ray analyzer (NPO Modern Technologies of Non-Destructive Control, Russia). Field trials were carried out at the validation test site of the Novokuban Branch of the Rosinformagrotech (Krasnodar Territory). The predominant soil type is typical chernozem, with medium humus content, heavy loamy. The sowing of winter wheat (*Triticum aestivum* L.) cv. Stan was performed on October 4, 2016 with a setting seed rate 240 kg/h. The assigned treatments were as follows: control (seeds without treatment), seed treatment with Fe NPs ($5 \times 10^{-4}\%$), Zn NPs ($1 \times 10^{-4}\%$), Cu NPs ($5 \times 10^{-7}\%$); Fe NPs + Zn NPs + Cu NPs ($5 \times 10^{-4}\%$ + $1 \times 10^{-4}\%$ + $5 \times 10^{-7}\%$). Soil samples were collected for chemical analysis. For phenological and biometric observations, plants were taken from three locations of 1 m² area from each experimental and control plot. Plant height, average root length, thickness of the main stem at the plant bottom, tillering and the depth of the tillering node were measured. Iron, zinc, and copper NPs were round single-crystal structures covered with a semi-transparent oxide film. Average diameter of Fe NPs was 27.0 ± 0.51 nm, Zn NPs 54.0 ± 2.8 nm, Cu NPs 79.0 ± 1.24 nm. X-ray phase analysis showed that iron NPs consisted of 53.6% crystalline metal phase, Fe₃O₄ content was 46.4 %, and the oxide film thickness was 3.5 nm. Cu and Zn NPs contained only crystalline metal phases with the similar oxide film thickness, 0.5 to 1.0 nm. Pre-sowing treatment of seeds with Fe NPs affected the height of seedlings, promoted the formation of a developed root system with total root length being 4.5% more ($p \leq 0.05$) than in the control group, and increased the seedling stand density by 9.96% ($p \leq 0.05$) vs. control. Pre-harvest monitoring of crops revealed an increase in the yield of wheat plant mass after pre-sowing seed treatment with Fe and Cu NPs. Stem length was larger than that of the control (81.3 ± 1.2 cm) by 3.8 and 8 cm, respectively,

the average thickness of the main stem at the plant bottom being larger by 6 mm (when processed with Fe NPs) and 5 mm (when treated with Cu NPs) in comparison with the control (44 mm). Plant stands productivity enhancement after Fe and Zn NPs treatments, higher resistance to pathogens (by 3.85 times vs. control) under Fe NPs, a tendency to an increase in the average 1000-grain mass when using NPs of Fe, Zn, and Cu were observed. The crop quality parameters had higher values as compared to the control: in terms of the content of wet gluten by 6.12 % when seeds were treated with Zn and Cu NPs or with NPs composition; the protein mass fraction was larger under treatment with Cu NPs and the NPs composition by 5.1 % vs. control. Pre-sowing treatment with Fe and Zn NPs reduced the prevalence of *Fusarium* infection in grain by 1.24 and 2.25 times respectively vs. control. Elemental analysis of the soil after harvesting showed a decrease in the content of mobile forms of phosphorus by 27 % and zinc by 48 % after seed treatment with Zn NPs in comparison with the control, and a decrease in the phosphorus mobile forms by 23 % and sulfur by 7 % after pre-sowing treatment with Cu NPs in comparison with the control. The data obtained demonstrate the effective influence of the pre-sowing treatment of seeds by metal NPs on the growth, development and grain quality of wheat.

Keywords: nanoparticles, iron, zinc, copper, yield components, grain quality, soil trace elements

Wheat is an essential food crop for countries with temperate climates; it is consumed by more than half of the world's population. The growing food demand is projected to double the demand for wheat by 2050 [1]. At the same time, wheat farming has ever more exacerbated issues, as new diseases and more aggressive pests appear, water resources are dwindling, arable land is deficient, the climate changes, and weather is unstable [2].

In addition to advanced agricultural technology, the wheat industry uses genetic and breeding breakthroughs, nanomaterials, and nanotechnology to improve yields [2-4]. The yield increase and better crop conditions enabled by nanotechnologically optimized plant nutrition and protection help address not only humanitarian problems such as yields and quality but also the environmental troubles [4, 5]. Agriculture commonly uses Zn, Cu, Fe, Mn nanoparticles (NPs) and oxides thereof. Zinc oxide (ZnO) and copper oxide (CuO) NPs are used in many commercial products including antimicrobials. Recent research proved their effectiveness as fungicides, on account of the ability to inhibit the growth of fungal plant pathogens. So it is not a coincidence that the use of NPs to boost growth and control diseases in plants is becoming a best practice [6-10].

The unique properties of NPs make them an interesting solution in crop production. The authors' long-term studies of dispersed systems and NPs have revealed the following biological effects of NPs. Metal NPs are 7 to 50 times less toxic than ionic metals. They have a prolonged and polyfunctional effect; they stimulate metabolism and easily reach all organs and tissues. Their biological activity stems from their structure and physicochemical properties. Metal NPs increase the effectiveness of natural polysaccharides when used in combination [11-14]. Due to their size, NPs actively penetrate into, and spread across, all issues, thus intensifying physiological, biochemical, and molecular processes of plant germination and growth. Once in a biological fluid, NPs function as a depot: their slow oxidation provides the plant with the micronutrients it needs to grow. These peculiar biological effects of NPs allow using them in crop production; effective NPs concentrations are two orders of magnitude lower than those of metal salts [15]. There is a special niche for research into the pretreatment of seeds with NPs, which increases the yield by 30-40% [16].

This paper is the first to show that the pre-sowing treatment (pretreatment) of seeds with metal NPs of specific physical and chemical properties affects the morphometric growth parameters of winter wheat at all phases of its development and influences the crop resistance to phytopathogens, the quality of grains, the extent of fusariosis-caused damage, and the content of elements in the soil after harvesting. The effects depend on which kind of metal is used.

The goal hereof was to find out how pretreatment of seeds with iron, zinc, and copper NPs, individually and in combination, would affect the growth of winter wheat in all phases, the quality of grains, and the levels of soil elements after harvesting.

Materials and methods. NPs of iron, zinc, and copper were produced by high-temperature condensation [17] on a Migen-3 unit (Institute of Problems of Chemical Physics of RAS, Russia) [18]. Weighed NP samples were dispersed in distilled water using a Scientz JY 92-IIN ultrasonic disintegrator (Ningbo Scientz Biotechnology Co. Ltd, China) at 0.5 A and 44 kHz over 30 s with a pause of 30 s, three repeated treatments; the dispersed mixture being cooled down in an ice bath.

The shape and size of NPs were estimated by transmission electron microscopy (TEM) on a JSM-7401F scanning electron microscope (JEOL Ltd., Japan) at 1 kV. To find out the average NPs diameter, microphotographs were processed in Micran 25 (<https://www.micran.ru/>), measuring at least 1000 particles across. The data were then used to plot NPs size distribution curves and estimate the average size.

X-ray crystallography (XRC) was run on an ADP-1 unit (Advanced Non-Destructive Testing Technologies, Russia). A cobalt tube was used as the radiation source. Scanning was done at increments of 0.05° with 8 to 10 minutes to accumulate the signal. To find out the phase composition, the obtained interference peaks were processed in Match 3.8.0.137 (<http://www.crystalimpact.com/>).

Research was carried out with the winter wheat (*Triticum aestivum* L.) variety Stan (Krasnodar Agricultural Research Institute) picked from the varieties allowed for use in the North Caucasus on moderately or highly fertile soils. The Stan is a strong, short-stalk variety resistant to lodging and shedding; it matures early and produces grain that has favorable traits for baking. The plants are about 95 cm tall and show immunity to loose smut when artificially infected. The Stan is resistant to brown, yellow and stem rusts, field resistant to powdery mildew, medium resistant to head smut, moderately susceptible to septoriosiis, and susceptible to fusarium ear blight; medium frost resistant, high heat resistant.

Field experiments were carried out at the test fields of the Novokubansk Branch of Rosinformagrotech, a site in the Krasnodar Territory where soil hydration is inconsistent. The farm mostly had typical chernozem with medium humus content and high loam content. The soil was sampled for chemical testing per GOST 17.4.4.02-84. Nature protection. Soils. Methods for sampling and preparation of soil for chemical, bacteriological, helminthological analysis (Moscow, 2018). Each average sample was made up of 10 individual samples taken at the test site by using the technique referred to as “the envelope method” in GOST (five sampling points: one in the center and four placed equidistantly around it forming a square — *translator’s note*); samples were taken from arable layer depth (30 cm). The soils had an enlarged concentration of humus, elevated or high nitrification capacity, low/medium/elevated phosphorus content, medium or elevated potassium content, and neutral or close-to-neutral exchangeable acidity. The sulfur content was low or medium; the manganese, zinc, and copper contents were low. On average, 1 kg of soil contained 37.1 mg of nitrate nitrogen, 20.0 mg of phosphorus, 297 of potassium, 5 mg of sulfur, 3.14 mg of manganese, 0.37 mg of zinc, 0.08 mg of copper, 4.56% of humus; pH 6.16.

Winter wheat variety Stan (RS-1) was sown for production at a time optimal for the central climatic zone: October 4, 2016; the sowing rate was 240 kg/ha. The monthly average temperature was +18.1 °C (September 2016), +10.7 °C (October 2016), +6 °C (November 2016), –2.6 °C (December 2016),

-1.3 °C (January 2017), +0.1 °C (February 2017), +8.3 °C (March 2017), +13.1 °C (April 2017), +17.6 °C (May 2017), +22.6 °C (June 2017), +26.6 °C (July 2017).

Several groups were planted: controls (untreated seeds), seeds treated with NPs of Fe ($5 \times 10^{-4}\%$), Zn ($1 \times 10^{-4}\%$), Cu ($5 \times 10^{-7}\%$), Fe + Zn + Cu ($5 \times 10^{-4}\% + 1 \times 10^{-4}\% + 5 \times 10^{-7}\%$). All the cultivation operations in both the experimental fields and in the control field were carried out on the same day with the same machinery.

Labile phosphorus and potassium compounds were detected per GOST 26204-91. Soils. Determination of mobile compounds of phosphorus and potassium by the Chiricov method modified by CINAO (Moscow, 1992). The extent of fusariosis-induced damage was estimated per GOST 31646-2012. Cereals. Method for determination of the scabby kernels content (Moscow, 2019).

To collect phenological and biometric data, plants were sampled from three sites sized 1 m² from each experimental and control field. Density, height, root length, tillering node depth, and tillering were measured in the third/fourth-leaf stage, as well as in the spring once vegetation resumed (6-8 leaves), and once the plants began tillering (parent shoot plus 2-3 tillers). Tillering was measured as the number of tillers per plant.

Two weeks before harvesting, the research team measured plant height, root length, main stem thickness at the bottom, number of grains per ear, number of productive and nonproductive stems. To that end, frames sized 50×50 cm were buried in the fields; all plants would be scooped out within a frame, sorted by yield structure, then counted and measured being triply repeated for each experimental scheme.

Grain quality was assessed in terms of wt.% of wet gluten (GOST 54478-2011. Grain. Methods for determination of quantity and quality of gluten in wheat. Moscow, 2012), wt.% of protein (GOST 10846-91. Grain and products of its processing. Method for determination of protein. Moscow, 2009)), vitreousness (GOST 10987-76. Grain and grain processing products. Methods for determination of vitreousness. Moscow, 2009), hectoliter weight (GOST 10840-2017 Grain. Method for determination of hectolitre weight. Moscow, 2019) and wt.% of moisture (GOST 13586.5-2015. Grain. Method of moisture content determination. Moscow, 2019).

Statistical analysis was made by Microsoft Excel 2010 and Statistica 20 (StatSoft Inc., USA). Means (M) and standard errors of the means (\pm SEM) were calculated. The significance of the differences between the groups was assessed by parametric tests (Student's t -test) and non-parametric tests (Wilcoxon's pairwise W -test). The significance threshold was $p \leq 0.05$.

Results. Requirements for nanopowders depend on the fields of their application, and that is why NPs characteristics are rather diverse, and so are the methods for their synthesis. The optimal biological activity of NPs depends on their physical and chemical properties such as size, phase and element composition, and oxide film thickness [13, 14]. Besides, the effectiveness of metal NPs depends on their concentration and application mode [15].

According to the TEM images, metal NPs tested herein were monocrystalline structures of regular shape covered with a translucent oxide film (Fig. 1). Thus, Fe particles were sized 5 to 80 nm, 27.0 ± 0.51 nm on average, Zn particles were 54.0 ± 2.8 nm on average, and Cu particles were 79.0 ± 1.24 nm. XRC of Fe particles showed that they were $53.6 \pm 4.2\%$ crystalline metal and 46.4% Fe₃O₄; the oxide film was 3.5 nm thick. Cu and Zn particles were crystalline metal only, the oxide film was of an identical thickness (0.5 to 1.0 nm).

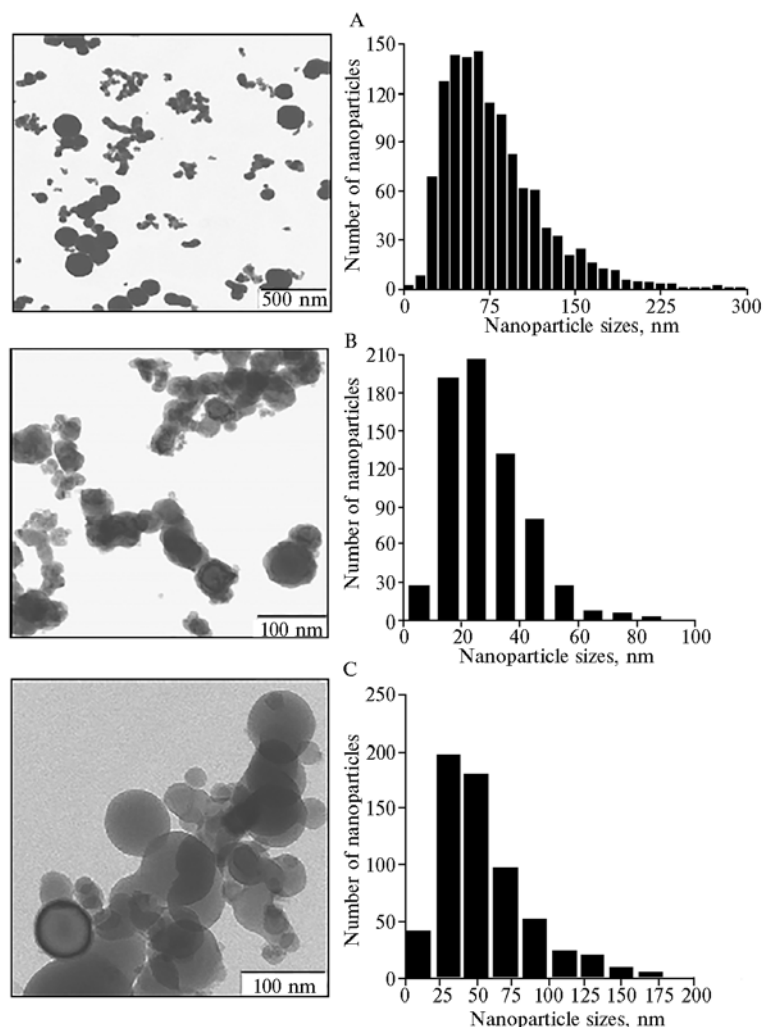


Fig. 1. Nanoparticles of copper (A), iron (B), and zinc (C) (to the left; images produced by transmission electron microscopy, JSM-7401F, Jeol Ltd., Japan) **and distribution by particle size** (to the right).

Field experiments represented a density of 320 ± 11 plants/m² when pre-treated, showing a 10% increase against the control (291 ± 9 per m²) ($p \leq 0.05$). Treatment with Fe NPs significantly increased the height ($p \leq 0.05$) by 2.0 ± 0.5 cm against the controls. Pretreatment with Cu NPs reduced the density by 17.9% ($p \leq 0.05$) whilst insignificantly increasing the height compared to the controls. Pretreatment with Zn NPs and an NP combination of Fe, Zn, and Cu somewhat delayed the growth of plants and the development of their roots; density virtually did not differ from the controls.

When early tillering in the autumn, Zn-pretreated specimens had the largest number of stems (1.5 times that of the controls, $p \leq 0.05$). This trend continued when the plants began tillering in spring (Fig. 2). Pretreatment with Cu NPs and with the combination intensified tillering as well. Tillering node was found at the greatest depth in Fe-pretreated plants in autumn (down to 4.3 ± 0.7 cm), closest to the surface in Zn-pretreated plants (2.35 ± 0.5 cm), compared to 3.13 ± 0.6 cm in the controls. In spring, the look was the same in all the groups (3.6 ± 0.1 cm).

The emergence of roots in the autumn and spring was intensified by pretreatment with Fe NPs, as the roots being respectively 4.5% and 3.8%

longer ($p \leq 0.05$) compared to the controls. Many authors have noted the boosting effect that Fe NPs have on the root system and its activity [15, 19, 20].

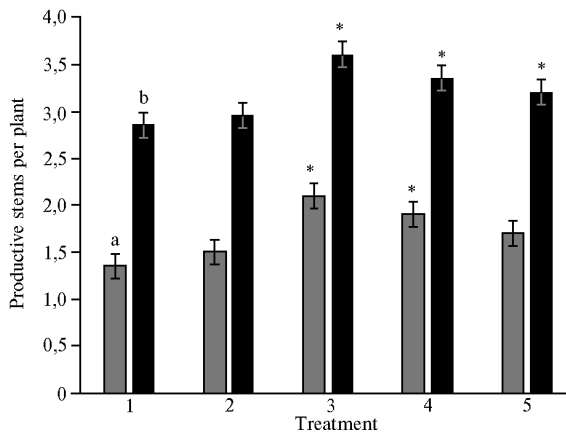


Fig. 2. Tillering of winter wheat (*Triticum aestivum* L.) cv. Stan in the autumn (a) and spring (b) after pre-sowing treatment with NPs: 1 — control (untreated), 2 — Fe NPs ($5 \times 10^{-4}\%$), 3 — Zn NPs ($1 \times 10^{-4}\%$), 4 — Cu NPs ($5 \times 10^{-7}\%$), 5 — Fe NPs + Zn NPs + Cu NPs ($5 \times 10^{-4}\% + 1 \times 10^{-4}\% + 5 \times 10^{-7}\%$) ($n = 290-320$, $M \pm SEM$; Krasnodar Territory, 2017). * Differences between treatment and control groups are statistically significant at $p \leq 0.05$.

Thus, before winter, wheat plants pretreated with metal NPs were well-rooted and tillered specimens that looked better than the controls in terms of stem height (Fe and Cu NPs), seedling density (Fe and Zn NPs, as well as the NPs combination), and the tillering node depth (Fe and Cu NPs, as well as the NP combination). Spring growth beginning in this particular case was complicated by the early cold snap and abundant snowing. But no dead or damaged plants were found in the field after the winter.

Pre-harvest monitoring showed NP pretreatment to increase plant height, with the most drastic increase of 9.1%

against the controls ($p \leq 0.05$) being observed in the Cu group (Table 1). The mean root length varied from 7.8 ± 0.9 cm (NPs combination) to 8.1 ± 1.0 cm (Cu), compared to 8.04 ± 0.5 cm in the controls. Stems were on average 13.6% or 11.4% thicker at the bottom when pretreated with Fe and Cu, respectively, compared to the controls (44.0 ± 1.0 mm), $p \leq 0.05$. Zn and combination-pretreated plants had the most stems, 0.8% and 2.0% more than the controls, respectively. Zn pretreatment increased the number of nonproductive stems by 1.3% ($p \leq 0.05$), whilst the combination reduced that number by 0.4% ($p \leq 0.05$) against the controls. Specimens pretreated with Fe, Zn, and Cu NPs had the least disease-affected stem number compared to the controls. Thus, there were four times less affected stems in Fe-treated plants ($p \leq 0.05$). In these groups, the 1000 grains mass exceeded by 1.9%, 1.3%, and 1.1% the control group value.

1. Pre-harvest monitoring of wheat (*Triticum aestivum* L.) cv. Stan pretreated with metal nanoparticles (NPs) ($n = 110-120$, $M \pm SEM$; Krasnodar Territory, 2017)

Group	Height, cm	Root length, cm	Stem thickness at the bottom, mm	Number of stems			1000 grain mass, g
				total, plants/m ²	nonproductive, %	disease-affected, %	
Controls (untreated)	87.8 ± 1.2	8.0 ± 0.8	44 ± 1.0	456.0 ± 11.0	1.5	2.4	44.2 ± 0.3
Fe NPs	91.6 ± 0.9	8.0 ± 0.9	50 ± 1.8	446.8 ± 10.1	1.5	0.6	45.0 ± 0.2
Zn NPs	88.3 ± 1.4	7.9 ± 0.6	46 ± 1.4	465.2 ± 9.6	2.8	1.1	44.8 ± 0.2
Cu NPs	95.8 ± 1.3	8.1 ± 1.0	49 ± 1.3	437.2 ± 10.3	1.2	1.6	44.7 ± 0.4
Combination of Fe, Zn, and Cu NPs	92.4 ± 1.0	7.8 ± 0.9	45 ± 0.9	460.0 ± 12.4	1.1	2.4	43.9 ± 0.3

2017 had excessive rainfall; high humidity is known to contribute to the growth of fungal pathogens, including fusariosis. Fungi are known to significantly compromise the yield and quality of grains in cereals; besides, they may end up in food raw materials, foods and feeds, contaminating them with fungal toxins [21]. Given the urgency of the issue, the presence of contaminated grains becomes an important quality metric. Pretreatment with Fe/Zn/Cu and

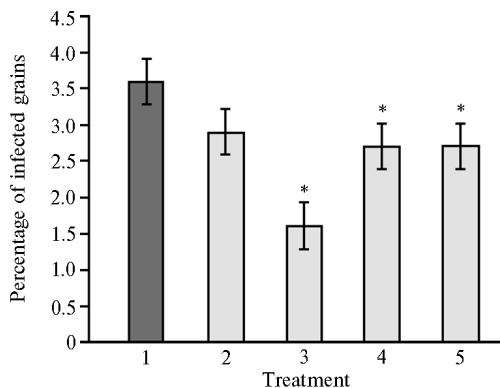


Fig. 3. Number of fusariosis-infected grains of winter wheat (*Triticum aestivum* L.) cv. Stan pretreated with metal nanoparticles (NPs): 1 — control (untreated), 2 — Fe NPs ($5 \times 10^{-4}\%$), 3 — Zn NPs ($1 \times 10^{-4}\%$), 4 — Cu NPs ($5 \times 10^{-7}\%$), 5 — Fe NPs + Zn NPs + Cu NPs ($5 \times 10^{-4}\% + 1 \times 10^{-4}\% + 5 \times 10^{-7}\%$) ($n = 1000$, $M \pm \text{SEM}$; Krasnodar Territory, 2017).

* Differences between treatment and control groups are statistically significant at $p \leq 0.05$

5.1% higher in Fe, Zn, Cu, and composition-pretreated plants, respectively. Vitreousness and bulk density increased by within 1% against the controls.

2. Grain quality of winter wheat (*Triticum aestivum* L.) cv. Stan pretreated with metal nanoparticles (NPs) ($n = 1000$, $M \pm \text{SEM}$; Krasnodar Territory, 2017)

Metric	Group				
	control (untreated)	Fe NPs	Zn NPs	Cu NPs	combination of Fe, Zn, and Cu NPs
Wt.% of wet gluten	19,6 \pm 1,2	20,1 \pm 1,4	20,6 \pm 1,3	20,8 \pm 1,4	20,6 \pm 1,2
Wt.% of protein (dry matter)	11,8 \pm 0,5	12,0 \pm 0,3	12,1 \pm 0,2	12,2 \pm 0,2	12,4 \pm 0,3
Vitreousness, %	45,7 \pm 5,4	46,1 \pm 4,3	45,9 \pm 4,6	46,2 \pm 4,3	45,7 \pm 3,2
Mass per volume, g/l	767 \pm 21	770 \pm 30	772 \pm 28	767 \pm 30	774 \pm 31
Moisture content, wt. %	13,4 \pm 2,4	13,5 \pm 1,8	13,2 \pm 2,1	13,2 \pm 2,2	12,8 \pm 1,8

Therefore, pretreatment of seeds with NPs of Fe ($5 \times 10^{-4}\%$), Zn ($1 \times 10^{-4}\%$), and Cu ($5 \times 10^{-7}\%$), individually and in combination, distinctly affect the germination of seeds, the biological yield of plant mass, the productivity of stems, the resistance to pathogens, and the quality of grains. The causative factor lies in the structural reorganization of leaves, the regulation of the growth of vascular bundles in leaves, stems, and roots, which boosts photosynthesis and improves the suction force of the roots [15]. Active research is going on into how these processes are regulated on the molecular level using transcriptome, proteome, and metabolome analysis [24, 25].

Postharvest soil composition testing revealed neutral soil pH in experimental groups; heavy metal concentrations were within the acceptable limits. The lead content (Pb) was 0.19 to 0.22 mg per kg of soil (the maximum permissible concentration (MPC) is 6.0 mg/kg), cadmium (Cd) was 0.023 to 0.028 mg/kg (MPC — 0.10 mg/kg). The availability of nitrate nitrogen (N-NO₃), labile phosphorus (P₂O₅), exchangeable potassium (as K₂O), labile sulfur (S), manganese (Mn), zinc (Zn), and copper (Cu) were largely the same as in the control field soil. However, some changes were observed, too. Pretreatment with Zn NPs reduced P₂O₅ content by 27% and Zn by 48%, bringing the soil into the “scarce” category. Cu NPs pretreatment reduced the availability of P₂O₅ by 23% and S by

their combination reduced contamination by a factor of 1.24 ($p \leq 0.05$)/2.25 ($p \leq 0.05$), 1.33 ($p \leq 0.05$) against the controls (Fig. 3). Zn NPs have been shown by other authors to effectively inhibit the growth of pathogens, including the fusariosis agent, not only in wheat but also in legumes [22, 23].

Per technical specifications, all the harvested grain from all the groups was Class IV soft wheat (Table 2). Grain quality was consistently higher in pretreated plants compared to the controls. Thus, the mass fraction of wet gluten was 2.6% higher in Fe NPs-pretreated plants, 5.1% higher in the Zn NPs and composition-pretreated plants, and 6.1% higher in the Cu NPs-pretreated plants than in the controls. The mass fraction of protein in grains was 1.6%, 2.5%, 3.4%, and

7% compared to the controls. Results of the analysis suggest that despite low concentrations, metal NPs may affect the content of elements in the soil. Further studies are expected to find out how exactly metal NPs affect the presence of micronutrients in the soil and its biome. Without a doubt, the fact that zinc and copper NPs may alter the micronutrient composition of the soil must be taken in mind when applying such NPs.

Thus, the finding is that the winter wheat variety Stan has better morphological indicators in all growth phases, better yield quality, and stronger phytophthorosis resistance after seed pretreatment with nanoparticles (NPs) of iron, zinc, copper, or a combination thereof. The best morphometric parameters of early growth and development were observed under the use of Fe NPs (a consistent 12% increase in seedling height, 4.5% in root length, 9.96% in density, and 9.3% in tillering node depth compared to the controls). Right before the harvest, the crops from experimental groups had high stems: 4.3% taller than the controls when treated with Fe, by 9.1% taller when treated with Cu. The main stem was 13.6% and 10.4% thicker at the bottom in these two groups than in the controls. Fe, Zn, and Cu NPs-pretreated plants had the least number of damaged from infections stems. The weight of 1000 grains was increased by 1.9%/1.3%/1.1% against the controls when pretreated with Fe, Zn, and Cu NPs. All the experimental groups had 2.5% to 6.1% more wet gluten, 1.6% to 5.1% more protein in grains (as wt.%). Vitreousness and bulk density improved as well. Fusariosis affected Zn-pretreated plants 2.25 times less than it did in the control; plants pretreated with other NPs or with the combination were 20% to 30% less affected. After harvesting, the soil had 27% less labile phosphorus, 48% less labile zinc in the fields after growth of Zn NPs-pretreated plants; pretreatment with Cu NPs reduced the content of P_2O_5 by 23% and that of S by 7%.

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EFFECTS OF NITROGEN-FIXING AND PHOSPHATE-SOLUBILIZING MICROORGANISMS FROM THE FAR EAST AGRICULTURAL SOILS ON THE CEREAL SEED GERMINATION

M.L. SIDORENKO^{1, 2} ✉, N.A. SLEPTSOVA¹, A.N. BYKOVSKAYA¹,
V.V. BEREZHNYAYA³, A.G. KLYKOV³

¹Federal Scientific Center of the East Asia Terrestrial Biodiversity, Far Eastern Branch RAS, 159, Prosp. 100-letiya Vladivostoka, Vladivostok, 690022 Russia, e-mail sleptsova-n@bk.ru, anastasia_n.boyko@inbox.ru;

²Far Eastern Federal University, Campus, 10 Ajax Bay, Russky Island, Vladivostok, 690922 Russia, e-mail sidorenko@biosoil.ru (✉ corresponding author);

³Chaika Federal Scientific Center of Agrobiotechnology in the Far East, Far Eastern Branch RAS, 30, ul. Volozhenina, Ussuriysk, 692539 Russia, e-mail bereg911@mail.ru, alex.klykov@mail.ru

ORCID:

Sidorenko M.L. orcid.org/0000-0002-4035-8395

Berezhnaya V.V. orcid.org/0000-0002-2086-0943

Sleptsova N.A. orcid.org/0000-0002-4226-1984

Klykov A.G. orcid.org/0000-0002-2390-3486

Bykovskaya A.N. orcid.org/0000-0002-0584-8882

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Abstract

Mineral fertilizers which can significantly increase crop productivity have an adverse effect on the soil and the environment as a whole when used for a long time. Microorganisms, as an alternative to mineral fertilizers, stimulate plant growth and development due to their ability to fix nitrogen, produce siderophores, phytohormones and enzymes, dissolve inaccessible elements of mineral nutrition, suppress plant pathogens, and increase consumption of water and nutrients. However, the effectiveness of such preparations highly depends on factors of a new environment. We believe that bacteria from soils that have long been exposed to various agricultural practices may be good plant stimulants. In the presented study, for the first time, we have isolated local active strains of nitrogen-fixing and phosphate-mobilizing bacteria from soils subjected to 74-year stationary intensive farming in the conditions of the Russian Far East and revealed isolates and their combinations which stimulate wheat and barley seed germination and seedling growth. The aim of the work was to study the plant-stimulating properties of nitrogen-fixing and phosphate-mobilizing bacteria from soils that have been actively exposed to mineral fertilizers for a long time. Bacteria were isolated from soils sampled in October 2015 (the experimental field 8, Federal Research Center for Agrobiotechnology FEB RAS, Ussuriysk, Primorsky Territory, Russia). The seeds of wheat *Triticum aestivum* L. cultivar Primorskaya 50, and barley *Hordeum vulgare* L. cultivar Tikhookeanskii (collection of the Federal Research Center for Agrobiotechnology FEB RAS) were treated. Of 68 bacterial isolates with different cultural and morphological properties, three isolates, the *Acinetobacter* spp. N1, *Azotobacter* spp. N2, and *Clostridium* spp. N3 were nitrogen fixers, and four isolates, the *Serratia* spp. P6, *Bacillus* spp. P7, *Arthrobacter* spp. P8, and *Pantoea* spp. P19 were phosphate-mobilizing bacteria. Tests with the monocultures of nitrogen-fixing and phosphate-mobilizing isolates and their different binary compositions showed a 13-51 % increase ($p \leq 0.05$) in wheat seed germination energy and 15-54 % increase ($p \leq 0.05$) in barley seed germination energy compared to the untreated control. Laboratory germination of wheat seeds increased by 2-32 %, barley seeds by 7-30 % compared to untreated control. The barley seedlings were 1.8 times longer, and the roots were 2.7 times longer. The binary combination N2P19, P6P19, and P8P19 caused the highest height of seedlings (120-140 mm, $p \leq 0.05$), and with P6P7, N2P19, and P6P19 the roots were the longest (120-130 mm, $p \leq 0.05$). These results allow us to conclude that short-term soaking seeds in the suspensions of the tested nitrogen-fixing and phosphate-mobilizing isolates improves seed germination energy and laboratory germination, and increases shoot and root length. Binary bacterial compositions have a greater effect on seed germination than monocultures. The strains N1 (*Acinetobacter* spp.), N2 (*Azotobacter* spp.), and P19 (*Pantoea* spp.) are the best stimulants. Species-specific differences in plant response to the treatment is probably due to lack of genetic, biochemical and physiological complementarities between specific plant species and the bacteria.

Keywords: biological fertilizers, diazotrophs, phosphate-mobilizing bacteria, soil, long-term chemicalization, *Triticum aestivum* L., wheat, *Hordeum vulgare* L., barley, seeds, germination energy,

Intensive exploitation of agricultural land is accompanied by the constant use of high doses of mineral fertilizers [1, 2], which can significantly increase productivity but with the long-term use leads to a decrease in the quality of crop production, environmental pollution, and violation of natural mechanisms of soil restoration [3-5]. Therefore, at present, mono, binary and multicomponent microbial preparations are increasingly used instead of mineral fertilizers. Unlike mineral fertilizers, they have some advantages: they do not pollute the environment, they are harmless to humans and animals, not phytotoxic, and do not have mutagenic activity since they are strains of natural soil microorganisms.

Microorganisms that serve as the basis of biological products, by entering into close interactions with the host plant and other participants in the microbiocenosis, stimulate the growth and development of plants by using direct and indirect mechanisms: the fixation of atmospheric nitrogen, the production of siderophores, phytohormones, and enzymes, the dissolution of inaccessible elements of mineral nutrition [6-8]. Some strains inhibit the development of phytopathogenic microorganisms living in the soil due to the production of antimicrobial metabolites [9]. The introduction of promising bacterial inoculates can reduce the phytotoxicity of soil contaminated with heavy metals [10, 11]. Through treatment with microbial preparations, it is possible to increase the stress resistance of plants in drought conditions by increasing the absorption of moisture and nutrients, as well as the production of exopolysaccharides [12, 13]. However, the effectiveness of the introduced preparations strongly depends on the factors of the new environment and the ability of the introduced microorganisms to survive among the indigenous representatives of the soil microflora [14].

The way out of this situation can be the creation of plant growth stimulants based on bacteria isolated from soils that have been exposed to various agricultural practices for a long time. Microorganisms that have preserved their viability in conditions of intensive farming may have high adaptability to the soil and climatic conditions of cultivated soils.

In this study, the authors first isolated local active strains of nitrogen-fixing and phosphate-mobilizing bacteria from the soils of long-term stationary experience in intensive farming in the Russian Far East and proved the possibility of creating plant growth stimulants based on such bacteria.

The work aims to study the effect of nitrogen-fixing and phosphate-mobilizing bacteria isolated from soils that have been exposed to active chemicalization for a long time on the germination of cereal seeds for the subsequent development of biological preparations of plant growth stimulants.

Materials and methods. Samples of agricultural soils involved in a long-term experiment (74 years) on fertilization were taken in October 2015 (the experimental field 8, the Federal Research Center for Agrobiotechnology of the Far Eastern Branch of the Russian Academy of Sciences) at a depth of 5-15 cm with sterile instruments and placed in sterile dishes.

The influence of soil microorganisms on seed germination was evaluated on wheat (*Triticum aestivum* L.) of the Primorskaya 50 cultivar and barley (*Hordeum vurlage* L.) of the Tikhookeansky cultivar (obtained from the collection of the Federal Research Center for Agrobiotechnology, the Far Eastern Branch RAS).

Strains of nitrogen-fixing and phosphate-mobilizing bacteria were isolated using standard methods used in soil microbiology [15]. A 1:10 soil suspension (10 g

of soil and 90 ml of water) was used to prepare a sequence of serial dilutions with inoculation on agar media: nitrogen-fixing microorganisms were isolated on Ashby's Glucose Agar (HiMedia, India), and phosphate-mobilizing microorganisms were isolated on a selective medium with tricalcium phosphate [16].

The strains of microorganisms of greatest interest were previously identified as representatives of *Acinetobacter* spp. (strain N1), *Azotobacter* spp. (strain N2), *Clostridium* spp. (strain N3), *Serratia* spp. (strain P6), *Bacillus* spp. (strain P7), *Arthrobacter* spp. (strain P8), and *Pantoea* spp. (strain P19).

Working suspensions of nitrogen-fixing and phosphate-immobilizing bacteria were obtained by cultivation in a nutrient broth (nutrient broth, Federal Budget Institution of Science State Research Center for Applied Microbiology and Biotechnology, Russia). The number was taken into account using a photoelectric colorimeter AP-101 (APEL Co., Ltd., Japan) at $\lambda = 600$ nm, and for calibration, the count of microbial cells in the Goryaev-Tom chamber (Axioscop 40 microscope, Carl Zeiss, Germany) was used [17]. After determining the initial counts of bacteria, a saline solution was used to dilute the suspension of microbial cells to a working concentration of 1×10^7 /ml.

The effect of isolates of nitrogen-fixing and phosphate-mobilizing bacteria on the germination of cereal seeds was evaluated by the synergy effect in binary compositions of monocultures. A sterile nutrient broth was used as a comparison control. Tap water was used as the experiment control.

Plant seeds (100 of each cultivar) were soaked for 30 minutes in the tested monoculture or binary composition of the studied bacteria. Then, the seeds were laid out in 10 pieces on filter paper moistened with sterile water, in sterile Petri dishes, and germinated at 23 °C. On day 3, the germination energy was determined, and on day 7, the laboratory germination rate, seedling length, and root length were determined [18]. The experiment was carried out in a 3-fold replication.

Statistical data processing (calculation of the mean values M , the standard error of the mean values \pm SEM, parametric comparison by the Student's t -test) was performed using the program SPSS v. 11.5 for Windows (<https://spss.software.informer.com/11.5/>). The differences were considered statistically significant at $p \leq 0.05$.

Results. The soil is a system of many different micro- and mesic environments, providing conditions for the development of a wide variety of soil microorganisms [19]. The ability to stimulate the growth and development of plants was noted in a large number of microorganisms isolated from soils, including representatives of the genera *Azotobacter*, *Azospirillum*, *Bacillus*, *Pseudomonas*, *Burkholderia*, *Pantoea* spp., *Enterobacter* [2, 20-22]. These microorganisms can synthesize vitamins, amino acids, polyhydroxy butyrate, phytohormones, siderophores, antibiotics, and enzymes, as well as fix molecular nitrogen and mineralize phosphates and other elements [23].

From the naturally formed microbial associations of soil fields of a long-term (74 years) stationary experiment in chemization (Federal Research Center for Agrobiotechnology of the Far Eastern Branch of the Russian Academy of Sciences), the authors isolated 68 bacterial strains with different culture-based and morphological properties. Based on these isolates, the authors created a collection of nitrogen-fixing and phosphate-mobilizing bacteria, screening of which by target characteristics (nitrogen fixation and phosphate mobilization) revealed seven of the most active strains, which were used in further experiments (Table 1).

1. Cultural and morphological properties of bacterial isolates from soils of the 74-year stationary chemicalization experiment (Federal Research Center for Agrobiotechnology of the Far Eastern Branch of the Russian Academy of Sciences, Primorye Territory, 2015)

Trait	Nitrogen-fixing bacteria			Phosphorus-mobilizing bacteria			
	strain N1	strain N2	strain N3	strain P6	strain P7	strain P8	strain P19
Colony description:							
size, mm	< 1	5	3	< 1	1	< 1	2
surface	Glossy	Glossy	Glossy	Glossy	Glossy	Glossy	Glossy
color	Yellow	Yellowish	Yellowish	White	White	White	Yellow
transparency	–	+	+	–	–	–	–
edge	Smooth	Laciniated	Smooth	Wavy	Laciniated	Smooth	Smooth
profile	Convex	Convex	Convex	Convex	Convex	Convex	Convex
center	+	–	–	–	–	–	–
surface	Crateriform	Smooth	Smooth	Smooth	Smooth	Smooth	Smooth
structure	Smooth	Homogeneous	Fine-grained	Homogeneous	Fine-grained	Fine-grained	Homogeneous
consistency	Easy to remove	Mucosa	Mucosa	Easy to remove	Easy to remove	Easy to remove	Easy to remove
Cell description:							
Gram staining	–	–	–	–	–	–	–
shape	Rods	Rods	Rods	Short rods	Short rods	Short rods	Rods
size, μm	1×0.5	0.8×0.4	0.8×0.3	1×1.5	1.2×1.8	1.2×1.8	1×0.4

Note. The strains were previously identified as *Acinetobacter* spp. (N1), *Azotobacter* spp. (N2), *Clostridium* spp. (N3), *Serratia* spp. (P6), *Bacillus* spp. (P7), *Arthrobacter* spp. (P8), and *Pantoea* spp. (P19); «–» and «+» — the absence or presence of the trait, respectively.

In this experiment, working suspensions of microorganisms were combined in a ratio of 1:1, resulting in 28 binary compositions and monocultures of nitrogen-fixing and phosphate-mobilizing bacteria (Table 2).

2. Scheme of combinations of nitrogen-fixing and phosphate-mobilizing bacteria used in the experiment

Strain	Nitrogen-fixing bacteria			Phosphate-mobilizing bacteria			
	N1	N2	N3	P6	P7	P8	P19
Nitrogen-fixing bacteria:							
N1	+	+	+	+	+	+	+
N2	-	+	+	+	+	+	+
N3	-	-	+	+	+	+	+
Phosphate-mobilizing bacteria:							
P6	-	-	-	+	+	+	+
P7	-	-	-	-	+	+	+
P8	-	-	-	-	-	+	+
P19	-	-	-	-	-	-	+

N o t e. The strains were previously identified as *Acinetobacter* spp. (N1), *Azotobacter* spp. (N2), *Clostridium* spp. (N3), *Serratia* spp. (P6), *Bacillus* spp. (P7), *Arthrobacter* spp. (P8), and *Pantoea* spp. (P19).

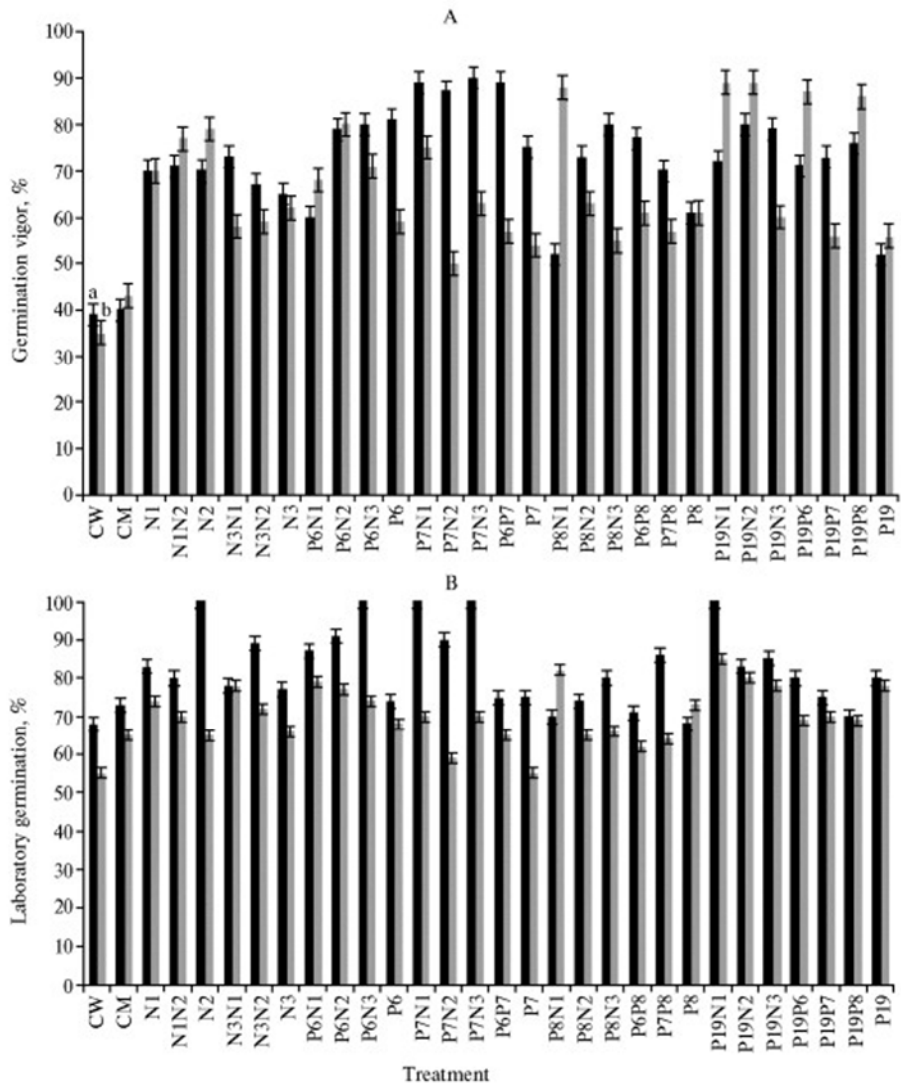


Fig. 1. Seed germination energy (A) and laboratory germination (B) in wheat (*Triticum aestivum* L.) cv. Primorskaya 50 (a) and barley (*Hordeum vulgare* L.) cv. Tikhookeansky (b) when treated with cultures of bacterial isolates: N1 (*Acinetobacter* spp.), N2 (*Azotobacter* spp.), N3 (*Clostridium* spp.) (nitrogen-fixing),

P6 (*Serratia* spp.), P7 (*Bacillus* spp.), P8 (*Arthrobacter* spp.), P19 (*Pantoea* spp.) (phosphate-mobilizing); CW — control (water), CM — control (nutrient medium) ($N = 3$, $M \pm \text{SEM}$). For all treatments, the differences are statistically significant at $p \leq 0.05$.

It was found that as a result of the treatment of seed material by the studied bacteria, the germination energy and laboratory germination rate varied depending on the experiment option and the type of plant. Thus, in wheat and barley, the seed germination energy in the control was 39 and 35%, respectively, in the comparison control — 40 and 43%, while when treated with nitrogen-fixing and phosphate-mobilizing bacteria, this indicator increased in wheat by 13-51%, in barley — by 15-54% compared to the untreated control. Wheat seeds had the highest germination energy in options N1P7, N3P7, and P7P6, while barley seeds had the highest germination energy in options N1P8, N1P19, and N2P19 (Fig. 1).

Laboratory seed germination in wheat and barley in the untreated control was 68 and 55%, respectively, in the comparison control 73 and 65%, when treated with nitrogen-fixing and phosphate-mobilizing bacteria, the laboratory seed germination increased by 2-32% and 7-30%, respectively, compared to the control without treatment. Full germination (100%) of wheat seeds was observed when using binary combinations of N3P6, N3P7, N1P19, as well as monoculture N2. The highest values of the laboratory seed germination index (80-85%) in barley were observed in the options N2P19, N1P8, and N1P19 (see Fig. 1).

The study of the influence of the microorganisms under investigation on the growth and development of wheat seedlings revealed the following significant ($p \leq 0.05$) morphometric changes relative to the control options (the difference between the controls was insignificant and lied in the range of the experimental error): an increase in the seedling length by 20.9%, in the root length by 83.7%. The maximum values of the seedling length were recorded for N2N3 (86.4 mm), N1P8 (87.4 mm), and N2P19 (88.3 mm) (Fig. 2), of the root length for N1N2 (165.4 mm), N2 (159.1 mm), and P19N3 (148.0 mm). In some treatments, the seedling length decreased by 5.2%, the root length by 17.4% compared to the controls ($p \leq 0.05$).

Treatment of barley seeds with strains of nitrogen-fixing and phosphate-mobilizing bacteria in most options also led to an increase in the seedling length by 1.8 times, and the root length by 2.7 times ($p \leq 0.05$). The greatest seedling length was observed in the options treated with combinations of bacteria N2P19 (140.0 mm), P6P19 (121.0 mm), P8P19 (120.0 mm), the root length was maximum when seeds were treated with P6P7 (119.2 mm), N2P19 (121.1 mm), and P6P19 (132.8 mm) suspensions (see Fig. 2). As with wheat, barley in some cases showed a decrease in morphometric characteristics: seedling length by 36.7%, root length by 46.7% compared to the control options ($p \leq 0.05$). It is interesting to note that the N3P7 combination, the treatment with which positively affected the germination energy and laboratory germination of seeds (especially in wheat), contributed to a reduction in the seedling and root length in both barley and wheat. In different cultures, the responsiveness of germinating seeds to treatment with the same combination of bacteria was not the same. Thus, a pair of P6P7 isolates significantly stimulated the growth of seedlings and roots in barley but did not significantly affect these characteristics in wheat.

For the N2P8 combination, the effect was reversed: in wheat germs, the length of the seedling and root increased markedly, while in barley seedlings — slightly and even in some cases decreased compared to the control ($p \leq 0.05$). Thus, we noted an increase in the seedling and root length in wheat compared to to both controls, while compared to one control (nutrient broth) it was longer (seedlings by 12 mm, roots by 50 mm), compared to the other (water) shorter

(seedlings by 10 mm, roots by 47 mm). In barley, these indicators generally decreased compared to both controls, but not equally: in comparison with the water, a 6 mm decrease in the seedling length and a 3 mm increase in the root length occurred and as compared to nutrient broth the seedlings were 4 mm longer and the roots were 3 mm longer.

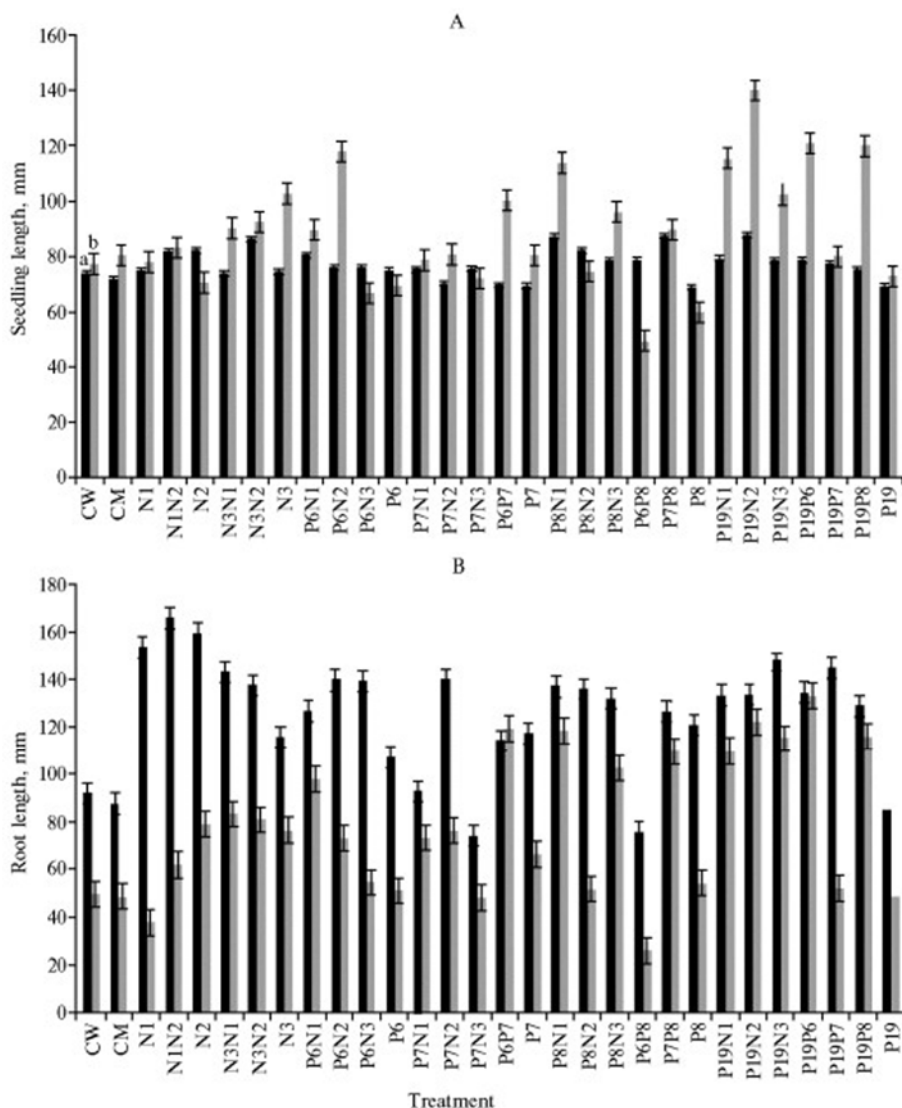


Fig. 2. Seedling length (A) and root length (B) in wheat (*Triticum aestivum* L.) cv. Primorskaya 50 (a) and barley (*Hordeum vulgare* L.) cv. Tikhookeansky (b) when treated with cultures of bacterial isolates: N1 (*Acinetobacter* spp.), N2 (*Azotobacter* spp.), N3 (*Clostridium* spp.) ((nitrogen-fixing), P6 (*Serratia* spp.), P7 (*Bacillus* spp.), P8 (*Arthrobacter* spp.), P19 (*Pantoea* spp.) (phosphate-mobilizing); CW — control (water), CM — control (nutrient medium) ($N = 3$, $M \pm SEM$, lab test). For all treatments, the differences are statistically significant at $p \leq 0.05$.

Germination energy and seed germination are important indicators of plant competitiveness, which affects their development and yield [24]. In our experiments, the treatment of wheat and barley seeds with suspensions of the studied nitrogen-fixing and phosphate-mobilizing isolates contributed to a significant and reliable ($p \leq 0.05$) increase in germination energy and laboratory germination. The most expressed stimulating effect was due to combinations of bacteria, including strains N1, N2, P7, and P19. Hahn et al. [22] treated rice (*Oryza sativa* L.) seeds

under sterile conditions with liquid cultures containing nitrogen-fixing bacteria of the genera *Burkholderia* and *Mesorhizobium*. Experiments have shown [22] that the initial germination, calculated as the percentage of seeds sprouted on day 3, from the number of seeds sprouted on day 6, in the options with treatment increased by 20-38% compared to the control. A significant increase in laboratory germination of corn was observed with the use of nitrogen-fixing bacteria of the genera *Pseudomonas*, *Bacillus*, *Enterobacter*, and *Pantoea* [21].

Laboratory germination and field germination may differ. For example, in the experiments of Batool and Iqbal [25], the increase in the germination of decontaminated wheat seeds treated with various strains of phosphate-solubilizing bacteria and germinated in Petri dishes concerning the control was 65-90%, while in the field when grown in vegetation vessels, the indicator increased by 20-80% compared to the control. Such discrepancies can be associated with sharply different environmental conditions and, as a result, with a change in the character of interactions in the plant—microorganism system. At the same time, fairly stable interactions are also possible: all the phosphate-solubilizing bacteria studied in the experiment had a positive effect on the development of wheat plants and were recommended by the authors in a monoculture or a combination as an alternative to mineral fertilizers [25]. It has been reported that inoculation of seeds with biological preparations containing live microorganisms has a positive effect on germination, while the effect depends on the weather conditions accompanying the beginning of the vegetation period [26]. It is evident that to ensure a positive result in microbial-plant interaction, the introduced microorganisms must not inhibit the growth and development of the partner plant. The stimulating or inhibitory effect of bacteria on the development of plants of barley *Hordeum vulgare* L., spring wheat *Triticum aestivum* L., rye *Secale cereale* could be detected even at the seed germination stage [2]. According to the literature, the effect of inhibiting plant development can be associated with a high concentration of the phytohormone indolyl-3-acetic acid, which in low concentrations stimulates growth [27]. Under laboratory conditions, the inhibitory effect of associative diazotrophs on germinating seeds could be manifested due to the absence or lack of nutrients in the environment necessary for the growth of this group of microorganisms. As it is known, rhizosphere bacteria consume mainly the metabolic products released by germinating seeds, but in the absence of photosynthesis, their number is small [20].

The combinations of the studied bacteria in most options contributed to an increase in the seedling length and the root length in the germs of both plant species to varying degrees ($p \leq 0.05$). An increase in the root length was observed when wheat seeds were treated with bacteria of the genus *Bacillus* [28]. The most expressed stimulating effect on these morphometric characteristics was exerted by combinations that included strains P19, N1, N2, and binary compositions that included both nitrogen-fixing and phosphate-mobilizing bacteria showed a generally more expressed effect than monocultures of the same microorganisms. Similar observations were made by Widawati and Suliasih [29], by studying the effect of bacteria of the genera *Azotobacter*, *Azospirillum*, and *Bacillus* on the germination of sorghum seeds *Sorghum bicolor* L., they found that the indicators of germination energy, seedling and root length decreased after inoculation with monocultures even relative to the control (the exception was the option with the treatment with *Azospirillum lipoferum*). At the same time, simultaneous treatment with nitrogen-fixing bacteria of the genus *Rhizobium* and phosphate-solubilizing bacteria of the genus *Pseudomonas* contributed to an increase in the mass of roots, seedlings, and wheat yield [30].

Let us note that the effect of the bacterial inoculates studied by the authors

on seed germination depended on the plant type. Thus, in the N1P8 option, the treatment did not have a noticeable effect on wheat seeds, but in the experiment with barley, the same binary composition provided the maximum values of germination energy and laboratory germination of seeds. A similar pattern was observed for the N1P7 combination but, in this case, the greatest stimulating effect was observed for wheat. It was reported [31] that barley yield was more responsive to treatment with the drug Rizoagrin than wheat. Moreover, for soybeans, varietal differences were noted when treated with *Bacillus subtilis* strains. It may be due to the synthesis of certain metabolites, the effectiveness of which directly depends on the plant genotype and its life cycle [32].

Thus, it was found that the treatment of wheat and barley seeds with suspensions of isolates of nitrogen-fixing and phosphate-mobilizing bacteria isolated from soils subjected to long-term (74 years) systematic treatment with mineral fertilizers contributed to a significant increase in the germination energy and laboratory germination of seeds, the seedling and root length. It was shown that the germination energy of wheat seeds in the options treated with nitrogen-fixing and phosphate-mobilizing bacteria increased by an average of 32% (from 13 to 51%) ($p \leq 0.05$), and of barley seeds by an average of 34.5% (from 15 to 54%) ($p \leq 0.05$) compared to the untreated control. At the same time, the laboratory germination of wheat seeds in the options with treatment with bacteria capable of nitrogen fixation and phosphate immobilization increased by 2-32% ($p \leq 0.05$), and barley seeds by 7-30% ($p \leq 0.05$) compared to the untreated control. The greatest seedling length was noted when using combinations of N2P19, P6P19, and P8P19, root length when using P6P7, N2P19, and P6P19. Binary bacterial compositions had a greater effect on the germination of plant seeds than monocultures. The combinations that included nitrogen-fixing strains N1 (*Acinetobacter* spp.), N2 (*Azotobacter* spp.), and phosphate-mobilizing strain P19 (*Pantoea* spp.) showed the most pronounced stimulating effect. Also, the differences in the responsiveness of plants to treatment depending on the species were observed, probably due to the lack of genetic and physiological and biochemical complementarity between a particular plant species and the studied isolate or their combination. Later, we plan to study the effectiveness of these bacterial inoculates in vegetation experiments and the field.

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NOVEL HYBRID MODULATORS OF PLANT IMMUNE RESPONSES BASED ON CHITOSAN AND BIOACTIVE ANTI-OXIDANTS AND PRO-OXIDANTS

E.V. POPOVA , N.S. DOMNINA, C.V. SOKORNOVA, N.M. KOVALENKO,
S.L. TYUTEREV

All-Russian Research Institute of Plant Protection, 3, sh. Podbel'skogo, St. Petersburg, 196608 Russia, e-mail elzavpopova@mail.ru (✉ corresponding author), ninadomnina@mail.ru, mymryk@gmail.com, nadyakov@mail.ru, mail@vizr.spb.ru

Popova E.V. orcid.org/0000-0003-3165-6777

Kovalenko N.M. orcid.org/0000-0001-9577-8816

Domnina N.S. orcid.org/0000-0002-7124-899X

Tyuterev S.L. orcid.org/0000-0003-2397-9656

Sokornova C.V. orcid.org/0000-0001-6718-4818

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Abstract

During the infection of plants with pathogens, the equilibrium between oxidative processes and antioxidant activity shifts toward an increase in the formation of ROS, which affects the course of the development of the disease and manifests itself differently in phytopathosystems of various types of parasitism. So, to protect plants from biotrophic pathogens, ROS generation is necessary, but the resulting high level of ROS does not protect, but, on the contrary, favors the pathological process caused by necrotrophs. Substances that can control not only ROS generation, but also their utilization, can become effective immunomodulators to protect plants when infected with pathogens of different lifestyles. For the first time, chitosan-based hybrid immunomodulators (Chit-Van+SA) were created, in the structure of which there are fragments of biologically active substances that have antioxidant and prooxidant effects. The basis of such systems is chitosan (Chit), containing covalently attached vanillin (Chit-Van), which reduces the ROS level by stimulating cellular antioxidant activity (antioxidant effect), and salicylic acid (Chit+SA), which is associated with a labile ionic bond, generating ROS (prooxidant act). It was shown that (Chit+SA) increases the wheat resistance to *Puccinia recondita* Roberge ex Desmaz f. sp. *tritici* biotroph and *Cochliobolus sativus* Drechs hemibiotroph, while (Chit-Van) effectively induces resistance only to *C. sativus* hemibiotroph. The effectiveness (Chit+CA) as an inducer of resistance is 1.2-2.0 times higher than the inducing effect of chitosan itself (Chit). It was found that hybrid immunomodulators (Chit-Van+SA) with respect to both the biotroph (brown rust) and the hemibiotroph (brown spot) with a molar ratio of vanillin and salicylic acid in them in the range 1:1-1:2 show a high immunomodulating activity, regardless of the molecular weight of chitosan, which is expressed in a decrease in the area of leaf damage to 5-10 % of the control. The results obtained indicate that the biological effectiveness of the hybrid polymer system (Chit-Van+SA) as an immunomodulator correlates with the content of SA in them, which confirms the participation of the signal salicylate system in the induction of resistance. It is established that specific enzymatic antioxidants superoxide dismutase, catalase and peroxidase, which regulate the relationship between oxidative processes and antioxidant activity, play an important role in the development of induced resistance when using the created immunoinductors. In general, the results of studying the effect of the hybrid immunomodulators (Chit-Van+SA) on the activity of antioxidant enzymes in the model system wheat—*C. sativus* suggest that the increase in plant resistance to pathogens is realized through the control of the intensity of redox processes in plants, which is caused by the presence in the structure of hybrid immunomodulators of a certain pro- and antioxidant balance.

Keywords: *Puccinia recondita*, *Cochliobolus sativus*, chitosan, induced resistance, salicylic acid, vanillin, wheat, dark brown spotting, brown rust

Increasing grain production of spring soft wheat remains an urgent task, since this crop occupies up to 70% of the grain area. Every year, in different regions of Russia, an unfavorable phytosanitary situation is noted on grain crops, which negatively affects the quantity and quality of the crop. The most common

and harmful leaf diseases of common wheat (*Triticum aestivum* L.) include brown rust (pathogen *Puccinia recondita* Roberge ex Desmaz f. sp. *tritici*) and brown spot (pathogen *Cochliobolus sativus* Drechs) [1, 2]. To protect plants from diseases, chemical methods are mainly used, among which treatment with fungicides prevails, which are highly effective, but at the same time have a negative impact on the environment [3]. In this regard, the development of agricultural technologies using the method of induced resistance, based on the activation of innate defense mechanisms of plants and the formation of resistance to pathogens in plants, is of particular relevance. The inducers of plant disease resistance created on the basis of these principles are an ecologically safe alternative to fungicides used both abroad and in Russia [4-6].

The development of molecular biology methods has led to a more complete understanding of the mechanisms of plant defense against pathogens and the identification of the role of various signaling systems in the development of defense reactions leading to a coordinated biochemical and physiological defense response to infection with a pathogen [4, 7-10]. The ability to control the pathological process is associated with the ability of inducers to direct metabolism in a direction unfavorable for the pathogen [11-13]. In this case, the formation of a protective response is due to the expression of genes under the action of signaling molecules [10, 14-16] and exogenous biologically active substances (BAS) – immunomodulators [7, 11].

Numerous studies have convincingly shown that in response to the action of factors of different natures, the pro/antioxidant balance is shifted, acting on which, with the help of BAS, it is possible to activate the genetic potential of protective reactions and form an immune response. In this regard, the hypothesis has recently become increasingly popular, according to which the adaptation of plants to the action of stressors of various natures largely depends on the functioning of the antioxidant system. It is known that infection of plants at the initial stage is accompanied by a sharp increase in the intensity of oxidative processes, expressed in the generation of reactive oxygen species (ROS) (oxidative burst), which include a chain of subsequent protective reactions [16-19]. ROS include superoxide radical anion, hydroxyl radical, hydrogen peroxide (H_2O_2), and nitric oxide. ROS play a dual role in the interaction of the host with the pathogen. First, they have a direct antimicrobial effect, strengthen the barrier properties of cell walls through the polymerization of phenolic compounds [20, 21]. Second, ROS, as signaling molecules, are involved in the formation of acquired systemic resistance to pathogens, regulating gene expression, the activity of defense systems [22, 23] and including the expression of genes of pathogenesis-related proteins, the synthesis of phytoalexins, and the triggering of the hypersensitivity response. It should be noted that among ROS, the role of hydrogen peroxide, the longest-lived transmembrane signal in plant cells, has been most studied [18, 19].

According to the currently available data [23-27], plant protection from biotrophic pathogens is aimed at generating ROS, while a high ROS content favors the pathological process caused by necrotrophs. In the case of hemibiotrophic pathogens characterized by a combined feeding strategy, information on the role of ROS is often contradictory and depends on the host-pathogen system [25, 26, 28]. It is assumed that the pathogen passes through the biotrophic and necrotrophic phases of development at different concentrations of hydrogen peroxide [26]. The important role of the balance between the formation and degradation of ROS (for example, H_2O_2) in the formation of plant defense reactions and their survival under stresses of various natures has been reported in many publications [26, 28, 29].

It is believed that maintaining the concentration of ROS already formed

in the cell at a sufficient level is possible due to the regulation of the activity of antioxidant enzymes [30–32]. Note that studies aimed at identifying the role of antioxidant enzymes in the formation of plant resistance to stress factors are being carried out quite intensively both in Russia and abroad [30, 31, 33]. It is known that under a strong infectious load, its own antioxidants cannot neutralize the overproduction of ROS, which leads to cell death and the development of necrosis. Therefore, an increase in the antioxidant activity of plants should increase their tolerance to the development of necrosis caused by pathogens or abiotic stresses [31–33]. Thus, by influencing in a certain way the induction of ROS and antioxidants, it is possible to regulate the susceptibility and resistance of plants to pathogens. To control these processes, it is advisable to use immunomodulators capable of controlling not only the generation of ROS but also their utilization. Despite the fact that intensive research on the development of effective immunomodulators is actively continuing all over the world, there is no information about the creation of inducers with the help of BAS of different natures (prooxidant and antioxidant) with the directed regulation of the formation of induced plant immunity, in particular when wheat is infected with pathogens with different types of nutrition (necrotrophic and hemibiotrophic). In this regard, it is important to obtain new inducers capable of regulating the relationship between oxidative processes and antioxidant activity in plant tissues when wheat is infected with pathogens with different trophicity.

We were the first to create hybrid immunomodulators based on chitosan, the structure of which contains fragments of BAS (vanillin and salicylic acid SA) that have anti- and prooxidant effects. The resulting hybrid immunomodulators are effective against pathogens of dark brown spot and brown rust of wheat (hemibiotroph and biotroph, respectively). It was shown that their modulating effect was manifested through the effect on the activity of the main antioxidant enzymes of plants – superoxide dismutase (SOD), catalase (CAT), and peroxidase (PO) during the development of induced resistance.

The purpose of this study was to develop immunomodulators – chitosan derivatives with prooxidant and antioxidant activity and to assess their effect on wheat resistance to brown spot (hemibiotroph *Cochliobolus sativus* Drechs) and to leaf rust (biotroph *Puccinia recondita* Roberge ex Desmaz f. sp. *tritici*).

Materials and methods. To create immunomodulators, chitosans with a molecular weight of 6.5, 60, and 100 kDa (Chit) were obtained by the method of oxidative destruction [34] from chitosan with a molecular weight of 150 kDa and a degree of deacetylation of 85% (Bioprogress, Russia). Chitosan derivatives containing 25 wt% of covalently bound vanillin fragments (Van) – (Chit-Van) were synthesized through a Schiff base followed by reduction with an excess of sodium borohydride [35]. The amount of introduced vanillin fragments was determined by UV spectra (spectrophotometer UV-2600, Shimadzu, Japan) at $\lambda = 280$ nm [35]. The ionic nature of the bond between chitosan and SA in chitosan derivatives (Chit+SA) (salt formation) was confirmed on the basis of IR spectra (Spectrum BX spectrometer, Perkin Elmer, Inc., USA) by the presence of characteristic bands 1552.92 and 1386.12 cm^{-1} from the COO^- carboxylate group, as well as a wide band in the region of 3100–2600 cm^{-1} , reflecting stretching vibrations from the NH_3^+ and OH functional groups [36].

Chitosan, containing simultaneously covalently attached vanillin and ionically bound SA, is a hybrid system (Chit-Van+SA). The synthesis of such systems was carried out by introducing SA into (Chit-Van) containing 25 wt% of vanillin, varying the amount of SA from 1:0.5 to 1:2 with respect to the attached vanillin.

The inducing activity of the test compounds was assessed by the detached

leaves method [37]. Seven-day-old wheat seedlings (*Triticum aestivum* L.) of the susceptible cultivar Saratovskaya 29 were sprayed with 0.1% (in terms of chitosan) solutions of immunomodulators 24 h before inoculation with the pathogen – hemibiotroph *C. sativus* (4000 spores/ml) or biotroph *P. recondita* (2000 pustules/ml). In each variant of the experiment, 100 seedlings were used. Leaf infection was assessed on the 4th day after infection with *C. sativus* and on the 7th day after infection with *P. recondita* according to the intensity of the disease development (the degree of damage to the leaf area) according to the description [37]. In the control, plants were treated with water. The infection of wheat leaves in the form of brown spots during infection with *C. sativus* and uredopustules during infection with *P. recondita* was taken as 100% in the control. The immunomodulatory activity of the test substances was assessed as a percentage as the extent of damage of plant leaves by the pathogen in relation to the control.

The effect of hybrid systems (Chit-Van+SA) (1:2) and SA on enzyme activity was assessed by analyzing samples of wheat leaves taken before infection and on days 1, 2, 3, and 4 after *C. sativus* infestation. Variants included plants without infection and treatments (control) and plants treated 24 h before infection with *C. sativus* with 0.1% solution (Chit-Van+SA) or 2 mM SA solution (experiment). To determine the enzyme activity, a weighed portion of 20 leaves was ground in 0.05 M phosphate buffer (pH 6.2) at a ratio of 1:5, extracted for 30 min at 4 °C, then centrifuged for 10 min at 8000 g (Eppendorf 5415R microcentrifuge, Eppendorf, USA).

CAT activity in wheat leaves was determined spectrophotometrically [38] on days 1, 2, 3, and 4 after infection with *C. sativus*, PO activity – colorimetrically according to Boyarkin [39]. SOD activity was assessed by a method based on measuring the inhibition of photochemical reduction of nitro blue tetrazolium [40].

All experiments were performed in 3 biological and 3 chemical replicates.

Analysis of variance was used for data processing (Statistica 6.0 (StatSoft, Inc., USA) and Excel 2016. In the calculations, the methods of parametric statistics were used (based on the mean M and their standard errors \pm SEM, 95% confidence intervals, the least significant difference (LSD) at $p < 0.05$).

Results. Analysis of the literature data suggests that the effectiveness of disease resistance inducers is determined by their ability to change the conditions of pathogen development in a plant at the stage of its introduction by regulating redox processes, which are the main ones in the dynamics of pathogenesis. In accordance with this approach, the created inducers of disease resistance should be compounds with multidirectional functions (hybrid) and contain two active centers in the structure: one will facilitate the generation of ROS, the other, on the contrary, will inactivate them. In addition, it is necessary to know the mechanism of the introduction and spread of the pathogen in plants. For example, for a biotroph, it is necessary to limit the time of its propagation, while for a hemibiotroph, it is desirable to increase the duration of residence in living plant tissues. In this regard, when interacting with a pathogen, the time order of the release of the administered BAS from the immunomodulator must be observed.

To create innovative hybrid immunomodulators, the authors have chosen the natural polysaccharide chitosan. Chitosan and preparations based on it are widely used in plant protection against diseases as inducers of nonspecific resistance [41–45]. In addition, chitosan attracts the attention of researchers by the presence of reactive functional groups, which make it possible to carry out various chemical modifications that enhance or impart new biological properties to this polymer [42, 46]. A classic inducer of disease resistance, SA, which plays a central

role in plant protection from biotrophic pathogens, was used as a BAS with prooxidant properties [14, 47, 48]. Among the BAS with antioxidant properties (usually phenolic compounds of natural and synthetic origin, for example, lilac, cinnamic acids, vanillin, etc.), vanillin was chosen.

Thus, the hybrid immunomodulator is chitosan, into the structure of which SA, which provides prooxidant activity, and vanillin, which has antioxidant properties, have been introduced. Due to the presence of a labile bond between chitosan and SA (Chit+SA), SA, an intense inducer of ROS generation, will be released first from the hybrid system [14, 15, 49, 50]. This effect is necessary at the initial stage of infection, when an increased concentration of ROS will promote necrosis and thereby limit the development of the biotroph. In the case of hemibiotrophic pathogens, for which the process of necrosis is favorable, sporulation requires, on the contrary, a decrease in the ROS content due to the work of the antioxidant system, which will lead to an increase in the duration of the stay of *C. sativus* necrotroph in living tissues at the biotrophic stage of its development. It is in this later process that the exogenous antioxidant vanillin, bound to chitosan by a strong covalent bond and gradually released under the action of enzymes, should be included.

When creating hybrid immunomodulators, the authors took into account the previously established [51] concentration dependencies of the inducing activity of SA and vanillin in relation to the hemibiotrophic pathogen *C. sativus*.

1. Wheat resistance to brown spot (*Cochliobolus sativus* Drechs) and brown rust (*Puccinia recondita* Roberge ex Desmaz f. *Sp. tritici*) when exposed to low molecular weight (6.5 kDa) chitosan and its derivatives based on bioactive substances (BAS) (method of separated leaves, cv. Saratovskaya 29)

Treatment	BAS, mM	Van:SA, mg/mg	Leaf damage, % of control	
			brown spot (LSD ₀₅ = 4.5)	brown rust (LSD ₀₅ = 9.0)
Control			100 ^a	100 ^a
Chit			25 ^a	25 ^a
Ван	1.5		60 ^a	100 ^a
CK	0.5		40 ^a	45 ^a
CK	1.0		50 ^a	35 ^a
CK	2.0		45 ^a	30 ^a
Chit-Van	1.5		15 ^a	50 ^a
Chit+SA	0.5		20 ^a	10 ^a
Chit-Van+SA	2.2	1:0.5	20 ^a	40 ^a
Chit-Van+SA	3.0	1:1	10 ^a	15 ^a
Chit-Van+SA	4.5	1:2	5 ^a	10 ^a

N o t e. The molecular weight of the initial Chitosan (Chit) is 6.5 kDa, BAS: vanillin (Van) and salicylic acid (SA). The concentration of the used sample solutions is 0.1% (based on chitosan).
^a — significant differences in the treatments compared to the control.

Table 1 shows that the original chitosan with a molecular weight of 6.5 kDa has good inducing activity, reducing the infection of wheat plants with leaf rust and brown spot to 25% in relation to the control. The presence of ionic-bound SA in chitosan increases its efficiency as an inducer in comparison with the initial chitosan, which is consistent with the literature data [20]. Treatment of wheat with a derivative (Chit-Van) reduced the infestation of leaves with brown spot by more than 6 times compared with the control. In the experiment with brown rust, the infection of leaves in wheat plants treated with the same derivative decreased by 2 times in comparison with the control. The results of studying three-component hybrid immunomodulators (Chit-Van+SA) showed that their inducing activity depended on the molar ratio of vanillin and SA. The highest inducing activity was observed for samples containing vanillin and SA in a molar ratio of 1:1 or 1:2. A further increase in the proportion of SA in the molar ratio to vanillin (1:3) in the hybrid derivative led to its phytotoxicity. The sample (Chit-Van+SA)

containing vanillin and SA in a molar ratio of 1:0.5 was even less effective as an immunomodulator than the original chitosan. The results obtained indicate that the SA concentration determines the biological activity of the hybrid system as an immunomodulator.

There is no unambiguously accepted opinion in the literature on a possible correlation between the molecular weight of chitosan and its inducing activity. The range of molecular weight values exhibiting high activity as an inducer of disease resistance is quite large, from 2 kDa to 300 kDa [4, 52]. In this regard, the authors carried out a comparative study of the inducing activity of hybrid immunomodulators obtained on the basis of chitosan with different molecular weights. Table 2 shows data on the assessment of the effect of chitosans with different molecular weights and their derivatives containing vanillin and/or SA on the resistance of wheat to brown spot and leaf rust.

2. Brown spot (*Cochliobolus sativus* Drechs) and brown rust (*Puccinia recondita* Rob-erge ex Desmaz f. sp. *tritici*) infestations (% of control) of wheat (*Triticum aestivum* L.) leaves when exposed to chitosan with different molecular weights and its derivatives based on bioactive substances (method of separated leaves, cultivar Saratovskaya 29)

Treatment	Chit 6.5 kDa		Chit 60 kDa		Chit 100 kDa	
	1	2	1	2	1	2
Control	100	100	100	100	100	100
Chit	30	20	25	20	20	15
Chit+SA	20	10	20	10	15	5
Chit-Van	15	40	15	25	10	35
Chit-Van+SA (Van:SA 1:2)	5	10	5	5	< 5	5

Note. Chit — Chitosan, Van — vanillin, SA — salicylic acid; 1 — brown spot (LSD₀₅ = 5.0), 2 — brown rust (LSD₀₅ = 8.0). The concentration of the used sample solutions is 0.1% (based on chitosan).).

Among chitosans with different molecular weights, chitosan with a molecular weight of 100 kDa had the highest efficiency as an inducer of disease resistance. In this variant of the experiment, the area of damage to the leaves of wheat *C. sativus* was only 20%, *P. recondita* — 15%.

Regardless of the molecular weight, chitosan containing SA (Chit+SA) in all variants of the experiment showed a higher immunomodulatory activity in comparison with unmodified chitosan, reducing the area of damage to leaves of *C. sativus* to 15-20%, and *P. Recondita* — to 5-10 % relative to the control. The inducing activity of chitosans with different molecular weights, containing only vanillin (Chit-Van), was significantly lower than that of unmodified chitosans in protecting wheat from brown rust and, on the contrary, higher in the case of brown spot. An innovative immunomodulator (Chit-Van+SA), regardless of the molecular weight of chitosan, effectively increased the resistance of wheat plants to leaf rust and dark brown spot, reducing the area of leaf damage to 5-10% of the control.

Thus, it has been experimentally confirmed that the high immunomodulatory activity of hybrid systems with a molar ratio of vanillin and SA in the range of 1:2 does not depend on the molecular weight of chitosan.

As noted above, antioxidant enzymes, primarily CAT, PO, and SOD, play an important role in the regulation of the amount of ROS in the cells of plants infected with the pathogen [28-31]. In order to assess the participation of antioxidant enzymes in the formation of induced resistance to brown spot in wheat seedlings under the action of a hybrid immunomodulator (Chit-Van+SA) containing vanillin and SA in a molar ratio of 1:2, the authors studied the CAT, PO, and SOD activity in the model system wheat—hemibiotroph of *C. sativus* when treated with this drug.

Analysis of the dynamics of changes in the activity of these enzymes in infected wheat seedlings showed that the process of infection of wheat plants with

the causative agent of brown spot *C. sativus* was accompanied by an increase in the activity of all antioxidant enzymes relative to control uninfected plants throughout the experiment. By the time of the strong development of the disease (day 4), when the entire leaf tissue was necrotic, their activity gradually decreased (Fig. 1, 2).

The experimental results revealed temporary differences in the increase in the activity of antioxidant enzymes during the development of the disease (see Fig. 1). On the 1st day, a sharp increase in the activity of PO, CAT and SOD was observed in the leaves of wheat infected with the pathogen, with a gradual decrease in their activity on day 4. The maximum increase in PO activity was observed on the 3rd day after infection, when clear signs of the disease appeared in the form of brown spots. As is known, the initial stage of plant infection is accompanied by the activation of the ROS generation system, and, first of all, a superoxide anion radical is generated, which is reduced by SOD to hydrogen peroxide. This enzyme forms the front line of defense against ROS, catalyzing dismutation of the superoxide radical anion to H_2O_2 , which leads to a significant increase in the activity of the enzyme. Hydrogen peroxide, being a CAT substrate, in turn induces an increase in the activity of this enzyme, which contributes to its increase on day 1 of infection. On day 2 of the development of the disease, the continuing increase in SOD activity led to further accumulation of hydrogen peroxide and was already accompanied by a significant increase in PO activity (day 3), which, along with CAT, was included in the control over the amount of hydrogen peroxide formed.

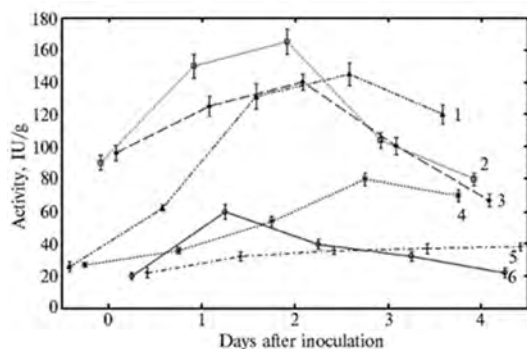


Fig. 1. Activity of antioxidant enzymes in wheat (*Triticum aestivum* L.) leaves infected with *Cochliobolus sativus* Drechs, when exposed to a hybrid immunomodulator based on chitosan (Chit) in combination with vanillin (Van, covalent bond) and salicylic acid (SA, ionic bond): 1 — peroxidase, 2 — superoxide dismutase, 6 — catalase (controls without Chit-Van+SA treatment); 3 — superoxide dismutase, 4 — peroxidase, 5 — catalase (with Chit-Van+SA treatment). $M \pm SEM$, the differences are statistically significant at $p < 0.05$ (lab test, cv. Saratovskaya 29).

Thus, an increase in the activity of SOD, CAT, and PO, observed in the initial asymptomatic biotrophic period (1-2 days) after infection of *C. sativus* plants, indicates a sharp increase in the amount of ROS and the involvement of antioxidant enzymes in the regulation of ROS accumulation in response to the introduction of a pathogen.

A gradual decrease in the activity of antioxidant enzymes with the development of the disease (4th day) and the transition of the pathogen to the necrotrophic phase led to an increase in oxidative processes, which in intensity exceeded the level of activity of the plant antioxidant system. As a result, intensive necrosis was initiated in the plant, which created a favorable habitat for the

pathogen *C. sativus*. The development of the disease during this period is visually manifested in the form of brown spots on the leaves. The consequence of this is an increase in the susceptibility of plants to brown spot, which corresponds to the literature data [53].

Treatment of wheat plants with a hybrid system (Chit-Van+SA) upon infection with *C. sativus* hemibiotroph decreased the activity of antioxidant enzymes, although the dynamics of its change remained the same as in control (untreated) infected plants. Thus, although the CAT activity increased on day 1 of infection, it was significantly lower than in the infected control plants (see Fig. 1). The PO

activity throughout the entire experiment was also significantly lower than that of the infected control plants. It should be noted that (Chit-Van+SA) insignificantly reduces the SOD activity at the first time (from the 1st to the 3rd day), while the dynamics of changes in activity remain the same as in infected plants. The latter means that due to increased SOD activity, superoxide anion-radicals are inactivated and peroxide accumulates in tissues within 1-2 days after infection. In turn, a decrease in CAT and PO activity in plants treated with (Chit-Van+SA) also leads to an increase in the amount of peroxide required to neutralize the phytopathogen in tissues and to restrain its spread during the biotrophic stage. Defense reactions are switched on, leading to the development of induced resistance to brown spot of wheat plants, which is manifested in a decrease in the development of the disease to 5% of the control.

It should be noted that the peculiarity of the created hybrid system is the presence in the working solution of the immunomodulator of SA, which is easily cleaved from chitosan, which suggests its participation in the manifestation of the inducing activity of immunomodulators at the initial stage of plant infection.

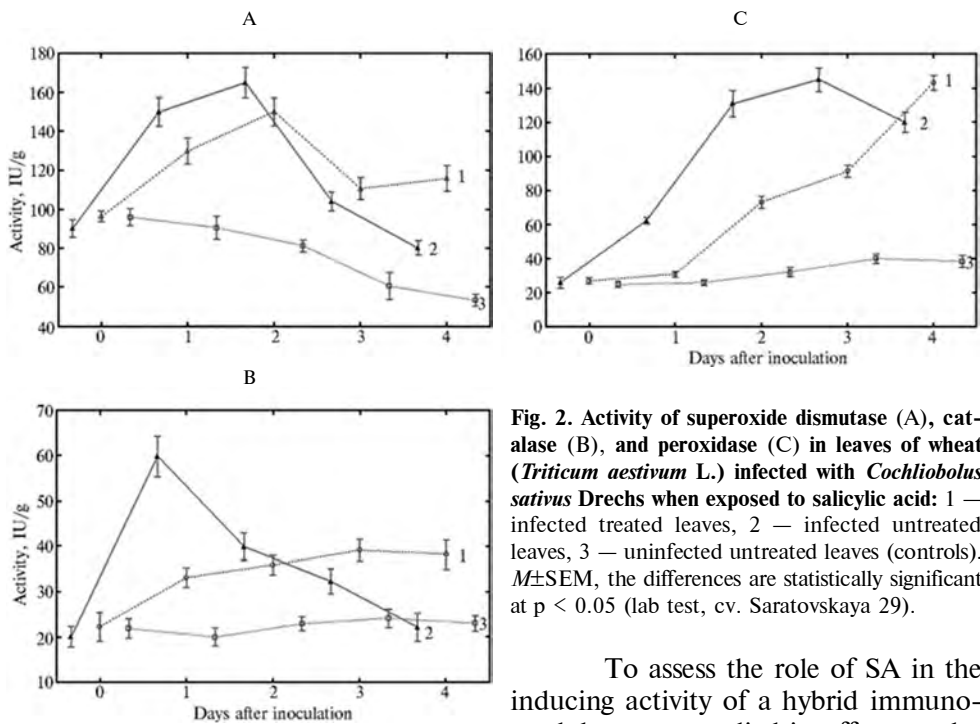


Fig. 2. Activity of superoxide dismutase (A), catalase (B), and peroxidase (C) in leaves of wheat (*Triticum aestivum* L.) infected with *Cochliobolus sativus* Drechs when exposed to salicylic acid: 1 — infected treated leaves, 2 — infected untreated leaves, 3 — uninfected untreated leaves (controls). $M \pm SEM$, the differences are statistically significant at $p < 0.05$ (lab test, cv. Saratovskaya 29).

To assess the role of SA in the inducing activity of a hybrid immunomodulator, we studied its effect on the activity of antioxidant enzymes upon infection of wheat with *C. sativus* hemibiotroph (see Fig. 2). It was found that the activity of SOD under the action of SA, although it decreased, was insignificant and remained high on the first day of the experiment. Similarly (as in the variant with wheat pretreatment Chit-Van+SA), in plants treated with SA and infected with the causative agent of brown spot, the activity of CAT (day 1) and PO (day 3) decreased 2-fold. That is, there is an analogy in the dynamics of changes in the activity of enzymes in plants treated with a hybrid immunomodulator and SA, upon infection with *C. sativus* hemibiotroph. The revealed analogy in the change in the activity of antioxidant enzymes (SOD, CAT, PO) in wheat leaves pretreated with SA and (Chit-Van+SA) indicates the participation of SA as a resistance inducer in the defense reactions of wheat plants activated by (Chit-Van+SA). Hence, it follows that SA, by reducing the activity of antioxidant enzymes, stimulates the accumulation of H_2O_2 in an amount sufficient to activate the protective mechanisms of induced immunity,

which is confirmed by the literature data [11, 54]. The observed inducing effect of SA is consistent with the data of phytopathological analysis, which showed that the effect of exogenous SA was manifested in the suppression of disease symptoms for hemibiotroph *C. sativus* up to 45% of the control (see Table 1). According to the literature, the inducing effect of exogenous SA in increasing plant resistance to phytopathogens is due to its ability to inhibit CAT, an enzyme that detoxifies hydrogen peroxide, which leads to the accumulation of ROS [14, 15, 30, 54]. The latter are considered as important signaling mediators in the formation of plant resistance. Taking into account these data, it can be assumed that the high inducing activity of hybrid systems (Chit-Van+SA), in which the Van:SA ratio is 1:1 or 1:2, is associated with the ability of exogenous SA to be first released from the polymer system and to induce the generation of H₂O₂ to an amount that leads to the activation of a whole spectrum of plant defense reactions that inhibit the development of pathogens. This increases the duration of the asymptomatic biotrophic stage of the development of the hemibiotroph and significantly slows down the development of the disease (the lesion is up to 5-10% of the control). The increased content of peroxide during this period also contributes to the formation of resistance to biotroph, and the development of *P. recondita* decreases to 10-15% of the control (see Table 1).

Treatment of plants with an immunomodulator (Chit-Van+SA) (Van:SA 1:0.5) with a smaller amount of exogenous SA (see Table 1) induces the appearance of a smaller amount of ROS. The exogenous antioxidant vanillin in the hybrid (Chit-Van+SA) also reduces the concentration of ROS. In this case, the amount of H₂O₂ decreases to a value that does not allow the development of resistance to the *P. recondita* biotroph, but is sufficient to induce resistance to the *C. sativus* hemibiotroph during the asymptomatic biotrophic period of the pathogen development. The results obtained indicate that the biological effectiveness of a hybrid derivative based on chitosan (Chit-Van+SA) as an immunomodulator correlates with the content of SA in them, which confirms the participation of the signaling salicylate system in the induction of resistance, which is consistent with the data of Sari and Etebarian [55], who, using the example of wheat lesions by the ascomycete *Gaeumannomyces graminis*, revealed the dependence of the effectiveness of SA as an inducer of resistance on concentration.

Thus, the research results suggest that the mechanism of action of new hybrid immunomodulators with covalently attached vanillin and ion-bound SA in the chitosan structure, leading to an increase in the resistance of wheat plants to pathogens with different nutritional strategies, is realized through the control of the activity of antioxidant enzymes (in particular, catalase CAT, peroxidase PO, and superoxide dismutase SOD), regulating the intensity of oxidative stress induced by the introduction of the pathogen. The high inducing effect of the created hybrid systems is apparently associated with the ability of exogenous SA, which is first released from the polymer system, to inhibit CAT and PO. As a result, cells accumulate hydrogen peroxide, which can enhance the expression of genes that determine protection against pathogens. Innovative hybrid immunomodulators as resistance inducers may be of practical interest in protecting plants from pathogens.

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STUDY OF BREAD WHEAT LINES WITH GENETIC MATERIAL OF *Triticum* SPECIES FOR RESISTANCE TO FUNGAL DISEASES

O.A. ORLOVSKAYA ✉, S.I. VAKULA, L.V. KHOTYLEVA

Institute of Genetic and Cytology of National Academy of Science of Belarus, 27, ul. Akademicheskaya, Minsk, 220072
Republic of Belarus, e-mail O.Orlovskaya@igc.by (✉ corresponding author), svettera@yandex.ru, L.Khotyleva@igc.by

ORCID:

Orlovskaya O.A. orcid.org/0000-0002-1187-1317

Khotyleva L.V. orcid.org/0000-0003-0295-5022

Vakula S.I. orcid.org/0000-0002-2242-7107

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Abstract

Triticum aestivum L. yield losses caused by phytopathogens can reach 40-80 % in epiphytotic years. Wild and cultivated relatives of common wheat are promising sources of broadening the genetic diversity of resistance loci for modern varieties. For the first time differences in the degree of infection by fungal pathogens of wheat lines obtained from crossing with *Triticum dicoccoides*, *T. dicoccum*, *T. durum*, *T. kiharae* were shown on the basis of long-term monitoring under conditions of the natural infectious background of Belarus. In our work, we studied the resistance of bread wheat lines with *Triticum* species introgressions to powdery mildew (*Blumeria graminis*), septoria (*Zymoseptoria tritici*), and leaf rust (*Puccinia triticina*) in a natural infectious background. We studied 30 introgression lines obtained in the Institute of genetics and cytology NAS of Belarus from crossing of six varieties of common wheat (Rassvet, Saratovskaya 29, Festivalnaya, Chinese Spring, Belorusskaya 80, Pitic S62) with accessions of tetraploid species *T. dicoccoides*, *T. dicoccum*, *T. durum* (AABB, $2n = 28$) and with hexaploid synthetic variety *T. kiharae* (A'A'GGDD, $2n = 42$). Twelve of the studied wheat lines were obtained with *T. durum* contribution, 7 — with *T. dicoccoides*, 6 — with *T. kiharae*, 5 — with *T. dicoccum*. The evaluation of resistance to the diseases was performed in a naturally occurring infectious background on the experimental fields of the Institute of Genetics and Cytology of the NAS of Belarus during 2012, 2014-2016, 2018, 2019 field seasons using Geshele's scale. We used the extent of flag leaf damage in the milky-wax ripeness phase as an indicator of resistance: 0-5 % — highly resistant plants, 5-10 % — resistant, 10-15 % — medium resistant, 15-25 % — medium susceptible, 25-40 % — susceptible. Statistica 10.0 software package was used for statistics (Kruskal-Wallis test, box-and-whiskers diagram, two-way ANOVA). The extent of flag leaf damage for the susceptible spring bread wheat variety Thatcher accounted 40-60 % by *B. graminis* and 15-25 % by *Z. tritici* during studied period. *P. triticina*, which causes wheat leaf rust, was detected only in 2012 and 2014 in a naturally occurring infectious background of Belarus (the degree of damage of susceptible control was 50 %). ANOVA confirmed the differences in the average degree of damage to wheat plants by fungal pathogens under weather conditions of six field seasons ($p < 0.01$). The highest sensitivity of wheat lines to powdery mildew and leaf rust was noted in 2014, to septoria blight — in 2012. The weather conditions of these years contributed to the intensive development of diseases (high precipitation, relatively low average daily air temperatures, Selyaninov's hydrothermal coefficient above 1.5). The least favorable conditions for the development of fungal pathogens were recorded in dry 2015 year (hydrothermal coefficient = 0.7). The species of genus *Triticum* were superior to common wheat varieties in resistance to fungal diseases. It was found that 60.0 % of introgression lines showed a high level of resistance to leaf rust, 56.7 % to septoria, and 36.7 % to powdery mildew. The highest number of highly resistant to powdery mildew and septoria genotypes were noted among the lines created on the base of *T. dicoccoides* (71.4 % and 85.7 %, respectively). Resistance to leaf rust (80 %) was common among lines with *T. dicoccum* genetic material. A significant statistical difference in the degree of damage by powdery mildew and septoria was shown between the groups of lines created using the species *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. kiharae*, and under different weather conditions. A total of 26.7 % of introgression lines demonstrated resistant to two diseases (lines 11-1, 13-3, 15-7-2, 16-5, 34-1, 34-2, 183/2-2, 184/1-6), 23.3 % — to three infections (lines 29, 8, 15-7-1, 1-3, 2-7, 19, 25-2). These lines are of interest in breeding for fungal disease resistance.

Keywords: genus *Triticum*, introgression lines, powdery mildew, septoria, leaf rust

Powdery mildew (pathogen *Blumeria graminis* (DC.) Speer f. sp. *tritici* March.), septoria (pathogen *Zymoseptoria tritici* (Desm.) Quaedvl. & Crous), and brown (leaf) rust (pathogen *Puccinia triticina* Erikss.) are the most common diseases of the aboveground organs of common wheat (*Triticum aestivum* L.). Their harmfulness is manifested mainly in a decrease in the leaf surface area (and, as a consequence, in the productivity of photosynthesis) and in a violation of the water balance, which causes premature death of the leaf apparatus, a decrease in grain yield, and a deterioration in its quality. Not only leaves are affected but also leafy sheaths, stems, and even an spike in years favorable for the development of powdery mildew [1].

Infection of plants with fungal pathogens is possible in a wide temperature range. For example, urediniospores of *P. triticina* and conidia of the fungus *B. graminis* germinate at 2.5–31 °C in the presence of drip moisture. Conidia of septoria pathogens spread mainly with raindrops and begin to germinate at temperatures from 5 to 35 °C [1]. Crop losses due to infection with these pathogens are usually 15–35%, but with severe epiphytotics, they can reach 40–80% [2–4].

The most effective method to control fungal wheat diseases has become the creation of varieties with long-term resistance to pathogens. Currently, genes are known that control resistance to powdery mildew (more than 90 *Pm* genes), leaf rust (more than 75 *Lr* genes), and septoria blight (more than 15 *Stb* genes) [5]. It should be borne in mind that the effectiveness of known resistance genes decreases over time, and new virulent biotypes of pathogens appear that can cause significant damage to crops of cultivated cereals [6, 7]. In this regard, the search for new genes for resistance to leaf-stem infections is an urgent task in wheat breeding.

It is known that wild and cultivated relatives of *Triticum aestivum* are a promising source of enlargement of the genetic diversity of modern cultivars by resistance loci [8–10]. In order to enrich and improve the common wheat gene pool, species of the *Triticum* genus (*T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. kiharae*) were involved in crosses with *T. aestivum* varieties. Previously, the authors analyzed the nature of foreign introgression in the obtained lines using SSR markers [11] and assessed their cytological stability in comparison with the parental forms [12].

In this paper, on the basis of long-term monitoring under conditions of the natural infectious background of the Republic of Belarus, for the first time, differences in the degree of infection by fungal pathogens of wheat lines created using the species *Triticum dicoccoides*, *T. dicoccum*, *T. durum*, *T. kiharae* were shown.

The research goal was to study the resistance to powdery mildew, septoria blight, and leaf rust of common wheat lines with introgression of the genetic material of the genus *Triticum* against a natural infectious background.

Materials and methods. The study included 30 introgressive lines obtained at the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus from crossing the varieties of common wheat Rassvet, Saratovskaya 29, Festivalnaya, Chinese Spring (CS), Belorusskaya 80, Pitic S62 with samples of tetraploid species *T. dicoccoides*, *T. dicoccum*, *T. durum* (AABB, $2n = 28$) and a hexaploid artificially synthesized species *T. kiharae* (A¹A¹GGDD, $2n = 42$). Samples of foreign donors were obtained from the All-Union Research Institute of Plant Breeding collection (VIR) (St. Petersburg, Russia), information on the genealogy of individual samples has not been preserved (the VIR catalog numbers are not indicated). Of the 30 lines studied, 12 were created with the participation of the *T. durum* sample (CS × *T. durum* — lines 183/2-2, 184/1-6; *T.*

durum × CS – 190/4-1, 190/5-3, 190/6-1, 191/6-3, 195-3, 196-1, 202-2, 200-3; *T. durum* × Belorusskaya 80 – 221-1 and 226-7), 7 lines – with the participation of *T. dicoccoides* (Rassvet × *T. dicoccoides* k-5199 – line 29; Saratovskaya 29 × *T. dicoccoides* – line 8; *T. dicoccoides* × Festivalnaya – lines 11-1, 13-3, 15-7-1, 15-7-2, 16-5), 6 lines – with *T. kiharae* (*T. kiharae* × Saratovskaya 29 – lines 19, 20-1, 25-2; *T. kiharae* × Festivalnaya – 28, 34-1, 34-2) and 5 lines – with *T. dicoccum* (*T. dicoccum* k-45926 × Festivalnaya – lines 1-3, 2-7; Pitic S62 × *T. dicoccum* k-45926 – 206-2, 208-3, 213-1).

The resistance of hybrid wheat lines, their parental forms, and the susceptible variety of spring bread wheat Thatcher to powdery mildew, septoria blight, and leaf rust was assessed in a natural infectious background on the experimental fields of the Institute of Genetics and Cytology of the National Academy of Sciences of Belarus in 2012, 2014-2016, 2018, 2019 according to the scale of Geshele. To describe the weather conditions, the authors used the values of meteorological parameters for April-August, provided by the website "Reliable Prognosis" (<http://rp5.by>). The authors used the extent of flag leaf damage in the milky-wax ripeness phase as an indicator of resistance: 0-5% – highly resistant plants, 5-10% – resistant, 10-15% – medium resistant, 15-25% – medium susceptible, 25-40% – susceptible, more than 40% are highly susceptible [13].

The Statistica 10.0 software package (StatSoft, Inc., USA) was used for statistical data processing. Significant differences between the means were tested using the Kruskal-Wallis test (non-parametric statistics module). To assess the degree of resistance, box-and-whiskers diagrams were used, categorized according to the year of experiment and the pathogen being investigated. Differences in resistance to fungal pathogens between groups of lines under conditions of a natural infectious background of 6 field seasons were determined using two-way analysis of variance. Histograms were built using Microsoft Excel.

Results. During the observation period, the infection of the susceptible cultivar Thatcher by the pathogen *B. graminis* was 40-60%, and that of *Z. tritici* was 15-25%. *P. triticina*, which causes leaf rust, was identified only in 2012 and 2014 (50% degree of damage of the susceptible controls). The maximum degree of damage by powdery mildew of the Thatcher variety was noted in 2012 – 60%, in other years it was 40%. The greatest septoria infection (25%) was also observed in 2012.

In 2012 and 2014, the most favorable weather conditions developed for the development of fungal diseases. The growing season of spring bread wheat was characterized by increased air temperatures, except for June, when the indicator was lower than the average long-term values. In both years, heavy rainfall was observed in June and August. The highest values of the hydrothermal coefficient (HTC) for 6 years were noted for the growing seasons of 2012 and 2014 – 2.2 and 1.6, respectively. The least favorable conditions for the development of fungal pathogens were formed in 2015: the highest temperature regime was recorded in June-August; the HTC was 0.8, which characterizes the growing season as arid. Especially low HTC values were noted in June (0.2) and August (0.1). In 2016 and 2018, the average monthly temperatures throughout the growing season were higher than the average annual values. In May, June, and August, the amount of precipitation was slightly lower, and in July – much higher than the average annual. The HTC was 1.5 in 2016 and 1.3 in 2018. In June 2019, an excess of air temperature and a lack of precipitation were observed in comparison with the norm. In July-August, the average monthly temperature was below optimal values and heavy rainfall fell, the HTC was 1.2.

Analysis of variance showed significant differences in the manifestation of fungal diseases in the studied wheat genotypes over 6 seasons ($p < 0.01$).

When assessing the resistance to leaf rust, only the degree of damage to the leaf blade in 2012 and 2014, when the pathogen was detected in the field, was analyzed. On average, for all genotypes, the highest sensitivity to *B. graminis* and *P. tritricina* was noted in 2014, to *Z. tritici* – in 2012, when weather conditions contributed to the intensive development of diseases (Fig. 1).

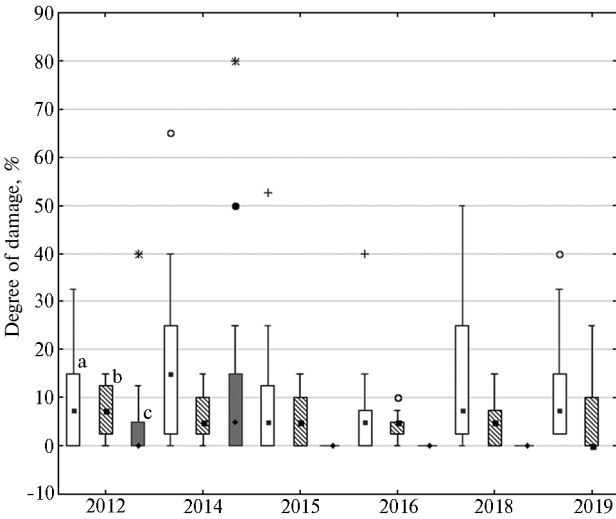


Fig. 1. The degree of damage by powdery mildew (*Blumeria graminis*) (a), septoria (*Zymoseptoria tritici*) (b), and brown rust (*Puccinia tritricina*) (c) to lines of spring bread wheat with introgression of genetic material of *Triticum dicoccoides*, *T. dicoccum*, *T. durum*, and *T. kiharae* during six field seasons: O, ● – spikes, +, * – extremes.

For powdery mildew, KW-H(5;234) = 17.93, $p = 0.003$; for septoria, KW-H(5;234) = 101.94, $p = 0.000$; for brown rust, KW-H(5;234) = 26.37, $p = 0.000$, where KW-H is the Kruskal-Wallis test, p is the significance level (the experimental field of the Institute of Genetics and Cytology NASB).

In 2014, in some samples, the incidence of powdery mildew exceeded 60% (lines 206-2, 213-1, 190/5-3), of brown rust 80% (line 206-2), which was significantly higher than in other years of field experiments. The smallest number of genotypes with high resistance to powdery mildew (27.8%) and leaf rust (44.4%) was also noted. Throughout the entire observation period, the number of genotypes highly resistant to septoria was 39.4-60.5%, their minimum number was detected in 2012.

1. Degree of damage (%) to parental forms of spring bread wheat introgressive lines caused by powdery mildew (*Blumeria graminis*), septoria (*Zymoseptoria tritici*), and brown rust (*Puccinia tritricina*) (the experimental field of the Institute of Genetics and Cytology NASB, 2012, 2014-2016, 2018, 2019)

Genotype	Average/maximum value		
	powdery mildew	septoria	brown rust
<i>Triticum aestivum</i> varieties			
Rassvet	0/5	5/10	15
Saratovskaya 29	15/40	10	15
Festivalnaya	15/25	15	0/5
Chinese spring	25/40	15	15
Belorusskaya 80	15/25	15/25	15
Pitic S62	25	15	10
Samples of genus <i>Triticum</i>			
<i>T. dicoccum</i> k-45926	0	0	0
<i>T. dicoccoides</i>	0	0-5/5	0
<i>T. dicoccoides</i> k-5199	0	0-5	0
<i>T. kiharae</i>	5/10	0	0

The damage to parental *T. aestivum* cultivars with brown rust did not exceed 15% (Table 1). The greatest sensitivity to septoria blight was shown by the cultivar Belorusskaya 80, the degree of damage of which reached 25% in the years most favorable for the development of the pathogen. The parental wheat varieties were susceptible to powdery mildew: in some years, the flag leaf infestation was 25-40%. The only exception was the Rassvet variety, which demonstrated high resistance to *B. graminis* (see Table 1). The species of the genus *Triticum* were characterized by immunity to fungal diseases throughout the entire

observation period (see Table 1). It can be noted that synthetic wheat *T. kiharae* was slightly affected by powdery mildew.

On average, for all the years of research, resistance to septoria was noted for lines with foreign genetic material: 56.7% of genotypes were highly resistant, 33.3% were resistant, and 10% were medium resistant. Among the lines with the genetic material of *T. dicoccoides*, highly resistant genotypes were the most numerous — 85.7% (lines 29, 8, 13-3, 15-7-1, 15-7-2, 16-5) (Fig. 2, A). No susceptible lines were identified (see Fig. 3, A).

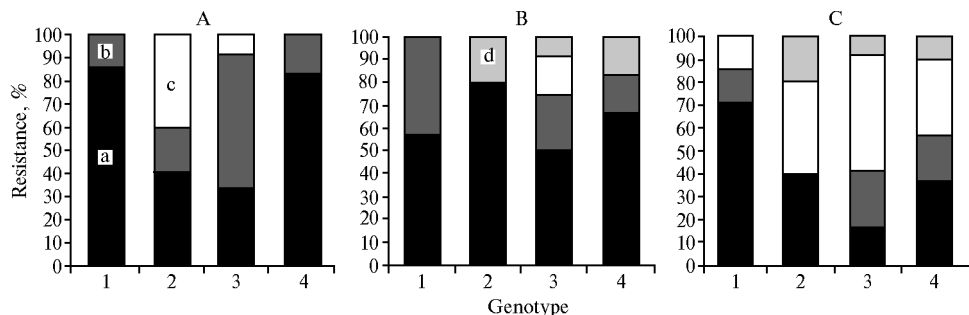


Fig. 2. Distribution of spring bread wheat lines with introgression of genetic material of *Triticum dicoccoides* (1), *T. dicoccum* (2), *T. durum* (3), and *T. kiharae* (4) by resistance to septoria (*Zymoseptoria tritici*) (A), brown rust (*Puccinia tritricina*) (B), and powdery mildew (*Blumeria graminis*) (C): a — highly resistant, b — resistant, c — medium resistant, d — susceptible (the experimental field of the Institute of Genetics and Cytology NASB, 2012, 2014–2016, 2018, 2019).

All the studied lines exceeded the parental wheat varieties in resistance to the Belarusian population of Septoria blight, which suggests the presence of genes that determine resistance to Septoria blight in foreign genetic material transferred into the common wheat genome. This fact is of great interest, since in recent years, there has been an expansion of the area of Septoria blight of spring wheat and an increase in the disease, and the resistance reserve of cultivated varieties is almost exhausted [14]. Currently, genes for resistance to this pathogen are predominantly mapped in the genome of soft and durum wheat [15]. Wild species as donors of genes for resistance to Septoria blight are not sufficiently involved in wheat breeding.

Introgressive lines showed a certain degree of resistance to leaf rust in 2012 and 2014: 60.0% were highly resistant, 23.3% were resistant, 6.7% were moderately resistant, and 10.0% were susceptible. Of the 30 studied hybrid genotypes, only 3 were susceptible, i.e., lines 226-7 (*T. durum* × Belorusskaya 80), 206-2 (Pitic S62 × *T. dicoccum*) and 20-1 (*T. kiharae* × Saratovskaya 29). In these genotypes, the sensitivity to the leaf rust pathogen was even higher than in both parental forms. Possibly, genes for resistance to leaf rust, which are effective in the genome of tetraploid wheat, lose their protective properties upon introgression into the genome of a hexaploid species [16].

Among the lines created with the participation of *T. dicoccum*, the largest number of highly resistant ones (80%) was found. The lines *T. kiharae*/*T. aestivum* were also characterized by a high value for this indicator and were distributed in terms of resistance to leaf rust as follows: 66.6% were highly resistant, 16.7% were resistant, and 16.7% were susceptible (see Fig. 2, B). Only highly resistant and resistant lines were isolated in cross combinations based on *T. dicoccoides* (see Fig. 2, B). It should be noted that lines 29 (Rassvet × *T. dicoccoides* k-5199) and 8 (Saratovskaya 29 × *T. dicoccoides*) showed high resistance to *P. tritricina*, despite the fact that the disease incidence of parental wheat varieties was 15%.

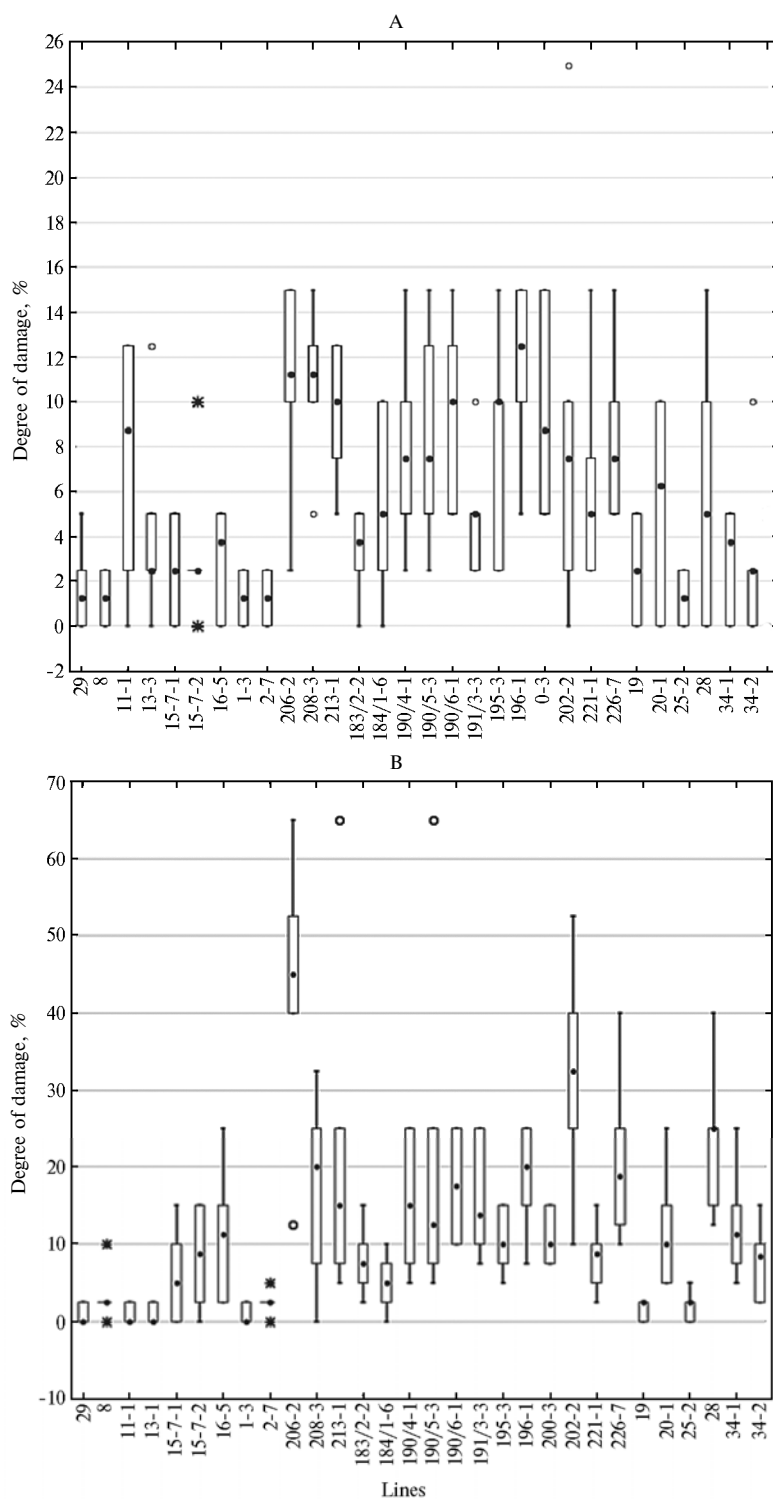


Fig. 3. Degree of damage (%) by septoria (*Zymoseptoria tritici*) (A) and powdery mildew (*Blumeria graminis*) (B) infection in lines of spring bread wheat with introgression of genetic material from *Triticum dicoccoides*, *T. dicoccum*, *T. durum*, and *T. kiharae*: ● — median, □ — 25-75%, I — maximum/minimum values, ○ — spikes, * — extremes. For septoria, KW-H(29;180) = 86.57, $p = 0.00$; for powdery mildew, KW-H(29;180) = 120.75, $p = 0.0000$, where KW-H is the Kruskal-Wallis test, p is the significance level (the experimental field of the Institute of Genetics and Cytology NASB, 2012, 2014–2016, 2018, 2019).

Species of the genera *Triticum* and *Aegilops* are often used as sources of new genes for resistance to leaf rust [17-19], since most of the *Lr* genes identified to date are alien [5]. For example, among the introgressive lines of spring wheat with high genetic diversity in resistance to this disease studied by Russian scientists, there were carriers of *Lr* genes transmitted from *T. durum*, *T. persicum*, *T. timopheevi*, *Aegilops tauschii*, *T. kiharae* [20].

Introgressive wheat lines were found to be the most susceptible to powdery mildew. In three samples out of 30 (10.0%), the damage to the leaf blade exceeded 25%. The line 206-2 (Pitic S62 × *T. dicoccum*) had the highest sensitivity: on average, over the entire study period, the disease incidence was more than 40%, and in some years, it reached 65% (see Fig. 3, B). Susceptibility to powdery mildew was also shown by lines 202-2 (*T. durum* × CS) and 28 (*T. kiharae* × Festivalnaya). The maximum disease incidence for these genotypes was 40-50% and, as a rule, only in 2014. In the remaining years of observation, the indicator was 10-25% (see Fig. 3, B).

Unequal resistance to powdery mildew was shown by 27 lines: highly resistant genotypes accounted for 36.7%, 20.0% were resistant, 33.3% were medium resistant. The resistant genotypes could be attributed to the lines with the genetic material of *T. dicoccoides*. i.e., 71.4% of the lines showed a highly resistant type (see Fig. 2, C). All lines created with the participation of this genus species exceeded the parental wheat varieties in resistance to powdery mildew. Lines 29, 11-1, and 13-3 should be particularly highlighted, which, as a rule, were not affected by the pathogen throughout the entire study period. According to the literature, the wild emmer *T. dicoccoides* is one of the most immune to leaf-stem infections among the common wheat related species. It serves as a source of valuable genes for resistance to powdery mildew, which are expressed both at the seedling stage (*Pm26*, *Pm42*, and *Pm49*) and in adult plants (*Pm16*, *Pm30*, *Pm31*, *Pm36*, *Pm41*, *Pm42*, *Pm49*, and *Pm50*) [21, 22]. More than 90% of *T. dicoccoides* samples from the VIR world collection have field resistance to powdery mildew [23].

2. Two-way analysis of variance for resistance to leaf-stem infections in lines of spring common wheat with introgression of genetic material from *Triticum dicoccoides*, *T. dicoccum*, *T. durum*, and *T. kiharae* (the experimental field of the Institute of Genetics and Cytology NASB, 2012, 2014-2016, 2018, 2019)

Trait	Factor	df	SS	MS	F
Resistance to powdery mildew (<i>Blumeria graminis</i>)	Genotype of the species of the genus <i>Triticum</i>	3	4175.65	1391.88**	9.90
	Weather conditions	5	2539.03	507.81**	3.61
	Genotype of the species of the genus <i>Triticum</i> × weather conditions	15	1405.61	93.71	0.67
	Error	156	21937.08	140.62	
Resistance to septoria (<i>Zymoseptoria tritici</i>)	Genotype of the species of the genus <i>Triticum</i>	3	737.33	245.78**	12.68
	Weather conditions	5	278.63	55.73*	2.88
	Genotype of the species of the genus <i>Triticum</i> × weather conditions	15	313.60	20.91	1.08
	Error	156	3022.62	19.38	
Resistance to brown rust (<i>Puccinia triticea</i>)	Genotype of the species of the genus <i>Triticum</i>	3	96.32	32.11	0.36
	Weather conditions	5	3707.39	741.48**	8.30
	Genotype of the species of the genus <i>Triticum</i> × weather conditions	15	457.16	30.48	0.34
	Error	156	13939.23	89.35	

Note. df — number of degrees of freedom, SS — sum of squares, MS — mean square, F — Fisher's test.

* and ** Statistically significant at $p < 0.05$ and $p < 0.001$, respectively.

The *T. kiharae*/*T. aestivum* lines were characterized by varying degrees of resistance to powdery mildew, i.e., 36.7% lines were highly resistant, 20.0% resistant, 33.3% moderately resistant. The lowest degree of resistance to powdery mildew was found for lines from combinations of crosses with the participation of *T. durum* (see Fig. 2, C): highly resistant genotypes accounted for only 16.7%

(lines 183/2-2 and 184/1-6). Introgressed resistance genes are increasingly being used to protect wheat varieties from powdery mildew. At present, it is known that 26 resistance genes from various species of the genus *Triticum*, 11 from *Aegilops* ssp., 5 from *Secale cereale* L., 6 from *Dasyphyrum villosum* (L.) Borbas, *Thinopyrum ponticum* (Popd.) Z.-W. Lin & R.-C. Wang, *Thinopyrum intermedium* (Host) Barkworth & DR Dewey, and *Agropyron cristatum* (L.) Gaertn. are introduced into the wheat genome [24].

A significant statistical difference in the degree of damage by powdery mildew and septoria was shown between the groups of lines created using the species *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. kiharae*, and under different weather conditions (Table 2).

When creating donors of genes for immunity to fungal diseases, samples with complex resistance to several pathogens are of particular value. Close relatives of wheat are often resistant to many fungal diseases. For example, at the Research Institute of Agriculture of the South-East (Saratov, Russia), more than 250 constant lines were created from the crossing of the wheat varieties Saratovskaya 29, Saratovskaya 55, and Saratovskaya 58 with *T. monococcum*, *T. dicoccum*, *T. timopheevii*, *T. persicum*, *T. militinae*. Of these, 178 lines with group resistance to diseases were selected against the background of strong natural epiphytotics and with artificial infection in a greenhouse [25]. Work on the creation of new varieties of wheat with complex resistance to stress factors of the environment by attracting wild relatives and other cultivated cereals is also carried out at the Lukyanenko Krasnodar Research Institute of Agriculture [9] and at the Siberian Research Institute of Agriculture [26].

In this study, 26.7% of introgressive lines (lines 11-1, 13-3, 15-7-2, 16-5, 34-1, 34-2, 183/2-2, 184/1-6) showed resistance to two diseases simultaneously, 23.3% (lines 29, 8, 15-7-1, 1-3, 2-7, 19, 25-2) to three diseases. The lines created with the participation of *T. dicoccoides* were characterized by group resistance. High resistance to three pathogens was found for lines 29, 8 and 15-7-1, to two – for lines 11-1, 13-3, 15-7-2, 16-5. The wild emmer *T. dicoccoides* is considered one of the ancestors of common wheat and is the source of genes for resistance to many diseases. Some genes for resistance to powdery mildew (*Pm16*, *Pm26*, *Pm30*, *Pm36*, *Pm41*), brown and yellow rust (*Lr53*, *Yr15*, *Yr35*, *Yr36*) have been mapped in *T. dicoccoides* using molecular markers [21, 27]. Currently, only a small part of these genes is introgressed into the genome of cultivated wheat. Also, group resistance to three pathogens was exhibited by two lines with *T. kiharae* genetic material (19 and 25-2) and two lines with *T. dicoccum* genetic material (1-3 and 2-7). It is known that *T. kiharae* isolated at VIR from the amphidiploid of *T. timopheevii* × *Ae. taushii* combines a complex of traits of both parental species, including resistance to most diseases and pests [28]. *T. dicoccum* (cultivated emmer) has been cultivated by humans since ancient times, and at present, its popularity is growing due to its unpretentiousness to growing conditions and valuable nutritional properties of grain [29]. The cultivated emmer is also of interest as a donor of genes for resistance to biotic and abiotic stresses. Thus, as a result of crossing with *T. dicoccum*, Russian scientists obtained 43 lines of spring bread wheat resistant to leaf rust and powdery mildew. With the participation of *T. dicoccum* and *T. persicum* species, 30 *T. aestivum* lines with complex resistance were obtained [30].

For lines with introgression of *T. durum* genetic material, high resistance was noted to only two pathogens, they are lines 183/2-2 and 184/1-6 (*CS* × *T. durum*), 221-1 (*T. durum* × Belorusskaya 80). In general, the lines created with the participation of this tetraploid species were characterized by the highest susceptibility to leaf-stem infections. A particularly high degree of damage was noted for

lines 202-2 (*T. durum* × CS) and 226-7 (*T. durum* × Belorusskaya 80). However, the influence of the variety should not be excluded either, since CS and Belorusskaya 80 were characterized by the highest susceptibility to fungal pathogens among the parent varieties of common wheat (see Table 1). The role of the recipient cultivar in the manifestation of resistance was also traced among the lines created with the participation of *T. dicoccoides*, *T. dicoccum*, and *T. kiharae*. Thus, for genotypes with genetic material *T. dicoccoides* and *T. kiharae* obtained on the basis of cultivars Rassvet and Saratovskaya 29, a higher resistance to powdery mildew and septoria was revealed than when using the Festivalnaya cultivar.

In addition to the fact that wheat varieties are characterized by unequal resistance to leaf-stem infections, they can affect the creation of distant hybrids: according to the literature, the fertility of hybrids, cytological stabilization, and the nature of recombination events in the hybrid genome depend on the parental wheat variety [31, 32]. All these processes lead to the creation of lines with different numbers, lengths, and localizations of fragments of foreign genetic material and, as a consequence, with different characters. In the works of a number of researchers, it was shown that the spectrum of substitutions and translocations was significantly different in introgressive lines resistant and susceptible to leaf rust and powdery mildew [33, 34]. It is obvious that the differences in resistance to fungal diseases revealed in the lines analyzed by the authors are also due to recombination events with the participation of chromosomes of wheat related species that occurred during the stabilization of hybrid genomes. The results of the authors' earlier assessment of the genetic diversity of the collection of introgression wheat lines using C-banding and SSR analysis methods indicate that most of the lines differ in both the number and localization of foreign fragments. It was found that introgression of foreign genetic material into the *T. aestivum* genome occurred both in the form of small regions and in the form of whole chromosomes (intergenomic substitutions) and their fragments (centric and terminal translocations). The highest frequency of introgression is shown for chromosomes 1A, 1B, 2A, 5B [11, 35]. In order to localize genetic factors that have a positive effect on resistance to fungal pathogens, a detailed analysis will be carried out in the future.

Thus, the lines of spring bread wheat containing the genetic material of *Triticum dicoccum*, *T. dicoccoides*, *T. durum*, and *T. kiharae* showed high resistance to brown rust (60.0% of lines), septoria (56.7% of lines), and powdery dew (36.7% of lines) against a natural infectious background in the Republic of Belarus. All studied lines, regardless of weather conditions, exceeded the parental varieties in resistance to the Belarusian population of the septoria pathogen, which suggests the presence of genes that determine resistance to septoria blight in the alien genetic material of the hybrid wheat genome. A significant statistical difference in the degree of damage by powdery mildew and septoria was shown between the groups of lines created using the species *T. dicoccoides*, *T. dicoccum*, *T. durum*, *T. kiharae*, and under different weather conditions. The largest number of genotypes highly resistant to powdery mildew and Septoria blight (71.4 and 85.7%, respectively) was found among the lines created with the participation of *T. dicoccoides*. Combined resistance to two diseases occurred in 26.7% of the studied introgression lines, to three diseases in 23.3%. These lines are of interest for breeding for resistance to fungal diseases.

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MOBILITY OF SILICON, FERTILITY OF SOD-PODZOLIC SOIL, BIOACCUMULATION OF SILICON AND YIELDS OF AGRICULTURAL CROPS UNDER THE INFLUENCE OF ZEOLITE

A.V. KOZLOV¹ ✉, A.H. KULIKOVA², I.P. UROMOVA¹

¹Minin Nizhny Novgorod State Pedagogical University, 1, ul. Ulyanova, Nizhny Novgorod, 603950 Russia, e-mail a_v_kozlov@mail.ru (corresponding author ✉), uromova2012@yandex.ru;

²Stolypin Ulyanovsk State Agricultural University, 1, bulv. Noviy Venets, Ulyanovsk, 432017 Russia, e-mail agroec@yandex.ru

ORCID:

Kozlov A.V. orcid.org/0000-0003-3034-6566

Uromova I.P. orcid.org/0000-0003-1000-3603

Kulikova A.H. orcid.org/0000-0002-7327-3742

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Abstract

In modern arable farming, beneficial properties of silicon-containing natural materials are of interest, including zeolites which have a structuring and moisture-retaining effect on soil, can optimize conditions for development of agronomically valuable microorganisms, replenish soil solution with available forms of potassium, phosphorus and trace elements, and show activity towards excessive acidity of soils. The provision of silicon makes it possible to increase adaptation of crops to stresses caused by agroecotope factors, leading to an increase in productivity and yield quality characteristics. For the first time, in the conditions of sod-podzolic soils of the Nizhny Novgorod region, we established the beneficial influence of various doses of zeolite rock of the Khotynets deposit on the main properties of effective fertility of sod-podzolic light loamy soil and content of biologically active silicon in it. The impact of the rock on accumulation of various silicon compounds in above-ground biomass of crops is evaluated. Increase of their yield due to zeolite action was revealed and optimization of quality indices of the main part of the crop was established. The purpose of the work was to determine mobility of silicon in sod-podzolic light loamy soil, to evaluate its physicochemical and agrochemical properties, and to identify patterns of bioaccumulation of various silicon compounds by above-ground parts of plants depending on the dose of zeolite rock as a high-silicon reclamation material. Studies carried out in 2015-2017 involved crop varieties zoned in the Volgav'yatka region, the winter wheat (*Triticum aestivum* L.) cv. Moscovskaya 39 and spring wheat cv. Kurskaya 2038, winter rye (*Secale cereale* L.) cv. Valdai, barley (*Hordeum vulgare* L.) cv. Veles, peas sown (*Pisum sativum* L.) cv. Chishminsky 95 and potato (*Solanum tuberosum* L.) cv. Red Scarlet. The design of the experiment included control (no treatment) and incorporation of 3, 6, and 12 t/ha zeolite of the Khotynetsky deposit (OOO Alsiko-Resurs, Russia) into the soil. The rock was introduced into soil once, manually, in the summer period of 2014. The soil of the field is a sod-podzolic medium-sod shallow-depressed ungelled light-ugly, formed on a cover loam. Plants were harvested upon complete ripeness (grain crops), the beginning (peas) and the end (potatoes) of drying of the tops. In above-ground plant biomass of all crops, the contents of organic, soluble mineral, insoluble polymer and general silicon compounds were determined. Soil was samples on harvest day from five points of each plot by envelope method and the content of mobile silicon compounds was evaluated. Also, in soil samples, the actual, exchange acidity, hydrolytic acidity, the content of exchange compounds of calcium and magnesium, the amount of exchange forms of potassium, the content of mobile phosphorus compounds according to Kirsanov and humus according to Tyurin were measures. It was shown that the use of 12 t/ha zeolite rock contributes to an increase in the content of water-soluble forms of silicon in the soil by 143 % ($p < 0.05$), acid-soluble forms by 2 times. The use of reclamation doses of zeolite for three years contributed to a reliable ($p < 0.05$) decrease of exchange soil acidity by 0.5 pH, hydrolytic acidity by 0.33 mg-equiv/100 g, significant increase of content of exchange compounds of calcium and magnesium (by 4.4 and 10.8 mg-equiv/100 g, respectively). In addition, there was a statistically significant ($p < 0.05$) increase in the amount of mobile phosphorus compounds (by 43 %) and potassium (by 46 %) vs. the control values. The accumulation of silicon in plant biomass depended on a crop, and in all tested cereals, it was higher in by-products than in the main yield. The use of zeolite led to an increase in absorption of silicon from soil, especially in

silicon accumulators. Under the action of material, in grain of spring wheat, barley and peas accumulation of total silicon exceeded control values 1.8-, 2.3-, and 3.6-fold, respectively ($p < 0.05$). The use of zeolite activated generation of organic and mineral soluble forms of element in the grain part of crop, but did not contribute to the accumulation of insoluble silicon compounds in the plant biomass. The main yield of winter wheat increased by 0.19 t/ha, of barley by 0.98 t/ha, of sown peas by 0.24 t/ha, of potatoes by 8.6 t/ha, of spring wheat by 0.92 t/ha, and of winter rye by 0.39 t/ha ($p < 0.05$). Doses of 6 and 12 t/ha of zeolite had best effect on all crops, while the ratio of main and by-products narrowed towards grain (tuberous) part of crop. Optimization of silicon nutrition of cultivated plants and mobility of element in soil due to application of high doses of zeolite had a positive effect on quality of the main yield. The accumulation of raw gluten in grain of winter and spring wheat reached 35.3 and 31.1 %, respectively, and the grain levels of protein in barley and peas was 12.7 and 20.6 %, respectively. Improved quality of potato tubers under the influence of the zeolite was expressed in enrichment with vitamin C (up to 22.2 mg%) and higher accumulation of starch (up to 16.3 %). Consequently, use of zeolite as a fertilizer and reclamation material was agronomically feasible and necessary under conditions of sod-podzolic soils.

Keywords: silicon, zeolite, crops, efficiency and quality of harvest, bioaccumulation of silicon in biomass, contents and mobility of silicon in soil

The crop yields largely depends on the availability of mobile forms of macro and microelements, the agrophysical properties of the arable horizon, as well as on the acid-base state of the soil solution [1, 2]. In addition, there are some external dynamic factors of the agroecotope that potentially affect the realization of the agrobiological potential of any crop. These include the dynamics of free water content in the root layer of the soil, the presence of easily soluble salts, aluminum compounds, and other phytotoxicants, the phytosanitary background, and the temperature conditions of the area [3, 4]. Increasing the adaptive potential of crops to such environmental parameters is one of the priority tasks in optimizing the functioning of agroecosystems [5, 6].

In the conditions of modern agriculture, it is necessary to search for effective and environmentally acceptable ways to increase the productivity of cultivated plants, which will ensure the maintenance of effective soil fertility, its acid-base regime, harmonious activation of saprotrophic and nitrogen-fixing associations of microorganisms, and also have a prolonged effect. Such methods include the use of natural high-silicon rocks of various genesis as fertilizers and ameliorants [7-9]. These materials contain a significant amount of nutrients and reclamation components available for plants, including phosphorus, potassium, and silicon compounds, as well as a calcium-magnesium ion exchange complex [10-12].

Silicon is one of the main elements assimilated by the plant organism but its participation in biochemical and physiological processes remains poorly understood [13-15]. It has now been established that silicon compounds are involved in the thickening of epidermal tissue cells, stimulate above-ground growth and root system activity, and increase the overall resistance of the plant organism to abiotic stresses (environmental and salt factors, loss of turgor from drying out and overheating), disease and pest damage [16-18].

Plants absorb silicon from the soil solution in the form of ions (SiO_3^{2-} and SiO_4^{4-}) and monosilicic acids (H_2SiO_3 and H_4SiO_4), after which these compounds are converted to silica gel ($\text{SiO}_2 \cdot n\text{H}_2\text{O}$) in the cell fluid. Then, a biochemical binding to the polymer components of the cell (polypeptides, proteins, and complex carbohydrates), accumulation in the cell walls and the round tissues (the epidermis of leaves and roots), as well as accumulation in phytoliths, occur. It was revealed that the double cuticular layer, which is a silicon cellulose membrane, was formed during the formation of the ground tissues of the plant [19, 20].

It was found [21, 22] that silicon in the plant cell was represented by orthosilicic esters of proteins, lipids, and phospholipids, simple amino acids, hy-

droxy-amino acids, polysaccharides, and lignin. The amount of silicon bound in organic structures is at least 40% of its total content. As a rule, the predominant form of organically bound silicon is polymer, its amount reaches 80-85%. In particular, in grain crops, up to 60% of silicon is associated with polypeptides and proteins, more than 11% with lipids and phospholipids, about 9% with fiber and hemicellulose, no more than 5% with pectin, less than 3% with lignin.

It is assumed that the essential compounds of silicon, which form bonds in the cell walls of plants with polysaccharides (pectin and fiber), as well as with proteins and lignin, form thermally insulating structures and, as a result, determine the frost resistance of plants, optimize overwintering, and accelerate the spring acclimatization of winter and perennial crops [23-25]. In the structure of fiber and hemicellulose, silicon acts as a crosslinking agent, by forming siloxane bonds (linkages) between sugar residues [26, 27]. Due to the formation of such bonds, the strength of the straw of cereal crops increases, as well as the resistance of grain crops to lodging and arid conditions of the area [28-29]. Some authors explain this phenomenon by an increase in the content of total and organically bound silicon in plant phytomass during vegetation [21, 30, 31].

Polymer substances containing silicon serve as components of the coronary cells of the root cap and mucus secreted by the root fibrils. Recent studies confirm that the external optimization of plant silicon nutrition contributes to an increase in the accumulation of root biomass, their branching, total and adsorbing working surface, and also improves root respiration of plants [32-34]. The use of silicon-containing fertilizers and silatrane growth stimulators in agrocenoses increases the overall adaptive potential of crops with increased content of phytotoxic substances in the soil reduces susceptibility to drought and increases resistance to oxidative and salt stress [35-37]. In addition, the deficiency-free balance of mobile silicon in soils normalizes the production of cytokinins and activates the formation of phytoalexins in plant cells [17, 18], which contributes to their natural resistance to eelworms, hyphae of phytopathogenic fungi, physiological secretions of bacteria, and the action of other root pests [38-40]. These facts can determine a more effective implementation of the agrobiological potential of the crop and its cultivar in specific soil and climatic conditions under the influence of the factors under consideration.

In general, it should be noted that other authors [41-43] note the multiple positive effects of silicon and its compounds in the soil-plant system. However, there is insufficient information in the scientific literature about the reserves of mobile silicon in soils, the reclamation effect of high-silicon rocks concerning excessive soil acidity, as well as about the features of the bioaccumulation of silicon in cultivated plants [44, 45]. In particular, there is very little data on the influence of reclamation high-silicon materials on the acid-base balance of soils, which is considered an acute and urgent problem in the conditions of agriculture in Russia.

Currently, acidification of arable lands in Russia has become almost ubiquitous [46], and this phenomenon applies not only to genetically leached and depleted soils, in particular, the soils of the podzolic series of the Non-Chernozem belt of the country [47]. One of the reasons for this situation is the irrational or extremely insufficient use of chemical ameliorants and organic fertilizers, which contribute to the optimization of soil acidity [48]. Some researchers emphasize the relevance of studying highly silicon rocks as an alternative to traditional ameliorants [36, 37, 45]. In addition, the fractional composition of silicon compounds in agricultural plants is one of the least affected aspects of physiology, which is important for the development of ideas about the distribution of these substances in the plant cell and the formation of physiological mechanisms

of adaptation to adverse conditions of the agroecotope. The solution of the above questions is necessary for understanding the mechanisms of silicon participation in the soil-plant system and, as a result, determines the interest in further studying the effects of the use of silicon-containing substances in agroecosystems.

For the first time in the conditions of sod-podzolic soils of the Nizhny Novgorod Region, the authors established the positive effect of various doses of zeolite rock from the Khotynets deposit on the effective fertility of sod-podzolic light-loamy soil and the content of biologically active silicon in it. The influence of the rock on the accumulation of various silicon compounds in the aboveground biomass of crops was characterized. The increase in their yield due to the action of zeolite was shown and the optimization of the quality indicators of the main part of the crop was established.

The work objective is to determine the mobility of silicon in sod-podzolic light-loamy soil, to evaluate its physicochemical and agrochemical properties, as well as to identify the features of bioaccumulation of various silicon compounds by above-ground phytomass of crops, depending on the dose of zeolite rock as a highly silicon reclamation material.

Materials and methods. The research was carried out in 2015–2017 on cultivars of crops zoned across the Volga-Vyatka region [49]: winter wheat (*Triticum aestivum* L.) of the Moskovskaya variety 39 and spring wheat Kurskaya 2038, winter rye (*Secale cereale* L.) Valdai, barley (*Hordeum vulgare* L.) Veles, peas sown (*Pisum sativum* L.) Chishminsky 95, and potatoes (*Solan tuberosum* L.) Red Scarlet. Each year, the results of the study were taken into account for two crops: in 2015, it was winter wheat and potatoes, in 2016, barley and spring wheat, in 2017, peas and winter rye.

Microplot trials with crops were laid in 2014 according to the rules generally accepted in agronomic practice [50] in the field conditions of the enterprise OOO Elitkhoz (Filippovskoye village, Bor municipal district, Nizhny Novgorod Region, 56°31'13.00" N 44°06'57.37"E). Plot (1 m²) allocation in the experiments was randomized (4-fold replication). The experimental design included group without the use of fertilizers and ameliorants (control), as well as the incorporation of zeolite into the soil (3, 6, and 12 t/ha, Z₁, Z₂, and Z₃, respectively). The rock was introduced into the soil once, manually, in the summer of 2014, when the section was divided into working plots.

The zeolite used in the work at the Khotynets deposit (OOO Alsiko-Resurs, Russia) was represented by clinoptilolite for more than 37% of the composition, and also contained more than 15% of opal-cristobalite, about 11% of hydrous micas, 10% of fine-grained quartz, and 8–10% of montmorillonite [51]. The total composition of the zeolite rock contained SiO₂ (56.6%), CaO (13.3%), MgO (1.90%), P₂O₅ (0.23%), K₂O (1.82%), Na₂O (0.23%), SO₃ (0.13%), Al₂O₃ (10.41%), FeO + Fe₂O₃ (3.87%), and other elements.

The soil of the field was a sod-podzolic medium-sod shallow-depressed ungelled light-ugly, formed on a cover loam. At the time of the experiment, the soil was characterized by a medium acid reaction (pH_{KCl} 4.8), hydrolytic acidity (H_g) of 2.83 mg-eq/100 g, an average content of exchange compounds of calcium (5.10 mg-eq/100 g) and magnesium (1.17 mg-eq/100 g), an average degree of saturation with bases (V_s 69%), low content of humus (1.21%), an average supply of mobile compounds of phosphorus (86 mg/kg) and potassium (110 mg/kg) according to Kirsanov, as well as the average deficit in the balance of actual (16 mg/kg) and potential (213 mg/kg) silicon compounds according to Matychenkov.

The plants were harvested in the phase of complete ripeness (grain

crops), as well as in the phase of the beginning (in peas) and the end (in potatoes) of the drying of the tops. The yield (biomass) of the above-ground part of grain crops, tops, and potato tubers was determined in the field on the day of harvest, and the yield (biomass) of grain was determined in the laboratory. In the aboveground phytomass of all cultures, the content of organic, soluble mineral, insoluble polymer, and general silicon compounds was determined; their extraction was carried out according to the method of Kolesnikov [21] with spectrophotometric termination according to the method of Barsukova [52] (PE-5400 VI spectrophotometer, OOO Ekroskhim, Russia). In winter and spring wheat grains, the content of raw gluten was also evaluated by washing according to GOST R 54478-2011 (Moscow, 2012); in barley and pea grains, the amount of protein was determined by wet digestion of the plant mass and protein nitrogen evaporation in the form of ammonia according to the micro-Kjeldahl method followed by conversion to the protein content [52]; in potato tubers, the starch content was determined by polarimetry [52] and the amount of vitamin C was determined fluorimetrically using a FLUORAT-02-5M analyzer (group of companies Lumex, Russia) following FR.1.31.2011.09380 [53].

The soil was selected on the day of harvesting from five points of the plot by the envelope method, prepared for analysis, and the content of mobile silicon compounds (soluble in distilled water and 0.1 n. HCl solution) was determined by spectrophotometric methods of Matychenkov et al. [54] (spectrophotometer PE-5400 VI, OO Ekroskhim, Russia). The actual, exchange acidity was also determined in the soil samples with the potentiometric method (pH meter-millivoltmeter MARK-903, OOO VZOR, Russia), hydrolytic acidity by titrimetry (GOST 26212-91. Moscow, 1992), the content of exchange compounds of calcium and magnesium by trilonometry (GOST 26487-85. Moscow, 1985), the amount of exchange forms of potassium by spectrophotometry (GOST R 54650-2011. Moscow, 2019), the content of mobile phosphorus compounds according to Kirsanov (GOST R 54650-2011), and humus according to Tyurin (GOST 26213-91. Moscow, 1992) by spectrophotometric methods (spectrophotometer PE-5400 VI) [55].

Chemical analyses of soil and plants were performed in 2015-2017. The obtained data were processed by the methods of variance and dispersion analysis [50] in the software package Microsoft Office Excel 2007. The arithmetic means and standard deviations ($M \pm SD$), the coefficient of variation (C_v , %), the error of the sample mean ($S_{x\text{mean}}$, %), the least significant difference (LSD_{05}), and Fischer's test (F_f) were calculated at a statistical significance level of $p < 0.05$ (the theoretical Fischer's criterion F_t at $n_l = 3$ and $p < 0.05$ is 3.86).

Results. The base-exchange complex of the used rock included a significant amount of exchange silicon compounds ($900 \text{ mg-eq}/100 \text{ g SiO}_3^{2-}$), calcium ($480 \text{ Ca}^{2+} \text{ mg-eq}/100 \text{ g}$), and magnesium ($\text{Mg}^{2+} 160 \text{ mg-eq}/100 \text{ g}$), as well as exchange compounds of phosphorus (up to $26 \text{ mg-eq}/100 \text{ g}$) and potassium (up to $25 \text{ mg-eq}/100 \text{ g}$), which determined the nutritional value of the material for agrophytocenoses. The relatively high solubility and content of the exchange forms of the major cations, as well as biogenic elements in the zeolite, determined its compliance with the gradual chemical decomposition in the soil and biochemical destruction by soil-inhabiting microorganisms, which can have a positive effect on the agrochemical and agrophysical properties of the arable layer.

The positive effect of zeolite on the content of mobile silicon compounds in the soil was established in experiments (Table 1). In particular, the accumulation of water-soluble silicon substances (monosilicon acids and silicate anions) significantly ($p < 0.05$) reached a maximum in 2016 (up to $40 \text{ mg}/\text{kg}$ of soil), and the effectiveness of the use of zeolite in 2017, when the increase in the

rate of excess removal of the element by crops varied from 82 to 209%, depending on the dose of the rock.

1. Mobile silicon compounds in sod-podzolic light-loamy soil upon treatment with zeolite from the Khotynets deposit (OOO Elitkhoz, Filippovskoye village, Bor municipal district, Nizhny Novgorod Province)

Treatment	Mobile silicon compounds, mg/kg soil						On average for 3 years
	2015		2016		2017		
	<i>M</i> ±SD	<i>Cv</i> , %	<i>M</i> ±SD	<i>Cv</i> , %	<i>M</i> ±SD	<i>Cv</i> , %	
Water-soluble forms of silicon							
Control	16±2	22	14±1	18	11±1	12	14
Z1, 3 t/ha	22±1	8	25±2	14	20±1	12	22
Z2, 6 t/ha	24±1	2	33±1	7	29±2	12	29
Z3, 12 t/ha	29±1	6	40±1	7	34±2	13	34
<i>F_f</i>	24.68		363.98		41.92		
Acid-soluble forms of silicon							
Control	213±9	8	201±4	4	206±5	5	207
Z1, 3 t/ha	281±12	8	306±3	2	292±5	4	293
Z2, 6 t/ha	330±21	13	399±7	4	376±7	4	368
Z3, 12 t/ha	409±26	13	429±4	2	402±6	3	413
<i>F_f</i>	15.56		371.65		330.94		

N o t e. *F_f* — Fischer's test.

In 2015 and 2016, the use of the minimum dose of zeolite (3 t/ha) statistically significantly ($p < 0.05$) contributed to an increase in the content of easily mobile silicon substances in the soil by 38 and 79%, respectively, relative to the control values, and on average for 3 years, the indicator increased by 57-143%, depending on the dose. The introduction of different doses of the material also had a significant but less expressed effect on acid-soluble forms of silicon. For example, increasing the dose of zeolite by 2 times additionally increased ($p < 0.05$) the content of silicon substances in the soil by 23-47%, depending on the year of the study. In general, the most expressed statistically justified effect was found in 2016, when the indicator increased ($p < 0.05$) by 52-113% from the control value. On average, over the years, the introduction of 12 t/ha of rock into the soil contributed to almost a 2-fold increase ($p < 0.05$) in the content of acid-soluble forms of silicon.

Such regularities were caused by the introduction of a significant amount of water-soluble silicon compounds into the soil in the form of silicates and monomers of silicic acids [56], which was facilitated by a sufficient amount of precipitation actively involved in the dissolution of the rock substance. The weather conditions of 2015 were characterized by an insignificant amount of precipitation, the year itself was generally hotter than the average climatic norms of the region (the hydrothermal coefficient of the HTI in the summer months varied in the range of 0.9-1.0). In 2016, on the contrary, no lack of precipitation was observed, the air temperature fluctuated within the normal range with its slight excess in August (HTI = 1.0-1.1). Weather conditions in 2017 were characterized by abundant precipitation in the spring and the first half of summer, the air temperature was within the average annual norms during the summer season (HTI = 1.1-1.2).

The obtained data are consistent with other reports. In particular, it was reported that when 3 t/ha of amorphous silica was introduced into the soil, the content of water-soluble forms of silicon increased by 7.3 mg/kg (36%) in ordinary chernozem and by 11.9 mg/kg (73%) in chestnut soil [19, 51]. However, the content of acid-soluble silicon compounds in these soils increased less significantly, by 15.5 mg/kg (22%) and 19.7 mg/kg (66%), respectively. The reason for a more significant increase in the content of mobile forms of silicon in sod-podzolic soil could be, on the one hand, low soil buffering, which does not prevent the release of dissolved compounds into the liquid phase, on the

other hand, the average acidity of the topsoil, which actively contributes to the chemical mineralization of the substance of ameliorants and the release of their soluble components into the soil solution [1, 11].

The use of zeolite in reclamation doses led not only to the replenishment of the soil solution with mobile silicon compounds but also to a decrease in soil acidity, as well as to the accumulation of the most important biogenic elements — calcium and magnesium (Table 2).

2. Fertility indicators of the of sod-podzolic light loamy soil upon treatment with zeolite from the Khotynets deposit (OOO Elitkhoz, Filippovskoye village, Bor municipal district, Nizhny Novgorod Province)

Treatment	Soil fertility						On average
	2015		2016		2017		
	<i>M</i> ± <i>SD</i>	<i>Cv</i> , %	<i>M</i> ± <i>SD</i>	<i>Cv</i> , %	<i>M</i> ± <i>SD</i>	<i>Cv</i> , %	
Actual acidity (pH _{H2O}), units							
Control	5.88±0.13	4	5.96±0.07	2	5.92±0.07	2	5.92
Z1, 3 t/ha	6.54±0.10	3	6.66±0.12	4	6.71±0.09	3	6.64
Z2, 6 t/ha	6.97±0.16	4	7.03±0.07	2	7.09±0.02	1	7.03
Z3, 12 t/ha	6.93±0.05	1	6.99±0.12	3	7.04±0.06	2	6.99
<i>F_f</i>	27.81		21.69		128.45		
Exchange acidity (pH _{KCl}), units							
Control	4.81±0.04	2	4.90±0.05	2	4.86±0.09	4	4.86
Z1, 3 t/ha	5.04±0.07	3	5.17±0.01	1	5.27±0.05	2	5.16
Z2, 6 t/ha	5.21±0.14	5	5.31±0.02	1	5.49±0.11	4	5.34
Z3, 12 t/ha	5.16±0.13	5	5.28±0.03	1	5.36±0.08	3	5.27
<i>F_f</i>	2.65		43.76		10.18		
Hydrolytic acidity (H _h), mg-eq/100 g							
Control	2.84±0.02	1	2.75±0.02	2	2.82±0.02	2	2.80
Z1, 3 t/ha	2.76±0.02	2	2.61±0.02	2	2.67±0.03	2	2.68
Z2, 6 t/ha	2.60±0.03	2	2.49±0.03	3	2.55±0.02	2	2.55
Z3, 12 t/ha	2.52±0.01	1	2.41±0.03	3	2.49±0.04	3	2.47
<i>F_f</i>	55.16		24.43		27.51		
Calcium exchange compounds (Ca ⁺⁺), mg-eq/100 g							
Control	5.12±0.04	2	5.23±0.05	2	5.19±0.11	4	5.18
Z1, 3 t/ha	6.43±0.05	1	8.56±0.05	1	9.11±0.12	2	8.03
Z2, 6 t/ha	10.30±0.02	2	13.67±0.07	1	15.83±0.28	4	13.27
Z3, 12 t/ha	18.04±0.03	1	23.89±0.13	2	26.60±0.14	1	22.84
<i>F_f</i>	298.80		341.86		614.74		
Magnesium exchange compounds (Mg ⁺⁺), mg-eq/100 g							
Control	1.19±0.02	3	1.26±0.02	4	1.22±0.01	2	1.22
Z1, 3 t/ha	3.18±0.02	1	4.49±0.05	2	3.20±0.04	3	3.62
Z2, 6 t/ha	5.76±0.02	2	8.11±0.05	1	6.85±0.14	4	6.91
Z3, 12 t/ha	10.92±0.08	2	15.33±0.10	1	13.31±0.05	1	13.18
<i>F_f</i>	950.83		432.97		450.47		
Organic carbon content (humus), %							
Control	1.21±0.01	2	1.24±0.01	2	1.22±0.01	2	1.22
Z1, 3 t/ha	1.21±0.02	2	1.25±0.01	2	1.23±0.03	5	1.23
Z2, 6 t/ha	1.22±0.01	1	1.26±0.02	3	1.24±0.02	4	1.24
Z3, 12 t/ha	1.22±0.01	2	1.26±0.01	1	1.23±0.02	3	1.24
<i>F_f</i>	0.44		0.96		0.11		
Mobile phosphorus compounds (P ₂ O ₅), mg/kg							
Control	90±2	5	86±1	3	81±2	6	86
Z1, 3 t/ha	99±4	7	103±3	5	114±4	6	105
Z2, 6 t/ha	117±2	3	122±3	4	131±3	4	123
Z3, 12 t/ha	112±5	9	119±3	5	125±4	6	119
<i>F_f</i>	19.20		41.91		194.91		
Potassium exchange compounds (K ₂ O), mg/kg							
Control	107±4	7	92±2	3	89±3	6	96
Z1, 3 t/ha	118±5	8	121±2	3	126±2	4	122
Z2, 6 t/ha	132±4	6	139±2	5	149±2	3	140
Z3, 12 t/ha	130±6	9	133±2	4	143±2	4	135
<i>F_f</i>	6.04		114.04		97.19		

Note. *F_f*— Fischer's test.

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For 3 years of zeolite application, the actual soil acidity decreased statistically significantly ($p < 0.05$) by more than 1 unit, and the exchange acidity by almost 0.5 units. This acid-base state of the soil solution was due to a significant ($p < 0.05$) decrease in hydrolytic acidity (by 0.33 mg-eq/100 g) due to a signifi-

cant ($p < 0.05$) increase in the content of exchange forms of calcium (4.4-fold) and magnesium (10.8-fold) in the soil, as well as a significant narrowing of Ca/Mg ratio towards the latter [46, 48]. These facts, certainly, have a positive significance not only for the acid regime of the soil but also for its granulometric composition, since the interaction of magnesium with organic matter more actively contributes to the formation of Mg-humate microaggregates in the soil [1, 7].

Amid maintaining the content of humus substances, the use of zeolite caused an increase in the amount of mobile phosphorus compounds in the soil by 22-43% and potassium exchange compounds by 27-46%, depending on the dose of ameliorant ($p < 0.05$).

According to modern studies, silicon compounds are necessary for cultivated plants both in nutrition [16-18] and in providing physiological protection against stress factors of the agroecotope [15, 30, 36]. In this regard, the content of silicon and its various fractions in the biomass is an important criterion for assessing the realization of the biological potential of agrophytocenosis and the adaptation formation [38, 44, 56].

3. Silicon bioaccumulation (MP/BP) in cultivars of various crops upon treatment with zeolite from the Khotynets deposit (OOO Elitkhoz, Filippovskoye village, Bor municipal district, Nizhny Novgorod Province)

Treatment	Crop					
	2015		2016		2017	
	winter wheat (Moscovskaya 39)	potato (Red Scarlet)	barley (Veles)	spring wheat (Kurskaya 2038)	peas (Chishminsky 95)	winter rye (Valdai)
Total silicon compounds (Si_{tot}), % per abs. dry weight						
Control	0.23/1.18	3.16/1.67	0.41/1.53	0.19/1.03	0.08/0.90	0.29/1.42
Z ₁ , 3 t/ha	0.26/1.22	3.20/1.78	0.68/1.69	0.21/1.08	0.19/1.11	0.35/1.50
Z ₂ , 6 t/ha	0.29/1.30	3.29/1.81	0.89/1.75	0.29/1.20	0.26/1.17	0.41/1.58
Z ₃ , 12 t/ha	0.34/1.44	3.37/1.85	0.95/1.79	0.34/1.26	0.29/1.19	0.48/1.66
Organic silicon compounds (Si_{org}), % per abs. dry weight						
Control	0.09/0.50	0.70/0.50	0.21/0.92	0.09/0.52	0.01/0.23	0.15/0.78
Z ₁ , 3 t/ha	0.11/0.59	0.74/0.59	0.41/1.28	0.11/0.59	0.04/0.32	0.20/1.02
Z ₂ , 6 t/ha	0.13/0.68	0.82/0.65	0.61/1.38	0.17/0.71	0.06/0.37	0.24/1.14
Z ₃ , 12 t/ha	0.16/0.84	0.88/0.72	0.66/1.47	0.20/0.81	0.07/0.42	0.32/1.31
Soluble mineral compounds of silicon (Si_{min}), % per abs. dry weight						
Control	0.06/0.20	1.30/0.32	0.13/0.26	0.04/0.11	0.05/0.19	0.05/0.16
Z ₁ , 3 t/ha	0.10/0.22	1.42/0.36	0.16/0.29	0.05/0.14	0.08/0.23	0.08/0.19
Z ₂ , 6 t/ha	0.11/0.24	1.48/0.37	0.20/0.30	0.05/0.14	0.15/0.25	0.13/0.20
Z ₃ , 12 t/ha	0.11/0.25	1.53/0.38	0.26/0.30	0.06/0.15	0.21/0.27	0.14/0.22
Insoluble polymer compounds of silicon (Si_{polym}), % per abs. dry weight						
Control	0.08/0.48	1.16/0.85	0.07/0.35	0.06/0.40	0.02/0.48	0.10/0.48
Z ₁ , 3 t/ha	0.05/0.41	1.04/0.83	0.11/0.12	0.05/0.35	0.07/0.56	0.07/0.29
Z ₂ , 6 t/ha	0.05/0.38	0.99/0.79	0.08/0.07	0.07/0.35	0.05/0.55	0.04/0.24
Z ₃ , 12 t/ha	0.07/0.35	0.96/0.75	0.03/0.02	0.08/0.30	0.01/0.50	0.02/0.13

Note. MP/BP — the content of silicon in the main (grain, tubers) and bypass (straw, tops) parts of the crop yield. Sx_{mean} (%) by Si_{tot} . (MP and BP): winter wheat vs. potato — 2 and 4 (MP and BP) vs. 3 and 2 (2015); barley vs. spring wheat — 4 and 6 vs. 3 and 3 (2016); peas vs. winter rye — 4 and 4 vs. 6 and 8 (2017). Sx_{mean} (%) by Si_{org} . (MP and BP): winter wheat vs. potato — 3 and 5 vs. 6 and 7 (2015); barley vs. spring wheat — 8 and 5 vs. 4 and 8 (2016); peas vs. winter rye — 3 and 4 vs. 6 and 4 (2017). Sx_{mean} (%) by Si_{min} . (MP and BP): winter wheat vs. potato — 3 and 4 vs. 4 and 5 (2015); barley vs. spring wheat — 3 and 4 vs. 3 and 3 (2016); peas vs. winter rye — 3 and 4 vs. 3 and 4 (2017). Sx_{mean} (%) by Si_{polym} . (MP and BP): winter wheat vs. potatoes — 4 and 6 vs. 8 and 9 (2015); barley vs. spring wheat — 6 and 5 vs. 5 and 7 (2016); peas vs. winter rye — 5 and 8 vs. 6 and 8 (2017).

First, it should be noted that in the plant bypass of all types, as a whole, more silicon compounds (Si_{tot}) accumulated than in the main part of the crop, except for potato: in this crop, the element content in the tubers was higher than in the tops (Table 3). However, the use of zeolite to the least extent contributed to an increase in the silicon content in potato phytomass, statistically significantly ($p < 0.05$) increasing the accumulation of Si_{tot} in tubers by 4-7% and in tops by 8-11%, organically bound forms (Si_{tot}) by 17-26 and 30-44%, respectively,

and soluble mineral forms ($Si_{min.}$) by 14-18 and 16-19%, depending on the dose of the rock.

For the remaining crops, increased efficiency in the silicon absorption was revealed when zeolite was added. In particular, in the grain part of the winter and spring wheat crop, the content of $Si_{org.}$ increased by 22-78 and 122%, respectively, in winter rye by 33-113%, in barley by 95-214% compared to the control values. In the pea seeds, the amount of $Si_{org.}$ increased 4-7 times ($p < 0.05$) amid the introduction of rock into the soil, which is probably due to its physiological characteristics. i.e., along with all grain crops, barley, as well as peas and potatoes are classified as silicophilic plants [19, 21, 25].

The content of mineral soluble forms of silicon in plant phytomass, which are orthosilicic acid monomers and metasilicates, also increased when the soil was fertilized with zeolite, and to a greater extent in the straw part of the crop than in the grain. The grain of winter rye and winter wheat, barley, and peas was characterized by the best and statistically justified responsiveness in the accumulation of silicon mineral compounds: amid the introduction of 12 t/ha of rock, the content of $Si_{min.}$ increased by 180, 83, 100, and 320%, respectively ($p < 0.05$).

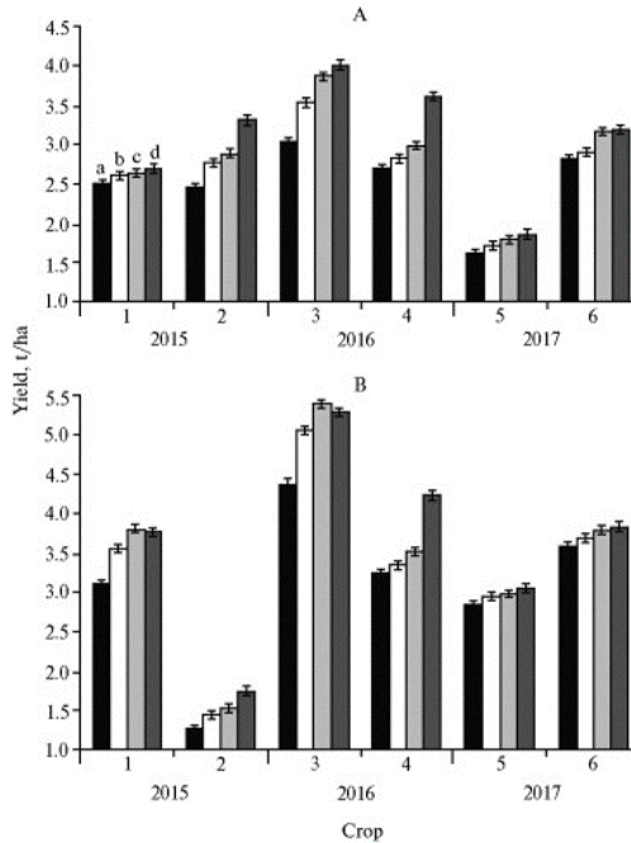
The accumulation of organic and mineral forms of silicon in the bypass part of the crop also increased under the influence of high doses of zeolite. The greatest increase in the amount of $Si_{org.}$ increased in the straw of winter crops (by 68%) and peas (by 83%), and the content of $Si_{min.}$ in straw of spring wheat (by 36%), winter rye (by 38%), and peas (by 42%) ($p < 0.05$).

The content in plant phytomass of the insoluble fraction of silicon compounds ($Si_{polym.}$), represented by polysilicon acids, amorphous silica, and opal microaggregates in phytoliths, in the options with the use of zeolite rock, as a rule, corresponded to the control values or decreased statistically significantly ($p < 0.05$). This phenomenon undoubtedly deserves a separate study and is of value both from the point of view of plant physiology and agronomic quality of the crop. Only in the grain of barley, spring wheat, and peas, as well as in its straw, a certain excess of the insoluble forms of silicon was detected, regardless of the zeolite dose.

By assessing the overall ratio of the accumulation of organic and mineral forms of silicon in plant phytomass, it should be emphasized that the use of zeolite contributed to a greater increase in the proportion of organically bound silicon in the straw of grain crops and potato tops than in grain and tubers (except for pea crop). The proportion of mineral soluble silicon compounds under the influence of zeolite increased in the grain (tuberous) part of the crop, except for barley. At the same time, depending on the dose of the rock, the increase in the proportion of $Si_{org.}$ from the total amount of silicon in the phytomass was more expressed and was traced, as a rule, in all options, and the accumulation of $Si_{min.}$ turned out to be less active and manifested only in the C_1 option. These patterns indicate the gradual binding of silicate ions by organic substances of the cell during the plant vegetation [18, 21].

By analyzing the yield of plants under the influence of high doses of zeolite (Fig.), it should be noted that there is a very significant positive change in indicators for all crops, and, first of all, for barley, a silicophilous plant. The grain biomass of this crop increased by 32% when introducing 12 t/ha of rock into the soil, and with a minimum dose (3 t/ha), the indicator increased by 17% compared to the control ($p < 0.05$). Approximately the same responsiveness to the introduction of silicon-containing material into the soil was characterized by the yield of winter wheat grain in 2015 and peas in 2017. The indicator increased by 4 and 6%, respectively, in the option with the minimum dose of the rock, and by 8 and 15% ($p < 0.05$) for the Z_3 . The biomass of the straw part of

the crop also increased amid the high-silicon material, but 1.5-2.0 times weaker than the main part, i.e., on average for all treatments by 6% in peas and by 19% in barley ($p < 0.05$) compared to the control. In winter wheat, the straw bio-mass, on the contrary, responded more actively when zeolite was introduced into the soil, on average, by 20% ($p < 0.05$) for the treatments.



Main (grain, tubers) (A) and bypass (straw, tops) (B) parts of the yield in winter wheat (*Triticum aestivum* L.) cv. Moskovskaya 39 (1), potato (*Solanum tuberosum* L.) cv. Red Scarlet ($\times 10$) (2), barley (*Hordeum vulgare* L.) cv. Veles (3), spring wheat cv. Kurskaya 2038 (4), peas (*Pisum sativum* L.) cv. Chishminsky 95 (5), and winter rye (*Secale cereale* L.) cv. Valdai (6) upon treatment with zeolite from the Khotynets deposit: a — control, b-d — zeolite (3 t/ha, 6 t/ha, and 12 t/ha, respectively) (OOO Elitkhov, Filippovskoye village, Bor municipal district, Nizhny Novgorod Province). F_f (the main part MP and bypass part BP): winter wheat vs. potato — 0.97 and 35.50 vs. 11.50 and 9.77 (2015); barley vs. spring wheat — 17.97 and 11.93 vs. 48.62 and 36.42 (2016); peas vs. winter rye — 12.35 and 3.10 vs. 11.73 and 3.19 (2017). Fischer's theoretical criterion (F_f) = 3.86 for $n_f = 3$ and $p < 0.05$.

In general, according to the experiments, it should be noted that the responsiveness of different parts of the crop when applying the rock partly depended on the particular crop. Thus, if in 2015 and 2016, the statistically justified effectiveness of the zeolite effect on the yield of potato tops and spring wheat straw was approximately the same (for potatoes, respectively, from 13 and 16% in Z_1 to 35 and 40% in Z_3 , for wheat from 3 and 5% in Z_1 to 31 and 34% in Z_3), then in 2017, when growing winter rye, the effectiveness of the rock was more pronounced with the grain part of the crop (in Z_3 , 14% of the reliable increase in grain weight vs. 6% increase in straw weight). In general, the best and most reliable ($p < 0.05$) efficiency was observed for grain crops when applying 6 t/ha of zeolite to the soil (Z_2), and for potatoes and spring wheat, 12 t/ha (Z_3).

The change in the quality indicators of the main products of cultivated

plants serves not only as a criterion for the agronomic efficiency of the use of fertilizers [1, 16] but also reflects the state of agrobiogeocenosis and allows assessing the implementation of the agrobiological potential of a crop or a cultivar in specific soil and climatic conditions of cultivation [3, 5].

We found that the content of raw gluten significantly ($p < 0.05$) increased in the Z₃ in winter wheat grain by 5% and in Z₁, Z₂, and Z₃ options in spring wheat grain by 9, 14, and 15%, respectively (Table 4). The greatest and statistically justified ($p < 0.05$) accumulation of protein in the main part of the crop of peas was revealed when 12 t/ha of rock was added to the soil (an increase of 16%), and in barley grain – almost equally when 6 and 12 t/ha were added (by 15–20%).

4. Quality indicators of the main part of the crop yields upon treatment with zeolite from the Khotynets deposit ($M \pm SD$; OOO Elitkhoz, Filippovskoye village, Bor municipal district, Nizhny Novgorod Province, 2015–2017)

Treatment	Gluten, %		Protein, %		Starch, %	Vitamin C, mg%
	winter wheat (Mosovskaya 39)	spring wheat (Kurskaya 2038)	Barley (Veles)	peas (Chish- minsky 95)	potato (Red Scalet)	potato (Red Scalet)
Control	33.7±0.3	27.1±0.4	10.6±0.1	17.8±0.2	14.7±0.2	17.0±0.1
Z ₁ , 3 t/ha	34.0±0.1	29.4±0.2	11.9±0.2	18.9±0.3	15.0±0.2	19.6±0.1
Z ₂ , 6 t/ha	34.9±0.3	30.8±0.3	12.2±0.2	19.4±0.5	15.5±0.1	20.3±0.3
Z ₃ , 12 t/ha	35.3±0.2	31.1±0.2	12.7±0.1	20.6±0.3	16.3±0.3	22.2±0.4
LSD ₀₅	1.1	1.7	1.5	1.4	0.6	2.8

A statistically significant ($p < 0.05$) increase in the starch content in potato tubers and the accumulation of vitamin C in them was found in Z₂ and Z₃, by 5–11% for starch and by 19–31% for ascorbic acid, respectively. Amid the minimum dose of high-silicon material (3 t/ha), trends in optimizing the quality indicators of the commercial part of the crop yield were mainly observed. Apparently, it is the high concentrations of mono and polysilicon acids in the soil solution, which may be due to the doses of ameliorant in 6–12 t/ha, that create the optimal background for nutrition and stability of the agrophytocenosis, which is expressed in the implementation of the cultivar (in improving quality indicators) [7, 28, 56].

In this work, the use of zeolite in high doses significantly ($p < 0.05$) contributed to the optimization of most of the considered indicators of the physical and chemical state of the soil and mineral nutrition of cultivated plants, including by replenishing the soil absorbing complex with exchangeable calcium and magnesium compounds, and the mobile nutrient fund of the arable horizon with mobile phosphorus, potassium, and silicon compounds. In turn, it increased the resistance of the agrophytocenosis to environmental factors, since it was expressed not only in increasing the overall productivity of the crop, but also in improving the quality indicators of its main part.

In many studies, the use of silicon-containing materials in a similar way contributed to an increase in crop productivity. In particular, in the work of Vasileva [7], the use of zeolite-containing tripolite at a dose of 1.5 t/ha on sod-podzolic soil led to an increase in the yield of barley by 0.8–2.4 dt/ha, depending on the year of the study, which was 6–12% of the control values. The effectiveness of the material influence on the productivity of potato reached 27.5–53.8 dt/ha (35–39% relative to the control). In joint studies of Russian and Chinese scientists [56] in the conditions of Hunan province (China) on rice soils formed on alluvial loams, a significant ($p < 0.05$) increase in the yield of rice grain (*Oryza sativa* L.) from the use of silicon fertilizers ranged from 0.95 to 14.9 t/ha. These fertilizers were amorphous fine-dispersed silicon dioxide, as well as organosilicon fertilizer with a high content of not only silicon available

for plants, but also organic matter. The obtained results confirm the positive effect of pure silicon preparations on the productivity of agrophytocenoses.

The combined use of high-silicon materials and full mineral fertilizer can have a more significant impact on plant productivity. Loboda et al. [41] studied the effect of zeolite from the Khotynets deposit and NPK fertilizers on the yield of various potato cultivars under similar soil conditions. In the option with the maximum of the studied doses of zeolite (1.2 t/ha) amid the complete mineral fertilizer (N₉₀P₆₀K₁₂₀), the efficiency of the combined action of fertilizers varied from 14.3 t/ha (47%) to 22.9 t/ha (68%) concerning the control, depending on the crop cultivar and weather conditions of the growing year. Despite the relatively low dose of zeolite, its combination with macro fertilizers contributed to a more significant increase in the yield of tubers of the crop.

Thus, when applying high doses of zeolite rock, silicon compounds are more actively ($p < 0.05$) absorbed by plants from the soil. This physiological process is activated by replenishing the soil solution with easily mobile forms of not only silicon but also calcium, magnesium, and other elements necessary for the growth and development of plants. Under the conditions of the microfield experiment based on sod-podzolic light loamy soil, the use of zeolite contributed to the increase in the implementation of the agrobiological potential of the zoned varieties of winter and spring wheat, winter rye, barley, seed sown, and potato, statistically significantly ($p < 0.05$) increasing their yield and improving the most significant quality characteristics. The interaction of the zeolite rock with the soil led to a significant and reliable decrease in its acidity, an increase in the content of exchange compounds of calcium and magnesium, as well as the main biogenic elements. The use of zeolite activated the plant uptake of silicon from the soil and its accumulation in the phytomass in the form of mineral soluble substances and organosilicon components. The established regularities allow considering zeolite as an alternative multi-component reclamation material that has a positive prolonged effect on the soil-plant system and recommending it for research in production conditions on soils of the podzolic series.

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NO-TILL TECHNOLOGY AS A FACTOR OF ACTIVITY OF SOIL INVERTEBRATE IN AGRICULTURAL CHERNOZEMS OF STAVROPOL REGION

O.V. KUTOVAYA¹ ✉, D.A. NIKITIN¹, A.P. GERASKINA²

¹Dokuchaev Soil Science Institute, 7/2, Pyzhyovskiy per., Moscow, 397463 Russia, e-mail langobard@mail.ru (✉ corresponding author), dimnik90@mail.ru;

²Center for Forest Ecology and Productivity RAS, 84/32 str. 14, ul. Profsoyuznaya, Moscow, 117485 Russia, e-mail angersgma@gmail.com

ORCID:

Kutovaya O.V. orcid.org/0000-0002-7977-2358

Geraskina A.P. orcid.org/0000-0002-8365-5787

Nikitin D.A. orcid.org/0000-0002-8533-6536

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Abstract

Soil macro- and mesofauna is highly sensitive to various methods of agricultural cultivation, therefore soil invertebrates are used as bioindicators of agroecosystems ecological condition. Since the macro- and mesofauna to a largely extent control the water balance of the soil and participate in the formation of humus, special attention should be paid to soil fauna in arid regions, primarily in the Cernozem Region. In this work, for the first time, an integrated estimation of the population density and ecological and functional diversity of macro- and mesofauna of Vorony-Calci Chernozem in the Stavropol region is given. The possibility of using these groups of invertebrates as bioindicators of the ecological status of agroecosystems has been shown. It is proved that the use of no-till technology stimulates the activity and number of all groups of macro- and mesofauna. The purpose of the work is to estimate the numbers and taxonomic diversity of ecological and functional groups of macro- and mesofauna with various technologies of soil cultivation (traditional plowing and no-till) with and without mineral fertilizers on the agrochernozems of the Stavropol region. Experiments on research no-till technology were carried out in 2012-2019 in an experimental farm of the North Caucasus Federal Scientific Agrarian Center (Shpakovsky district of the Stavropol region). In 2019 we studied plots of fields with three types of factors: tillage (plowing and no-till technology); presence/absence of fertilizers; agricultural crops. The soil is Vorony-Calci Chernozem. Crop rotation: maize (*Zea mays* L.) variety Mashuk, soybean (*Glycine max* L.) variety Duniza (until 2018), which was replaced later by peas (*Pisum sativum* L.) variety Phaeton, winter wheat (*Triticum aestivum* L.) variety Deya, sunflower (*Helianthus annuus* L.) variety Bagrat. Deposit soil near the experimental fields served as a control. Fertilizers were applied at the time of sowing (N₁₆₀P₉₀K₆₀ for winter wheat, N₇₂P₅₈K₃₂ for sunflower, N₈₀P₄₈K₄₈ for corn, and N₆₀P₆₀K₆₀ for soybeans and peas). The soil macrofauna was registered by the method of excavation of areas 25×25×30 cm and manual analysis of soil samples. Soil mesofauna was isolated from the soil monolith by the method of ektectors, identified and counted using a microscope Biomed-5 PR LUM (Russia) at a magnification of ×40. Most abundant among the macrofauna were centipedes (*Myriapoda*), adults and larvae of coleopterans (*Coleoptera*), spiders (*Araneae*) and earthworms (*Lumbricidae*). *Aporrectodea caliginosa* dominated among earthworms, while single of *A. rosea* were found only in deposit lands. The minimum number of *A. caliginosa* (32 ind/m²) was recorded under peas and sunflower with traditional plowing, the maximum — under corn on no-till plots and on plowed plots (556 and 512 ind/m², respectively). In general, the number of earthworms was higher in no-till fields under all crops (excluding sunflower) in comparison with plowed plots. Among other groups of soil macrofauna, the most numerous were centipedes (up to 1500 ind/m²), as well as spiders (up to 500 ind/m²) and beetles (up to 500 ind/m²). Woodlice (*Oniscidea*) and molluscs (*Gastropoda*) were also encountered. The density of centipedes, spiders, coleoptera and earthworms was always higher for no-till options than for plowed fields, regardless of crop. The application of mineral fertilizers, as a rule, reduced the number and diversity of the macrofauna representatives. Among the mesofauna, ticks (*Acar*) and collembolans (*Collembola*) prevailed in terms of abundance and diversity. Mesofauna of no-till fields was taxonomically more diverse than plowed plots. The minimum number

of mesofauna representative was found under peas and corn, the maximum — under winter wheat and sunflower. In general, the distribution of soil invertebrates (macro- and mesofauna) was significantly influenced by the method of soil cultivation, however, the agricultural culture often influenced the abundance indicators. The use of fertilizers reduced the biodiversity of macrofauna and decreased its number in all plots, regardless of the method of soil cultivation.

Keywords: no-till, plowing, chernozems, soil invertebrates, *Lumbricidae*, macrofauna, mesofauna, bioindication

Intensive agriculture based on conventional tilling (plowing) often degrades not only fertility [1, 2] but also the ecology of agricultural biocenoses (agrocenoses) [3]. Irrational farming drastically alters the physical properties of soil [2, 4] and kills soil macrofauna and mesofauna en masse; the geobionts it destroys are critical for agriculture as they additionally loosen, mix, and structure the soil, disintegrate and homogenize plant residues, convert matter, deoxidize the soil, and kill phytopathogens [5-7]. Since invertebrates largely control the water balance of soils by making runs and redistributing pore space [3], geobionts are crucial for arid regions, in particular Chernozem areas. Notably, macrofauna and mesofauna are directly involved in the formation of humus [8-10], and a severe decline in, or nullification of, detritophagous population may cause uncontrolled soil degradation [4-6]. Unfortunately, some farms fail to see that intensive agriculture needs to be not only cost-effective but also reasonably eco-friendly [8]. However, some farms have undertaken to protect the environment by minimizing the tilling, in particular by adopting a no-till approach [11, 12]. Data on the benefits or hazards of both plowing and no-till is contradictory and needs to be verified [11]. Since invertebrate geobionts are the quickest to respond to tilling [5, 13, 14], a comprehensive “census” of macrofaunal and mesofaunal communities is imperative in order to project the effects of this or that soil tillage method [6, 15, 16].

This paper is the first to present an integrated density and ecofunctional diversity assessment of macrofauna (earthworms, spiders, beetles, myriapods, woodlice, and mollusks) and mesofauna (mites, springtails, proturans, nematodes) found in typical chernozem within the experimental farm in the Stavropol Territory. The paper further demonstrates these groups of invertebrates can indicate the ecological status of agrocenoses. No-till is shown to boost the activity and population of all macrofauna and mesofauna.

The goal hereof was to evaluate the population and taxonomic diversity of macrofaunal and mesofaunal ecofunctional groups in southern chernozems of the Stavropol Territory as affected by different tillage methods (conventional plowing vs no-till) with and without mineral fertilizers.

Materials and methods. No-till experiments with/without mineral fertilizers were run in 2012-2019 at an experimental farm operated by North Caucasus Federal Agricultural Research Center (45°07'48"N 42°01'39"E, Shpakovsky District of the Stavropol Territory). In 2019, land plots were tested by three factors: plowing vs. no-till, fertilized vs. unfertilized, and sown crops (pea, corn, sunflower, and winter wheat). Each plot was 300 m² (50×6 m), 90 m² the declared area. Experiments were run three times.

The soil was typical medium low-humus heavy-loam chernozem upon luss-like carbonate loams [17]. The arable horizon was relatively low on humus (3.87%) and nitrate nitrogen (11.9 mg/kg), had medium concentrations of labile phosphorus (18.7 mg/kg, Machigin's test) and exchangeable K (245 mg/kg); pH 6.32. Deeper sampling was associated with a reduction in all the parameters. Horizon C located at 126-175 cm down had 0.65% humus, 0.5 mg/kg N-NO₃, 3.4 mg/kg P₂O₅, 155 mg/kg K₂O; pH 8.3 [17]. In some plots, the soil was subject to conventional plowing (with overturning); other plots utilized a no-till approach. Such distinct practices were applied over the course of eight years.

Crop rotation was corn (*Zea mays* L.) variety Mashuk, soybean (*Glycine max* L.) variety Duniza (until 2018) later replaced with pea (*Pisum sativum* L.) variety Phaeton, winter wheat (*Triticum aestivum* L.) variety Deya, and sunflower (*Helianthus annuus* L.) variety Bagrat. For control, the research team tested soils from near-field long fallows. Mineral fertilizers were applied by sowers when sowing. The estimated dosage was: N₁₆₀P₉₀K₆₀ for winter wheat, N₇₂P₅₈K₃₂ for sunflower, N₈₀P₄₈K₄₈ for corn, N₆₀P₆₀K₆₀ for soybean and pea.

Pre-harvest soil density was 1.23 g/cm³ in conventionally tilled land plots, 1.24 g/cm³ in no-till plots within 10 cm of depth. Macrofauna estimates relied on sampling from sites sized 25×25×30 cm that were manually sorted into layers (0-10, 10-20, and 20-30 cm) [13]. Samples were kept in 96% ethanol prior to testing. Earthworms were classified by species per Kasprzak [18]; other groups were classified by supra-species taxa [19].

Mesofauna was extracted from monoliths (cylinders sized 101.1 cm³) sampled from upper soil (down to 5.1 cm in depth) by means of eclectors; estimation involved a Biomed-5 PR LUM microscope (Biomed, Russia) at a magnification of ×40.

Diagrams were plotted in Microsoft Excel by calculating the means (*M*) and standard deviations (±SD). Cluster analysis was run in Statistica 10 (StatSoft Inc., US). A dendrogram was plotted by Ward's method based on analysis of variance for inter-cluster distance measurement.

Results. In soil macrofauna, we found and recorded earthworms (family *Lumbricidae*), spiders (order *Araneae*), larvae and imagoes of beetles (order *Coleoptera*), myriapods (subphylum *Myriapoda*), woodlice (suborder *Oniscidea*), and mollusks (class *Gastropoda*). The most numerous taxa were the subphylum *Myriapoda*, the orders *Coleoptera* and *Araneae*, and the family *Lumbricidae*.

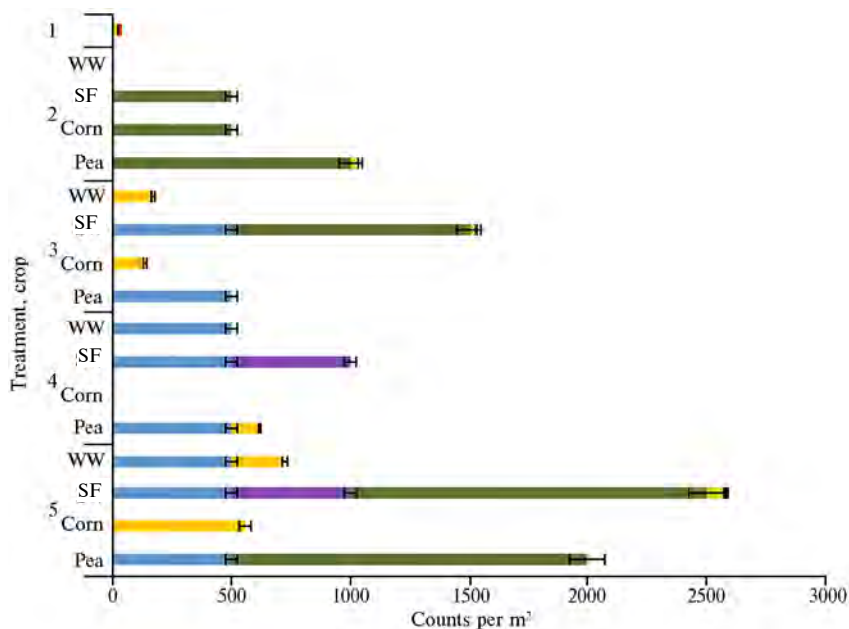


Fig. 1. Population of core macrofaunal groups as a function of soil tillage method (1 for fallows, 2 and 3 for plowing, 4 and 5 for no-till), use (2, 4) or non-use (3, 5) of fertilizers, and the crop: WW — winter wheat *Triticum aestivum* L. cv. Deya, SF — sunflower *Helianthus annuus* L. cv. Bagrat, Corn — *Zea mays* L. cv. Mashuk, Pea — *Pisum sativum* L. cv. Faeton; ■ — order *Coleoptera*, ■ — order *Araneae*, ■ — superclass *Myriapoda*, ■ — *Aporrectodea caliginosa*, ■ — *A. rosea* (Shpakovsky District, the Stavropol Territory, 2019).

The taxonomy of earthworms in the experimental soils had only one endogeic species — *A. caliginosa*. Only fallows contained another endogenic

species, the *A. rosea* (Fig. 1).

The smallest population of *A. caliginosa* (32 ind/m²) was observed under fertilized pea and unfertilized sunflower in tilled soils; the largest populations were observed under corn on non-till plots, as well as in plowed unfertilized soils (556 and 512 ind/m²). In general, the earthworm population was higher in no-till fields for any crop except sunflower. It was low at 40 ind/m² in fallows; however, only fallows contained *A. rosea*, the relative abundance of which was as high as 40%. The general earthworm population in the Center's fields was on the order of data collected in other studies carried out in the steppes of Central Fore-Caucasus [13].

Such low earthworm diversity might be due to two factors. First, soil was sampled in late May, when Stavropol soils are relatively dry [20]. Invertebrates usually tend to be more prevalent and diverse in wet soils [10]. Second, agricultural activity (plowing in particular) is known to reduce the diversity and population density of earthworms [5]. Fallows, on the other hand, are transitioning to natural biogeocenosis that is more diverse in terms of invertebrate population compared to fields [10, 21, 22].

In some cases, the crop and fertilization had a greater effect on the worm population than the tillage method. This pattern has also been observed in other zoological studies [8, 10, 23]. No-till plots had a greater earthworm population due to lesser mechanical agitation of soil and the abundance of plant residues, which contribute to the well-being of all *Lumbricidae* species [15, 23]. Earthworms were not found under fertilized winter wheat, corn, or sunflower regardless of the tillage method. Mineral fertilizers are well-known to kill macrofauna [24], especially earthworms, since they lack chitin or lime carapaces that most other invertebrates have [25]. Only pea plots had earthworms even when fertilized. Adding legumes to crop rotation is known to boost worm population [26]. *A. caliginosa* were found in nearly all unfertilized plots under most crops, whether tilled or not tilled.

A. caliginosa being the single dominant endogeic species is not untypical for agrocenoses [27-29]. *A. caliginosa* is less vulnerable to drought compared to other earthworms [27]. Adult and juvenile *A. caliginosa* are capable of "taking" a summer diapause, whereby they dehydrate their tissues, adult specimens shed their tubercula pubertatis, and worms "curl up" alone or together with others to mitigate moisture loss [30]. This sustainability mechanism is especially relevant in the Stavropol Territory, where spring and summer droughts are not unheard of [20].

Myriapods, spiders, and beetles constituted other populous macrofaunal groups. Woodlice and mollusks were rather rare.

Unfertilized no-till fields had as diverse as possible macrofauna. Beetles were found in all but corn-sown plots at up to 500 ind/m²; however, spiders were only found on sunflower plots at up to 500 ind/m², see Fig. 1. Myriapods on unfertilized no-till plots had populations of up to 1,500 ind/m², but they were detected only under pea and sunflower. Beetle and spider populations were the same in fertilized no-till soil as on unfertilized no-till plots; however, myriapods were not found in such soil, see Fig. 1.

Plowed unfertilized fields were devoid of spiders; however, sunflower and pea fields had beetles (up to 500 ind/m²) and myriapods (up to 1,000 ind/m²), see Fig. 1. Plowed fertilized fields totally lacked any macrofauna except myriapods (up to 500 ind/m²) found under corn and sunflower, see Fig. 1.

Beetles had a higher population density in no-till fields compared to plowed fields, where they were only numerous on unfertilized pea and sunflower plots, which might be due to the abundance of plant mulch in no-till fields [31]. Ecologically, these fields are closer to steppe biogeocenoses as they preserve litter

(a horizon that provides habitat to predators and myxophages) and plant residues, which are a trophic resource for saprophages, phytophages, and myxophages [32].

Dense spider populations of up to 500 ind/m² were only found in sunflower fields. Perhaps the biotope affinity of these obligate carnivores to sunflower was due to the abundance of potential prey, in particular *Coleoptera*, in such fields [33].

Myriapods were the most common group in unfertilized no-till fields of sunflower and pea ind/m²). They also were the only group that inhabited fertilized conventionally tilled fields. Since the subphylum Myriapoda has saprophages (*Diplopoda*) and predators (*Chilopoda*), their density depends on the quality and quantity of post-harvest plant residues and potential invertebrate prey [34].

In general, winter wheat and corn fields were the least favorable habitats for soil macrofauna. In some cases, these fields did not contain any macrofauna at all when fertilized. It is only logical to assume that post-harvest cereal residues are harder to decompose due to their wider carbon-to-nitrogen ratio as compared to legumes such as pea or soybean, or composite flowers such as sunflower; this makes such fields less attractive for saprophages. Decomposition rates of cereals are only half those of legumes: wheat only loses 23.8% of its post-harvest weight over a year, whilst alfalfa loses 45.5% [35].

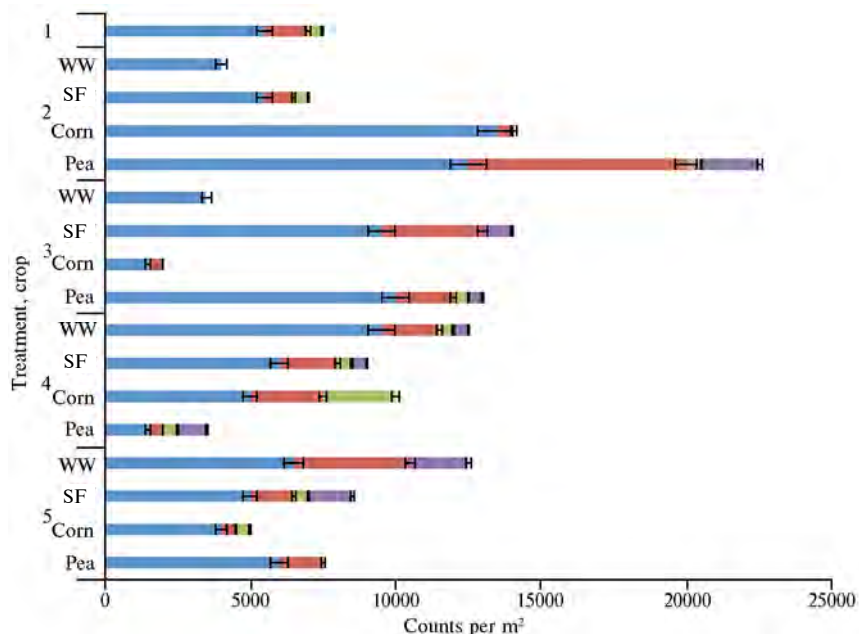


Fig. 2. Population of core mesofaunal groups in soil as a function of soil tillage method (1 for fallows, 2 and 3 for plowing, 4 and 5 for no-till), use (2, 4) or non-use (3, 5) of fertilizers, and the crop: WW — winter wheat *Triticum aestivum* L. cv. Deya, SF — sunflower *Helianthus annuus* L. cv. Bagrat, Corn — *Zea mays* L. cv. Mashuk, Pea — *Pisum sativum* L. cv. Faeton; ■ — subclass *Acari*, ■ — subclass *Collembola*, ■ — type *Nematoda*, ■ — order *Protura* (Shpakovsky District, the Stavropol Territory, 2019).

Mesofauna was dominated by oribatid mites and gamasina (subclass *Acari*) and springtails (subclass *Collembola*), see Fig. 2. Proturans (order *Protura*) and nematodes (type *Nematoda*) were found as well. No-till fields had a far richer mesofauna (total population density of 9.5×10^3 ind/m² and 12 morphotypes) compared to plowed plots (4.0×10^3 ind/m² and 7 morphotypes). Pea was associated with a minimum mesofaunal population whilst winter wheat had the

maximum numbers.

As for unfertilized no-till fields, corn was associated with the smallest population and lowest diversity (up to 4.0×10^3 ind/m² for mites, up to 500 ind/m² for nematodes and springtails), whilst sunflower and winter wheat had the largest populations and the best diversity (up to 6.5×10^3 ind/m² for mites, up to 4.0×10^3 ind/m² for springtails, up to 500 ind/m² for nematodes, up to 2.0×10^3 ind/m² for proturans), see Fig. 2. Proturans were only found under sunflower and winter wheat, whilst nematodes were only found under sunflower and corn. The proportionate population of springtails depended on the crop, increasing in the following order: corn, sunflower, pea, fallow, winter wheat.

In fertilized no-till fields, the mesofaunal population was minimum at up to 5.0×10^3 ind/m² for mites, up to 2.5×10^3 ind/m² for springtails and nematodes under corn; maximum at 9.5×10^3 ind/m² for mites, up to 2.0×10^3 ind/m² for springtails, up to 500 ind/m² for nematodes and proturans under winter wheat, see Fig. 2. Regardless of the crop, mites were the most common mesofaunal group followed by springtails, nematodes, and proturans (the latter were only absent in corn fields).

As for plowed unfertilized fields, corn was associated with minimum mesofauna (mites at up to 1.5×10^3 ind/m², springtails at up to 500 ind/m²), and so was winter wheat (mites at up to 3.5×10^3 ind/m²), whilst maximum mesofauna was associated with pea (mites at up to 1.2×10^4 ind/m², springtails at up to 7.5×10^3 ind/m², nematodes at up to 500 ind/m², proturans at up to 2.0×10^3 ind/m²) and with sunflower (mites at up to 9.5×10^4 ind/m², springtails at up to 3.5×10^3 ind/m², proturans at up to 1.0×10^3 ind/m²), see Fig. 2. Proturans were only found under pea and sunflower, nematodes were only found in pea fields. Wheat fields only had mites. Springtails accounted for 33% of the total mesofaunal population of pea and corn fields, 25% in sunflower fields.

Plowed fertilized fields had the least diverse taxonomy. Wheat was associated with minimum population and diversity of mesofauna at up to 4.0×10^3 ind/m² for mites, whilst pea was associated with maximum figures at up to 1.25×10^4 ind/m² for mites, 7.5×10^3 ind/m² for springtails, 500 ind/m² for nematodes, and 2.5×10^3 ind/m² for proturans, see Fig. 2. Wheat fields were found to only contain mites. Proturans were only found in pea fields, and nematodes only in pea and sunflower fields. Pea fields were most springtail-populous at up to 7.5×10^3 ind/m².

The general mesofaunal population in the Center's fields was on the order of data collected in other studies carried out in the steppes of Central Fore-Caucasus [36]. The dominance of mites in the tested soils was due to the use of eclectors, a method specifically designed to count oribatids [13].

The more numerous and diverse mesofaunal taxonomy of no-till fields was mainly due to the abundance of plant residues and the lack of mechanical impact on soil, which is known to kill invertebrates [24]. The low population of mesofauna under pea and the maximum figures under winter wheat and corn could be due to the better preservation of post-harvest cereal residues thanks to their slower decomposition by saprophagous invertebrates and microorganisms [35]. This preserves a larger horizon that provides habitat to mesofauna, especially the one found in litter, i.e., mites and springtails [37, 38]. The use of mineral fertilizers also had a negative impact on mesofaunal population and diversity, which is in line with what Prasanthi *et al.* reported [39].

The fact that the full taxonomic spectrum of mesofauna was found in unfertilized no-till fields confirms what other researchers reported: plowing and abundance of mineral fertilizers depopulate agricultural landscapes of

invertebrates [8, 23, 26]. At the same time, fertilizers may actually boost the growth of some soil invertebrates in plowed fields. The increased mesofaunal population and diversity in fertilized plowed fields might be due to the boosted growth of microorganisms that benefit from the excess of nitrates and phosphates [14].

Springtails are common in agroecosystems, as are mites. In this particular study, springtails were abundant on virtually any plot. Other authors have also shown springtails to be highly adaptable to various agricultural techniques in no-till, plowed, fertilized/unfertilized fields [40].

Proturans are fairly rare and have a rather specific ecology [41]. Their substantial presence in all the tested fields might be due to the good porosity of soil, since proturans themselves are incapable of digging and instead occupy the airspace between soil aggregates that larger invertebrates create. An early study showed a positive correlation between the density of endogeic worms and that of proturans [42].

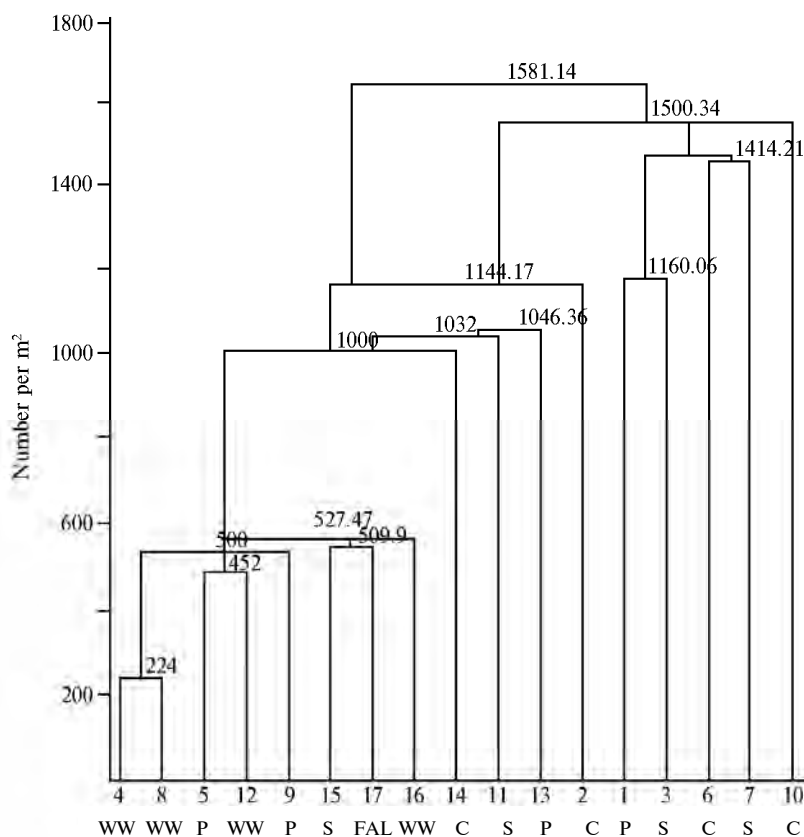


Fig. 3. Dendrogram of similarity between experimental fields in terms of macrofaunal and mesofaunal density as affected by tillage, presence or absence of fertilizers, and the crop (data based on population figures): 1 to 4 for unfertilized no-till fields; 5 to 8 for fertilized no-till fields; 9 to 12 for unfertilized plowed fields; 13 to 16 for fertilized plowed fields; 17 for fallows; WW — winter wheat (*Triticum aestivum* L.) cv. Deya, S — sunflower (*Helianthus annuus* L.) cv. Bagrat, C — corn (*Zea mays* L.) cv. Mashuk, P — peas (*Pisum sativum* L.) cv. Faeton; FAL for fallows (Ward's method) (Shpakovsky District, the Stavropol Territory, 2019).

When clustering plots by the density of macrofauna and mesofauna (Fig. 3), plots that were both plowed and fertilized formed a separate cluster (Nos. 14 to 16). The same cluster also included a fallow field (No. 17) that had similar macrofaunal and mesofaunal density. This could be due to the fact that this fallow was young and had earlier been used as plowed fertilized land. The cluster of

highest invertebrate density contained no-till plots, both fertilized and unfertilized (Nos. 1, 3 and 6, 7), where sunflower and pea were farmed. The smallest invertebrate populations were observed on fertilized no-till pea and winter wheat plots (Nos. 5 and 8), as well as on plowed unfertilized pea and winter wheat fields (Nos. 9 and 12). The same cluster contained an unfertilized no-till plot with winter wheat (No. 4). Therefore, single-crop wheat cultivation prevented invertebrates from populating even no-till fields, i.e., had the same effect as fertilization or plowing. In general, the distribution of soil invertebrates (macrofauna and mesofauna) was greatly affected by the tillage method; however, the crop itself often influenced the population figures, too.

Thus, this paper presents an integrated assessment of population density and taxonomic diversity of macrofauna and mesofauna in agricultural chernozems in the Shpakovsky District of the Stavropol Territory, as affected by tillage method, use of fertilizers, and the crop of choice. It shows that the energy-saving no-till technology had a positive effect on macrofaunal and mesofaunal biodiversity whilst mitigating the dominance of specific groups and lowering the risks of pest outbreaks. Tested macrofaunal groups had their numbers increased by a factor of 2.5; the populations of earthworms, myriapods, spiders, and beetles rose. Mites and springtails constituted the most populous mesofaunal groups, whilst proturans were the least populous group. Therefore, these groups of soil invertebrates could serve as biological indicators of the ecofunctional status of agroecosystems. Mineral fertilizers were found toxic for macrofauna, having a negative impact on its biodiversity and population regardless of the tillage method. Fertilized land plots had no earthworms but a larger myriapod population. Fertilization did not have a profound impact of mesofauna; rather, it was affected by the crop in rotation. Winter wheat was associated with the largest mesofauna population.

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