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Parthenium argentatum A. Gray, *Taraxacum kok-saghyz* L.E. Rodin, AND *Scorzonera tau-saghyz* Lipsch. et Bosse AS ALTERNATIVE SOURCES OF NATURAL RUBBER: DO WE REALLY NEED THEM?

(review)

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Abstract

Natural rubber (NR) is a strategic raw material essential to the manufacture of 50,000 different rubber and latex products. In most cases, e.g., in automobile and aviation industries, it cannot be replaced by synthetic rubber alternatives. There are several important reasons why should we care about alternative sources of NR. Among them are a strong allergic reaction to products made from Hevea latex and a danger of spread of South American late blight (South American Leaf Blight, SALB) in Southeast Asia. The latter would cause irreparable damage to the production of natural polymer. At present, the only commercially significant source of NC is *Hevea brasiliensis* (Willd. ex A. Juss.) Müll. Arg. — an evergreen tree growing in tropical regions. It is not surprising that the research aimed at finding and creating alternative sources of NR by genetic engineering is intensively developing in Europe and North America. On this issue, there are numerous reviews of leading researchers in this field, in particular, the Dr. K. Cornish's team. Thus, back in 2000, one of the first detailed reviews devoted to the problem of alternative NC sources was published (H. Mooibroek et al., 2000). A year later, NR biosynthesis in evolutionarily distant rubber plants was described in detail (K. Cornish, 2001). This problem has been further developed in later works of this researcher (K. Cornish, 2017). Detailed reviews of alternative rubber producers have also been published by other leading groups in the field (J. van Beilen et al., 2007; S.C. Gronover et al., 2011; D.T. Ray et al., 2005). We have recently published two review articles describing in detail the biochemical and molecular genetic aspects of NR biosynthesis (A.Y. Amerik et al., 2018; A.Y. Amerik et al., 2021). In this review, we pay special attention to the historical aspects of this problem, which, in our opinion, have not received sufficient consideration in the literature, describe the state of the industry at the present time, and characterize three rubber plants that are promising producers of NR. As alternative sources of NR, two plants are receiving increased attention. These are Mexican guayule shrub (*Parthenium argentatum* A. Gray) and kok-saghyz or Russian dandelion (*Taraxacum kok-saghyz* L.E. Rodin). We certainly should also mention the undeservedly forgotten, but very promising alternative producer of NR tau-saghyz (*Scorzonera tau-saghyz* Lipsch. et Bosse), which, in our opinion, is currently not given enough attention. *T. kok-saghys* is the most promising alternative rubber plant. For biochemical and molecular genetic studies of the plant, modern molecular biological approaches were used, such as improved transformation protocols, RNA interference (silencing) approaches, and analysis of EST libraries to identify new genes. As a result, the key proteins responsible for NR biosynthesis, cis-prenyltransferase 1-3 (CPT1-3) (T. Schmidt et al., 2010) and CPT activator (RTA) (J. Epping et al., 2015), were identified. It should be noted that the intracellular concentration of CPT regulates NR biosynthesis in cells of *Taraxacum brevicorniculatum*, the closest relative of *T. kok-saghyz*. Transgenic lines in which expression of all three CPT genes was suppressed by RNA interference (RNAi) demonstrated almost complete suppression of NR biosynthesis (J. Post et al., 2012). However, more research is needed before *T. kok-saghyz* NR becomes a commercial alternative to *H. brasiliensis* NR. Research on *P. argentatum* is also rapidly developing. In particular, the work carried out in the laboratory of D.K. Ro should be noted. Researchers have

identified and characterized a protein complex that includes CPTs and plays a key role in NR biosynthesis (A.M. Lakusta et al., 2019). Unfortunately, research on tau-saghyz (*S. tau-saghyz*) is not so successful. This species was critically undermined during intensive harvesting in the 1940s. Nevertheless, work on the restoration of this unique species, the concentration of NR in roots of which under favorable conditions reaches 40 % (dry weight), is currently being carried out at the Kazakhstan National University (S.K. Turasheva et al., 2016). Thus, there is a need for alternative rubber crops and technologies for processing raw materials into final products. Thermostable derivatives, e.g., epoxidized rubber from alternative crops can enter the market to significantly reduce the carbon footprint.

Keywords: natural rubber, *Hevea brasiliensis*, South American Leaf Blight, SALB, latex, allergy, *Parthenium argentatum*, *Taraxacum kok-saghyz*, *Scorzonera tau-saghyz*.

Natural rubber (NR) is one of the most important biopolymers synthesized by higher plants, which is widely used in industry and medicine. It has unique physical properties (elasticity, resilience, impact resistance, efficient heat dissipation) and is able to maintain plasticity at low temperatures [1-4]. Despite the scientific and technological progress in the development of rubber synthesis technologies, at present there is no synthetic rubber that would correspond to NR in terms of its main characteristics.

With the development of the industrial production of synthetic rubber (SR), many innovations have been introduced into the technology that have a positive effect on the consumer properties of rubber. SC is an artificial elastomer derived from various monomers; it is synthesized using different raw materials (oil, coal, natural gas and acetylene). Some of the most commonly used synthetic rubbers are ethylene-propylene-diene, polyisoprene, polybutadiene, styrene-butadiene and iso-butylene-isoprene. They are widely used in the manufacture of tires, conveyor belts, belts, hoses, various seals, floor coverings and shoes. High-tech production of synthetic rubbers has also been created in Russia. When using various highly efficient catalysts - synthesis initiators (conventionally called lithium and titanium), polyisoprenes are formed containing up to 93-98% cis-1,4-units. However, both these types of SR are inferior in terms of microstructure homogeneity to NR, whose macromolecules contain up to 100% cis-1,4-units attached exclusively in the 1.4-1.4 type ("head to tail"). Imperfections in the microstructure of synthetic polyisoprenes manifest themselves primarily in their lower ability to orientate and crystallize compared to NR, which affects the strength and dynamic characteristics. Nevertheless, it should be noted that some types of synthetic rubber are superior to natural rubber in a number of technical properties [5].

The demand for NR is determined by two-thirds of the production of automotive industry. First, we are talking about tires for the primary equipment of new cars. A tire is made of a variety of materials, including several rubber components, each with a specific and unique purpose. NR is used in tire carcasses requiring high strength, while synthetic rubbers are used in tread materials to provide tire grip. At present, the share of NR used in the tire industry is approximately 50% of all types of rubber used [6].

The natural polymer is becoming more and more in demand with the development of high technologies. For example, the rubber components of aircraft tires, designed to operate with enormous loads and speeds at the smallest possible size and weight, are made only of NR. To produce oversized tires, NR is also mainly used. Another example of the exceptional use of NR is the production of mining truck tires and tires with solid steel cord in the carcass. However, compared to SR, NR is less resistant to oils, some chemicals and oxygen. It is also more susceptible to aging, erosion, and retains plasticity in a smaller temperature range compared to SR [7].

It should be noted that these shortcomings could be largely leveled by the epoxidation of NR [8]. Epoxidized natural rubber (ENR) is a molecular structure

that carries an epoxy group that replaces the double bonds in the main chain of the NR rubber polymer. ENR has consumer properties (lower gas permeability, better oil resistance), which allow it to be widely used in industry [9].

The fresh latex is approx. 60% water, 35% cis-1,4-polyisoprene and 5% non-isoprene molecules. NR, in turn, is a hydrocarbon from the group of isoprenoids, in the structure of which the monomers are C_5H_8 isoprene molecules. The hydrocarbon component of NR contains up to 99.5% or more of 1,4-cis-isoprene units (Fig. 1). Also, in latex there are 5% of other organic compounds. These are mainly proteins, lipids, carbohydrates, and their distribution in the latex fractions is not uniform. Although these substances make up a minor part of the latex, some of them remain in the NR after processing and are considered to play a critical role in the properties of the NR. In fact, these impurities probably account for the better mechanical properties of NR compared to its synthetic counterparts, but they also cause unstable NR quality. More than 2500 plant species synthesize NR [10], but only a very few of them can produce economically significant amounts of high-quality polymer with a molecular weight of more than 10^6 Da [11-13].

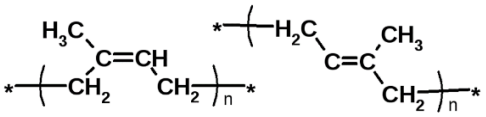

Fig. 1. The structure of plant polyisoprenes: rubber (left) is cis-1,4-polyisoprene, guttapercha (right) is trans-1,4-polyisoprene.

Interestingly, the group of plants synthesizing high molecular weight polyisoprene in the trans configuration (see Fig. 1) is very limited. These include *Palaquium gutta*, *Mimusops balata* and *Eucommia ulmoides*. The polymers they form (respectively gutta-percha, balata, and Chinese gutta-percha) [14-16] are not NR.

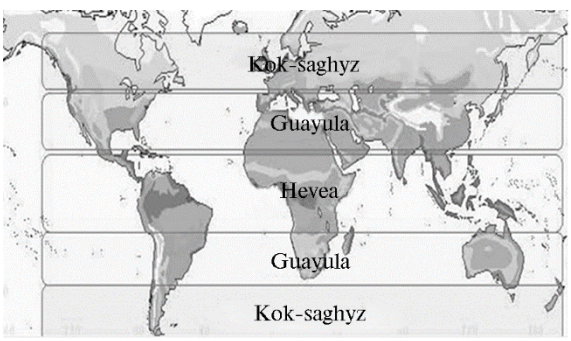

Fig. 2. Geographic distribution of *Hevea brasiliensis* (Willd. ex A. Juss.) Mьll. Arg., guayules *Parthenium argentatum* A. Gray and Kok-saghyz *Taraxacum kok-saghyz* L.E. Rodin. Figure is taken from article by K. Cornish [17].

Even though many plants can synthesize NR, the only commercially significant source of NR currently remains *Hevea brasiliensis* (Willd. ex A. Juss.) Mull. Arg. (Brazilian rubber tree, hevea) [17]. The demand for natural rubber is constantly increasing. According to preliminary estimates, the world production of NR is expected to increase by 1.8% (up to 13.836 million tons), while during 2021, global demand was projected to grow by 8.3% (up to 14.028 million tons). According to forecasts, by 2023 it will amount to about 16.5 million tons per year and will grow in the future [18]. Of course, there are concerns that modern plantations of hevea trees will not be able to meet the increasing needs for this product. The process of collecting NR is very time-consuming and does not lend itself to mechanization. Plants begin to produce significant amounts of NR from the age of 5-7 years [19]. In addition, hevea can grow in a narrow climatic zone of tropical forests (Fig. 2) [17].

It should also be noted that the production of NR is at particular risk because only a few closely related clones were used for the cultivation of hevea (unlike other agricultural crops) [17]. Thus, a single clone could form the basis of plantations with an area of hundreds of thousands of hectares. As a result, many phytopathogenic fungi infect genetically homogeneous plants, exposing hevea

plantations to great danger. So, in Brazil, South American late blight (South American Leaf Blight, SALB) is a lethal disease for *H. brasiliensis* caused by the fungus *Microcyclus ulei* that has led to an almost complete cessation of NR production (Fig. 3).

Fig. 3. Symptoms of South American Leaf Blight (SALB, causative agent ascomycete *Microcyclus ulei*) **on leaves** (above) **and stem** (below) **of *Hevea brasiliensis* (Willd. ex A. Juss.) Müll.** Figure is taken from J. Guyot et al. [20].

The former leader in the supply of natural rubber currently produces only 1.5% of its global volume, while many times more is needed for its own needs. As a result, Brazil itself depends on imports of NR from Southeast Asia. Currently, studies are being conducted to obtain *H. brasiliensis* genotypes resistant to SALB, but it will take at least 25 years to replace existing plants with clones immune to the disease [17, 20, 21]. It should also be noted that repeated contact with some hevea latex proteins leads to allergic hypersensitivity of the first type [17]. Thus, the diversification of NR producers becomes a primary task to meet the needs for this polymer.

The purpose of this review is a comparative analysis of potential alternative producers of natural rubber that can replace the only commercially significant source of polymer to date — *H. brasiliensis*. Currently, *Parthenium argentatum* A. Gray (guayula) and *Taraxacum kok-saghyz* L.E. Rodin (kok-sagyz, Russian dandelion) are considered as such producers. *P. argentatum* is a perennial shrub growing in the Mexican Chihuahua desert and Southern Texas [22]. *T. kok-saghyz* is an herbaceous plant growing naturally in Kazakhstan, Southern Siberia, Uzbekistan and China [23, 24]. The review also pays attention to the perennial semi-shrub tau-sagyz (*Scorzonera tau-saghyz* Lipsch. et Bosse), a representative of the genus *Scorzonēra*.

The history of natural rubber production. The fact that NR can be obtained from some trees and used for various purposes was known back in the days of the ancient civilizations of Central and South America long before the appearance of Europeans there. The Aztec chronicles tell us that the NR was collected as a tribute from the conquered peoples and was used in religious ceremonies. It was also used to make balls and waterproof clothing. For Europeans, NR was discovered in 1743 by Charles-Marie de la Condamine, one of the first explorers of the Amazon, who compiled a fairly accurate map of it. He learned about rubber and quinine from local Indians and in one of the expeditions described the process of making rubber products and the treatment of malaria with quinine. The ability of NR to erase pencil inscriptions was noticed by Joseph Priestley, who in 1770 introduced the English word “rubber” into European usage. The unique hydrophobic properties of NR were due to the first attempts to use it in Europe for the manufacture of waterproof clothing and shoes [25].

However, the large-scale application of NR in industry was extremely difficult until in 1818 James Syme discovered that benzene can dissolve NR, and Charles Mackintosh used this discovery to create a special fabric containing a waterproof layer of NR. Raincoats made of this fabric were called mackintoshes [26]. It should be noted that products made of natural rubber had serious

disadvantages. They became soft and sticky at elevated temperatures in summer and hard and brittle in winter. Therefore, interest in NR products fell until 1839 when Charles Goodyear, after 5 years of research that almost led him to bankruptcy, discovered that exposure to high temperature and sulfur stabilizes NR and leads to its unique properties being preserved over a wide temperature range. Later this process was called vulcanization [27]. It was vulcanization with subsequent modifications that expanded the possibilities of using NR on an industrial scale. Vulcanized NR (rubber) can be stored for a long time and transported to any point of the globe. Rubber quickly became an integral part in the aviation and automotive industry (tires), the manufacture of electrical appliances (insulators) and various medical devices.

Fig. 4. Collection of hevea latex (https://derevo-s.ru/drevesina/listvennye/geveya).

Large-scale harvesing NR in the Amazon basin began at the end of the 19th century near the Brazilian Atlantic port of Para and eventually spread to the east of the South American continent. The rapid development of the automotive, medical industry, and electric power industry has led to a rapid increase in demand for NR. For 12 years (from 1890 to 1910), the production of NR increased 6-fold [25].

Several rubber producers have been investigated as potential industrial sources of NR. These are different species of *Sapium* (caucho blanco) [28], *Castilla* (caucho negro) (29) and *H. brasiliensis* [17, 29-31]. It has been shown that it is the latter plant that produces high-quality natural rubber. A close relative of *H. brasiliensis*, the *H. guianensis* also synthesizes NR, but of low quality [32]. Hevea *H. brasiliensis* has been found only in the Putumayo River basin (a tributary of the Amazon). *H. brasiliensis* NR is obtained by cutting the bark (Fig. 4), but the collection of milky juice is possible only 6 months a year, since trees grow in lowlands prone to flooding during the rainy season.

Initially, NR was also obtained year-round, using trees of the genus *Castilla — C. elastica* and *C. ulei*, growing on non-flooded elevations. However, in this case, mechanical destruction and deep processing of wood were required, and as expected, the raw material base quickly dried up [29]. Thus, *H. brasiliensis* was finally chosen as the optimal source of NR.

In 1857, Thomas Hancock, the founder of the British company Thomas Hancock's clothing, proposed to create *H. brasiliensis* plantations. During the 1870s, three collections of *H. brasiliensis* seeds from South America were delivered to the Royal Botanic Gardens in London (Kew Gardens). One of them belonged to Henry Wickham, who lived at that time in the upper part of the Amazon in one of the largest right tributaries of the Amazon — Tapajos [17]. In 1876, he brought to London about 70,000 seeds, of which 2,700 germinated. A significant number of seedlings were sent to Malaysia and Ceylon, several to Indonesia and Singapore. Thanks to the hard work of the local population and British settlers, by 1907, about 10 million hevea trees had been grown on plantations in Southeast

Asia. In 1912, latex exports from Malaysia and Indonesia amounted to 8,500 tons, but this was significantly less than exports from the Amazon basin — 38,000 tons. Commercial production in Southeast Asia continued to expand, and in 1917, the Asian colonies of Great Britain, France and the Netherlands exported 370,000 tons of NR. This led to a sharp drop in prices for NR, which made its production in the Amazon unprofitable [25]. In the 1920s, Henry Ford tried to resume the collection of NR on Fordlandia plantations in the Amazon basin, but SALB (see Fig. 3), caused by the fungus *Microcyclus ulei*, nullified these efforts [20, 21].

It is not surprising that the United States was interested in an independent source of NR. An alternative rubber carrier — guayula (*P. argentatum)* was first used in Mexico and somewhat later in the USA. However, due to the depletion of raw materials in Mexico, the Mexican Revolution and the Great Depression, the production of NR stopped quite quickly. At its peak, it produced about 20% of the total amount of polymer consumed in the USA [10, 22].

The next attempt to find alternative sources of NR was caused by Japan's seizure of *H. brasiliensis* plantations in Southeast Asia in 1942 during World War II. This led to the fact that the countries of the anti-Hitler coalition lost their sources of NR. At this time, the production of SR began to develop intensively in the USA and interest in alternative sources of NR was again manifested. In addition to the already mentioned guaiula (*P. argentatum*) and Russian dandelion (*T. kok-saghyz*), rubber vine (*Cryptostegia grandiflora* R. Br.) [33] and golden rod (*Solidago leavenworthii* Torr. & A. Gray) [34] was considered as such sourses.. However, after the end of World War II, relatively cheap NR from Southeast Asia became available on the world market again. This circumstance, as well as the expansion of the production of synthetic rubber, led to the fact that by the mid-1950s alternative sources of NR practically ceased to be of commercial interest. However, some studies related to *T. kok-saghyz* were conducted in the Soviet Union until its collapse in 1991 [10, 35, 36].

Interest in guaiula resumed after the global spread of the deadly type 1 allergy to latex proteins of *H. brasiliensis* [37]. Studies have shown that latex from *P. argentatum* does not contain proteins that cause allergies, and products from it can be used by people who are sensitive to hevea latex proteins [38, 39]. But in general, *H. brasiliensis* remains the only commercially significant rubber carrier.

Synthesis of rubber in rubber carriers. Structurally, polymers produced by different rubber carriers are cis-1,4-polyisoprenes, the synthesis of which begins with the formation of two or three trans-initiator units [40-44]. However, the molecular weight, macromolecular structure, intermolecular bonds, and chemical composition of the molecule depend on the specific rubber carrier and affect the properties of NR [10, 44-46]. Plants produce many different cis-polyisoprenes, but NR only includes polymers containing at least 100 isoprene units, and at least 15,000 units are required for the polymer to be classified as high-quality NR [17].

NR is an elastic material that returns to its original size and shape after deformation. This is because NR can undergo deformation crystallization [47, 48]. Crystallization is a phase transition (from an amorphous to a crystalline state), accompanied by the release of heat, a change in specific volume and physico-mechanical properties. When rubber is stretched, crystallization occurs quickly and is accompanied by the orientation of the molecular links along the direction of stretching. Under the influence of special reagents (sulfur, peroxides, metal oxides, amine-type compounds), the vulcanization of rubber occurs with the

crosslinking of molecules into a single spatial grid [27]. The strength characteristics of rubber, its hardness and elasticity increase, but the plastic properties, the degree of swelling and solubility in organic solvents decrease. The rate of vulcanization and the density of crosslinking critically depend on the components of NR that are not related to polyisoprenes. These components are specific to each rubber carrier, which leads to significant differences in the properties of the final product [17].

The synthesis of NR occurs in cytoplasmic rubber particles (Fig. 5) [49, 50]. Such particles are often formed in the multinucleated cells of the bark or roots, called laticiphras [51, 52]. This statement is true for the bark of *H. brasiliensis* and the roots of *T. kok-saghyz*. Interestingly, in *P. argentatum*, rubber particles are formed in the cytosol of parenchymal bark cells [53]. It is likely that the differences in the chemical composition of NR from different sources are due to the specific features of the cytosols containing rubber particles [54].

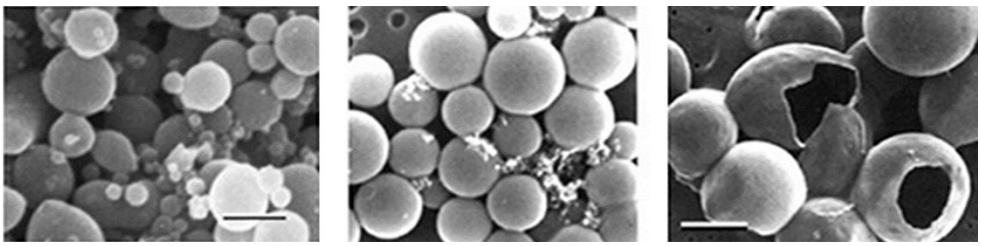

Рис. 5. Micrograph of rubber particles from *Hevea brasiliens* (A), *Parthenium argentatum* (B) and *Ficus elastica* (C), obtained using a scanning electron microscope. Scale bars are 1 μm (A and B) and 2 μm (C). The figure is taken from the article by K. Cornish [17].

Alternative sources of natural rubber and their comparative assessment. Although the genetic resistance of hevea to SALB has been studied quite actively recently, according to the forecast, it will take at least 25 years to create highly productive plantations based on plants immune to SALB [17]. Therefore, biodiversification of NR sources remains an extremely important task.

Currently, *P. argentatum* (guayula) and *T. kok-saghyz* (kok-sagyz, Russian dandelion) are considered as alternative rubber carriers. It should be noted that three rubber-bearing plants — *H. brasiliensis*, *P. argentatum* and *T. kok-saghyz* grow in different geographical areas, the Central and South America; northern Mexico and southeastern USA (mainly Texas); Kazakhstan, southern Siberia, Uzbekistan and Northwestern China, but together their ranges cover almost all the world's areas available for agriculture (see Fig. 2) [17]. Both alternative sources of NR are being intensively studied in the USA and Europe to ensure the security and price stability of the NR market. It is assumed that guayula will become a new or alternative crop for arid and semi-arid areas of the southwestern United States, north-central Mexico, and regions with a similar climate around the world [22, 25].

Guaiula (*Parthenium argentatum*). Among the potential alternative sources, NR gvayula (Fig. 6) stands out because, as already noted, it has a relatively long history of commercialization and even short-term periods of critically important intensive research (22). Unfortunately, not enough attention was paid to these studies in the future, as a result, the genetic material obtained and the experience of breeding work were lost.

Selection of *P. argentatum*. Fragmentary breeding studies of guayula during the XX century led to partial domestication of *P. argentatum*. The state of these works was analyzed twice, in 1991 [55] and in 2005 [56]. The selection of

P. argentatum is greatly facilitated by very high variability between and within the lines for each analyzed trait (in particular, the amount and quality of NR, dry weight, number of resins, yield of NR [11]. But at the same time for breeders of *P. argentatum* and *H. brasiliensis* are complex objects, since they are perennial plants, besides, relatively large areas are required to perform the corresponding programs. The guayula reaches the generative phase of development by about 2 years of age and reproduces mainly asexually — by apomixis [10, 11]. Thus, the selection is mainly reduced to the isolation of plants that give a higher yield of NR. Significant progress has been made in this regard, since several new lines that were completed as a result of work funded by the United States Department of Agriculture (USDA) produced five times more NR than the lines used in the 1940s and 1950s years [56]. Unfortunately, the success was not complete, since the descendants of the selected lines were unable to reproduce the results of highly effective parents [10, 56]. Nevertheless, such an approach certainly has potential. Similar studies performed on H. brasiliensis, over 40 years have led to an increase in the productivity of lines by 10 times — from 300 kg/ha per year to 3000 kg/ha per year [10; K. Cornish, personal communication].

Fig. 6. Guayule (*Parthenium argentatum*): plant in natural growth (A) and plantation cultivation of guayule (B). Figure is taken from articles by J. van Beilen et al. [11, 12].

Molecular genetic studies of P. argentatum. Wild guaiula is represented in nature by diploids ($2n = 2\times = 36$), triploids ($2n = 3\times = 54$) and tetraploids ($2n = 4\times = 72$). Interestingly, plants with the number of chromosomes reaching the octaploid ($2n = 8\times = 144$) were identified under cultivation conditions. It should be noted that diploids reproduce mainly sexually, while polyploids by facultative apomixis. Guaiula also has a sporophytic self-incompatibility system, and many plants contain B- or supernumerary chromosomes [57, 58].

A sufficiently large number of *P. argentatum* genes have been cloned that co-generate enzymes and proteins that participate in the biosynthesis of NK, including a gene encoding the major guayule rubber particle protein, RPP [59]. A gene encoding a protein with a molecular weight of 24 kDa has also been cloned, strongly associated with the so-called small rubber particle protein (SSRP). In vitro functional analysis using heterologous expression in *Escherichia coli* cells showed that the SSRP gene can participate in the synthesis of the polyisophene chain [59]. Several other proteins associated with rubber particles have been isolated and studied, but their functions have yet to be established [61]. The main protein associated with rubber particles in *P. argentatum* is cytochrome P450 with a molecular weight of 53 kDa. It is a member of the CYP74 family and has a high degree of homology with allene oxide synthase (AOS). It accounts for approximately 50% of the total protein of rubber particles. Despite the fact that it is catalytically active (converts 13(S)-hydroperoxy-octadecadenoidic acid into α-

and γ-ketolic fatty acids) and is the main protein of washed rubber particles capable of synthesizing NR, its role in this process is unclear. Moreover, it has no structural homology with cis-prenyltransferases [60, 61].

Significant progress has been made in molecular biological studies of the protein complex responsible for the synthesis of NR in *P. argentatum*. This complex includes cis-prenyltransferases (CPT) directly involved in the synthesis of the polyisoprene chain and proteins necessary for their activity (CBP/RTA) [62]. The transcriptome of the guayula diploid is available in the NCBI database (National Center for Biotechnological Information, GenBank: PI1478640) [63]. Transcriptome analysis using tissues of roots, leaves, flowers, and stems (a total of 51,947 transcripts collected from 983,076 fragments) compared with previously identified sequences of the *Lactuca satиva* lettuce genome [64] showed that the genes of three CPT proteins (PaCPT1-3) and one CBP protein (CPT-Binding Protein, CPT binding protein) [62, 65].

In eukaryotic cells, a short oligoisoprenoid dolichol with several monomeric units from 8 to 18 is necessary for the transport of sugar molecules for post-translational glycosylation [66]. Therefore, all eukaryotes have at least one pair of CPT and SVR. The absence of one of these proteins is lethal to the cell. The yeast *Sacharomyces cerevisiae* has one SVR homologue (Nus1) and two SRT homologues (Rer2 and Srt1).

To study the function of the SRT/SVR complex, a double mutant *rer2 str1* was constructed, which is viable only in the presence of URA3 plasmid carrying the *RER2* gene [62]. On a medium containing 5-fluorotic acid (5-FOA), *Ura3* expression converts 5-FOA into a toxic derivative — 5-fluorouracil. Thus, only cells in which URA3 plasmid is lost and *er2*Δ *srt1*Δ mutations are complemented by the PaCBP/RaSRT1-3 combination can grow on media containing 5-FOA. Complementary analysis in yeast showed that PaCBP is necessary for the enzymatic activity of PaCPT1-3. The PaCBP and PaCPT1-3 genes alone or in combination were expressed in the double mutant *rer2*Δ *srt1*Δ. Indeed, it turned out that only PaCBP together with one of the PaCPT can complement a double mutant. Separately, PaCBP and PaCPT1-3 are not able to support the growth of yeast cells on media containing 5-FOA [62] (Fig. 7).

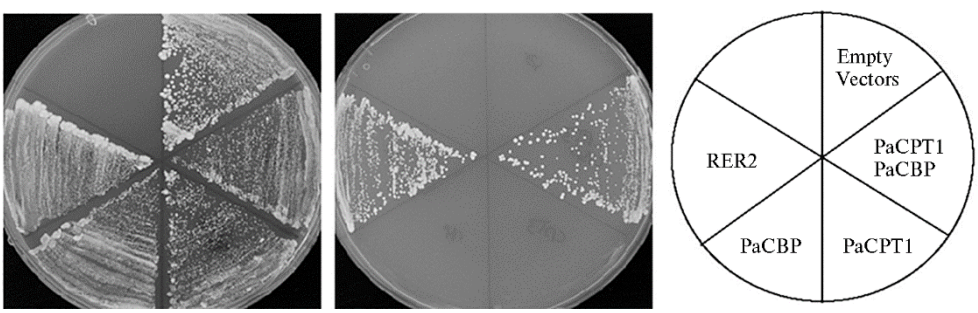

Fig. 7. Functional complementation of the *rer2*Δ *srt1*Δ mutant (*Sacharomyces cerevisiae*) with plasmids encoding PaCPT1 and PaCBP. The yeast strain *rer2*Δ *srt1*Δ is lethal but is maintained by expression of *RER2* in a URA selective plasmid. This strain was used to transform plasmids expressing both *PaCPT1-3* and *PaCBP*. Successful transformants were streaked onto selection plates with 5-FOA-xontaining medium to remove the *RER2*-containing URA plasmid. Yeast growth by selection for 5-FOA was observed only for PaCPT/PaCBP pairs or retransformed RER2 in the TRP plasmid. No growth was observed when PaCPT alone or PaCBP alone was expressed. The figure is taken from the article by A.M. Lokusta et al. [62].

Moreover, extracts from yeast cells growing on media with 5-FOA have been shown to exhibit cis-prenyltransferase activity. It should also be noted that the results of a two-hybrid analysis using the technology of split ubiquitin and

coimmune precipitation convincingly showed that PaCBP and PaCPT1-3 interact with each other [62].

Cultivation of *P. argentatum*. Guayula is a perennial herb growing in the hilly areas of the Chihuahua Desert in Mexico and the Big Bend area in South Texas. The temperature in these regions varies from –18 to +50 °C. The high temperature does not seem to have a negative effect on the growth of the plant, but at values below +4 °C, the gum falls into suspended animation. Prolonged presence of the plant at negative temperatures can lead to its death [11]. One of the problems arising during *P. argentatum* cultivation is root disease, especially in stagnant water [67]. Well-drained limestone and sandy-clay soils with a relatively low content of nutrients are optimal for the cultivation of guayula. In general, the plant is quite unpretentious, it has been successfully grown in desert and semi-desert conditions, rainforest and middle belt with moderate temperatures and precipitation typical for these areas [11].

P. argentatum prefers regions where 280 to 640 mm of precipitation falls annually. It is shown that intensive irrigation is necessary for the maximum yield of NR. Interestingly, the formation of NR and resins increases in proportion to the availability of water. Even though a significant amount of water is required for intensive growth and production of NR, the plant is resistant to arid conditions, the periods of which can be long, but the synthesis of NR at the same time ceases [68].

The NR output varies greatly between lines. Moreover, in plants of the same line, it often differs markedly depending on the region, soil, and weather conditions. The season and the age of plants also affect the yield of NR and resins. In some varieties, the content of NR varied significantly depending on the time of year, in others the effect was not so obvious. For a set of eight varieties of *P. argentatum*, it was shown that the amount of biomass increases with the age of plants, but the dynamics of accumulation of NR and biomass differ [69]. Some studies show that old plants can contain very large amounts of NR. Thus, the biomass yield can reach 20 t/ha per year, whereas the NR is 2 t/ha per year (K. Cornish, personal communication).

Guayula synthesizes and accumulates rubber particles primarily in the epithelial cells of parenchymal tissue. The technologies for obtaining NR from the biomass of guayula are described in detail in the literature. Three methods of obtaining NR from guayula have been developed and applied. The first and oldest method is flotation. Crushed plants are placed in a large container with an alkali solution, wood tissue absorbs water and sinks to the bottom, and resinous rubber floats to the surface in the form of so-called “worms”. In the future, the rubber is cleaned of resins using acetone [70]. The second method is sequential extraction, in which the resin is first extracted with acetone or another polar organic solvent, and then the rubber is extracted with hexane [71]. The third treatment method is simultaneous extraction, which uses a mixture of solvents, usually acetone and hexane or pentane. After the initial extraction, acetone is added to coagulate high-molecular-weight rubber [72].

According to economic forecasts, for guaiula to become a competitive crop without subsidies, it is necessary to increase the yield of rubber and/or identify and develop commercial use of by-products of processing [73]. One of the potentially valuable by-products is the low molecular weight fraction of rubber, which accounts for about 25% of its total yield. These low molecular weight rubber compounds are of great importance as a special rubber not used in tires [73]. Another by-product of processing, the resins is characterized only partially, but mainly represents triglycerides of fatty acids and terpenoids. Resins are successfully used as preservatives for wood, raw materials for special chemicals (coatings and additives

to rubber), as well as high-quality fuel without ash [11, 73].

Advantages and disadvantages of natural rubber from P. argentatum. The molecular weight and properties of NR from guayula are very close to those of NR from hevea. However, unlike NR from *H. brasiliensis*, natural rubber from guayula does not contain proteins capable of causing a severe allergic reaction [38]. This important pre-property has revived interest in NC from *P. argentatum*. For example, the companies Yulex (Solana Beach, CA, USA) and PanAridus (Casa Grander, AZ, USA) produce such NC, which practically does not contain allergenic proteins. It can be used for the manufacture of hypoallergenic gloves and other medical products, in which the strength and elasticity of NC is combined with the absence of dangerous allergenicity.

Isolated directly from the plant and dried, the NR of the guaiula can contain from 20 to 40% resin. If NR was extracted with solvents and then treated to remove the resin, then the viscosity of such NR is significantly lower than the viscosity of NR from *H. brasiliensis*. Selective coagulation is necessary to obtain a high-molecular fraction of NR from guayula, which is similar in properties to NR from hevea (74). NR from *H. brasiliensis* contains proteins and, consequently, reactive groups capable of crosslinking. This leads to the formation of branched polymer chains, and as a result, the viscosity of NR increases during storage for a long time. In contrast, irreversible chain cleavage induced by temperature occurs in the protein-free NR from *P. argentatum*. That is, such NR is less resistant to elevated temperature than NR from *H. brasiliensis*. Moreover, the triglycerides of unsaturated fatty acids present in the resin contribute to the oxidation of polymer chains. To prevent the oxidation process and increase the stability of NR from guayula, a combination of antioxidants and zinc dialkyldithiocarbamate [74] is used.

Kok-sagyz, Russian dandelion (*T. kok-saghyz*). An ideal rubber carrier should yield annually, grow rapidly, and produce a large amount of biomass. Plants yielding an annual harvest can be quickly planted and harvested depending on the needs of the product and the market situation. Kok-saghyz (*T. kok-saghyz*) meets these criteria to a greater extent than guayula.

T. kok-saghyz (Fig. 8) was discovered in Kazakhstan, in the Tien Shan valleys and was first described by the botanist L.E. Rodin in 1932 during the implementation of the strategic program of the USSR for the development of its own production of NR. The study of 1048 species from 316 genera and 95 families of the native flora showed that 609 species synthesize rubber and rubber-like substances.

The development of koksagyz occurs in harsh conditions of a sharply continental climate on saline soils, with a lack of moisture and strong winds [72]. Based on koksagyz seeds collected in places of natural growth, a collection of plants was created at the All-Union Institute of Plant Breeding named after N.I. Vavilov (VIR, St. Petersburg), a botanical description and determination of the intraspecific diversity of plants were carried out, which made it possible to select the best genotypes for cultivation. The biological and morphological features of the plant were studied and it was found that the crop is moist, requires at least 420-600 mm of precipitation per year with their uniform distribution, needs highly fertile soils (floodplains of rivers, cultivated peat bogs, black steam). Agrotechnics of culture for various types of soils were developed, diseases and pests were studied, breeding work was carried out [36, 72, 75, 76].

The roots of wild koksagyz accumulate from 4 to 12% of high-quality rubber synthesized in laticifera — elongated secretory cells found in the leaves and stems of those plants that produce latex and rubber as secondary metabolites [51, 52].

T. kok-saghyz was actively cultivated in the USSR from 1930 to 1952. In

1941, 67 thousand hectares of plantings covered about 30% of the country's need for NR [11]. With the outbreak of the Second World War, there was an acute shortage of NR, and several countries independently began to implement emergency programs to develop technologies to produce NR from *T. kok-saghyz*. Among them, the USA [77], Great Britain [78], Germany [79], Sweden and Spain [80] should be mentioned. If the best result in the USA was 110 kg/ha, then in the USSR it was possible to exceed the indicator of 200 kg/ha [36].

Fig. 8. Plants of kok-saghyz (*Taraxacum kok-saghyz* L.E. Rodin): A — general view, cultivation in the soil; B — roots of a plant grown in the soil: C — growth of kok-saghyz plants under phytotron conditions (aeroponics cultivation) (authors' photo).

Unfortunately, the cultivation of *T. kok-saghyz* is laborious and expensive. The seedlings of the plant are very small, it is difficult for them to compete with the weeds, which makes constant intensive weeding necessary. After the resumption of supplies of cheap NR from Southeast Asia to the world market after the end of World War II, the cultivation of *T. kok-saghyz* in the USSR continued until the early 1950s, but then these works were stopped in the USSR for economic reasons [36].

Selection of T. kok-saghyz. For kok-sagyz to become economically competitive, the rubber content in latex needs to be increased. The main goal of all breeding programs is to increase the yield of rubber per unit area. It should be noted that the programs for the selection of koksagyz were carried out from the moment of its description, study, but were conducted inconsistently, with long

time intervals. In addition, breeding was complicated by the fact that this species has a system of self-sterility (self-incompatibility) that prevents self-fertilization [81]. The genetic material used in the USA during the implementation of the emergency rubber program was essentially improved samples of wild type koksagyz, obtained from the USSR. If in the most productive plants the yield of NR was about 5-6% of the dry weight of the roots, then in most cases it did not exceed 2-3% [10, 36]. It is noteworthy that, according to published data, in the USSR, the yield of NR reached 15% (82). In 1953, by the method of multiple crosses, it was shown that the size of the koksagyz crust and the accumulation of NR in them could be significantly increased [83]. Based on these studies, it was assumed that the selection of cocsagyz would potentially increase the yield of NR to 15-25% [84].

The yield of rubber can be increased by increasing the biomass and/or the content of rubber in it. An increase in the rubber content is more desirable since this increases the efficiency of plant processing. The increase in biomass is associated with additional costs associated with harvesting, transportation, and processing. To turn *T. kok-saghyz* into a commercially attractive product, it is necessary to significantly improve its agronomic properties, for example, the growth rate. This is possible by crossing *T. kok-saghyz* with the common dandelion *T. officinale*. In experimental fields in New Zealand, the yield of *T. officinale* dry roots was 6-9 t/ha after 6 months of growth [85]. Thus, theoretically, a hybrid of koksagyz and ordinary dandelion could produce NR in a quantity of about 1200-1800 kg/ha.

Several features make *T. kok-saghyz* an exceptionally attractive model system for studying NR biosynthesis, including for breeding purposes. It has a very short life cycle (6-8 months) compared to other rubber carriers. For example, in the case of *H. brasiliensis*, it takes an average of 7 years to assess the phenotype of a plant by its ability to produce NR. For the shrub *P. argentatum*, the same period is 2 years. Moreover, *T. kok-saghyz* can be genetically modified relatively easily (for example, transformed to produce transgenic plants). The analysis of the NR content in the roots of kok-sagyz can be carried out already 3-6 months after transformation [41, 42].

Molecular genetic studies of T. kok-saghys. To use *T. kok-saghyz* as a model organism in the study of NR biosynthesis, modern molecular biological approaches are required - improved transformation protocols, the use of RNA interference (silencing) to suppress gene expression and EST (Expressed Sequence Tag) libraries. In molecular biology, the expressed sequence label (EST) is a short cDNA subsequence. EST identification is carried out quickly, and now there are about 74.2 million EST in publicly available databases (for example, GenBank as of January 1, 2013, all types). Earlier, we described in detail molecular genetic approaches to the study of NK biosynthesis in *T. kok-saghyz* cells [42].

The key enzymes in NK biosynthesis are cis-prenyltransferases associated with rubber particles (rubber transferases, CPT, RT); they synthesize the polyisoprene chain and can be isolated into a separate subfamily (CPT) [62]. CPT classes differ in cellular localization, the ability to bind substrate molecules, and the size of the reaction products formed. It is noteworthy that only RT-class enzymes can synthesize high-molecular polyisoprene ([41, 42, 86]. For cloning CPT *T. kok-saghyz* used degenerate primers corresponding to conservative sites of *H. brasiliensis* HRT1 and HRT2 [87], *Arabidobsis thaliana* ACPT [88] and *S. cerevisiae* Rer2 [89] enzyme sequences. RT-PCR (reverse transcription polymerase chain reaction) analysis performed using total latex RNA (milky juice rubber-bearing

plants) as a matrix, led to the identification of three cDNAs encoding structurally related CPT1-3 [90]. It is extremely important to note the fact that the intracellular concentration of CPT regulates the biosynthesis of natural rubber in cells *T. brevicorniculatum* is the closest relative of *T. kok-saghyz*. For a more complete understanding of the role of CPT1-3 in latex, transgenic plants of *T. brevicorniculatum* were obtained in which the expression of all three CPT genes was suppressed using the RNA interference method (RNAi) [91]. Transgenic lines demonstrated almost complete suppression of NK biosynthesis. It is noteworthy that transgenic plants were morphologically indistinguishable from wild-type plants.

Proteins functionally related to CPT have been identified relatively recently. One of them is SRPP, an acidic protein (pI 4.8) found in H. brasiliensis latex with a molecular weight of 23 kDa [92)]. The important role of SRPP in the biosynthesis of natural rubber has been reported [42]. Comparative analysis of *T. kok-saghyz* EST sequences using the known SRPP sequence from *H. brasiliensis* led to the identification of five cDNAs encoding potential SRPP1-5 ([90]. Studying the proteome *T. kok-saghyz* showed that three of these proteins (TkSRPP3, TkSRPP4 and TkSRPP5) are associated with rubber particles [93]. The main isoform associated with rubber particles, TkSRPP3, was studied in more detail. To characterize the functional role of SRPP in NK biosynthesis, the TkSRPP3 protein gene was overexpressed in the transgenic *T. kok-saghyz*. Real-time RT-PCR analysis showed that the number of transcripts of the TkSRPP3 gene in transgenic lines was increased (by more than 2 times). The Western blot also confirmed an increase in the level of TkSRPP3 in overexpressing transgenic lines. Measurement of the NR content in these lines demonstrated its increase (for example, by 30%) compared to the control. The molecular weight of natural rubber in the overexpressing lines practically did not differ from that in the control line and varied in the range of $1.0\text{-}1{,}2\times10^6$ Da [95]. Phenotypically transgenic plants did not differ from wild-type plants. To study the role of SRPP in NK biosynthesis, the expression of the TkSRPP3 gene in *T. kok-saghyz* was suppressed by RNA interference (RNAi). Several transgenic lines were obtained in which the mRNA level of the TkSRPP3 protein was significantly lower than in the control line. Western blot showed that the accumulation of TkSRPP3 protein in these lines was also reduced (by 60%). The molecular weight of the rubber in transgenic lines was also significantly lower than in the control [93]. Thus, the suppression of the expression of the TkSRPP3 gene in *T. kok-saghyz* cells significantly affects the amount of synthesized NR and its molecular weight.

The CPT family includes not only enzymes responsible for NR biosynthesis, but also other CPT capable of synthesizing polyisophene chains with a maximum length of up to 50 monomers [94, 95]. In eukaryotes, these enzymes synthesize dolichol, which is necessary for glycosylation of proteins, and other polyisoprenoids that perform various functions, including adaptation to stress [96, 97]. In humans, CPT, responsible for the biosynthesis of dolichol containing 22 isoprene units, interacts with Nogo-B receptor protein (NgBR). This protein stabilizes the enzyme through direct protein-protein interactions. It is also necessary for the enzymatic activity of CPT [98, 99]. Studying whether proteins related to NgBR can stabilize cis-prenyltransferases responsible for the biosynthesis of NR, which are part of the transferase complex on the surface of rubber particles, such a protein was found in *T. brevi-tacorniculatum* cells. It contains three conservative sites (motifs I, II and III), which are characteristic of NgBR plants and mammals. Based on the assumed functional analogy with NgBR, the authors named this protein a cis-prenyltransferase activator (TbRTA) [100]. RT-PCR analysis showed

that the concentration of mRNA encoding TbRTA in latex is much higher than in plant tissues. This correlates with the expression level of the CPT1-3 gene and suggests that TbRTA is involved in NR biosynthesis. To study the role of TbRTA in this process, the expression of the TbRTA gene in *T. brevicorniculatum* cells was suppressed using RTA-RNAi. The obtained transgenic lines showed pronounced inhibition [100]. It is noteworthy that the suppression of the expression of the TbRTA gene did not affect the growth and development of transgenic plants, they were indistinguishable from wild-type control plants. The effect of TbRTA gene expression pressure on NR synthesis was also studied. In wild-type plants, latex formed a foam-like upper layer containing rubber particles after centrifugation, while in transgenic plants the upper layer was absent. The absence of NR in transgenic lines was confirmed by ^{1}H-NMR analysis [100]. To find out whether the absence of natural rubber in transgenic plants is associated with the inhibition of TbCPT1-3 gene expression or there is a posttranslational loss of TbCPT1-3 proteins, RT-PCR analysis and Western blot were performed. It was shown that the mRNA levels of TbCPT1-3 genes are approximately the same in transgenic RNAi lines and a wild-type control plant, however, Western blot did not detect TbCPT1-3 proteins in transgenic lines. These results suggest that TbRTA is necessary to maintain the active formation of TbCPT1-3 as part of the transferase complex on the membrane of rubber particles and explain the absence of polyisoprene in TbRTA-RNAi transgenic lines. Thus, TbRTA not only activates TbCPT1-3, but also protects transferases from degradation. Moreover, because TbCPT1-3 do not have a transmembrane domain, TbRTA may play an important role in the localization of transferases on the surface of rubber particles (100). Thus, TbRTA is a key component of the transferase complex.

The technology of obtaining NR from *T. kok-saghyz* biomass is described [77, 79]. It is constantly being modernized and improved, however, for new rubber-bearing crops to become economically competitive, effective complex processing of by-products from leaves and residues of root biomass after isolation of rubber, resins and inulin is also necessary [80].

Advantages and disadvantages of natural rubber from T. kok-saghyz. Laboratory studies of the physical and chemical properties of NR from *T. kok-saghyz* have shown that this natural rubber has excellent quality and is in many ways like NR from *H. brasiliensis.* It is noteworthy that automobile tires made from this material are better in all characteristics than tires made from NR *P. argentatum* [6]. The high molecular weight (2.2×10^6 Da) fully confirms this conclusion [13, 17, 101]. One of the potential problems associated with NR from Russian dandelion is the high protein content, which is even higher than in rubber from *H. brasiliensis* [11]. Consequently, people sensitive to NR from hevea may also be allergic to the polymer from *T. kok-saghyz* [37, 38], therefore, NR from kok-sagyz is preferable to use in areas not related to medicine, for example in the automotive industry.

Tau-sagyz (*Scorzonera tau-saghyz*). The perennial semi-shrub kozelets tau-sagyz (*Scorzonera tau-saghyz*) (Fig. 9) is certainly one of the most promising alternative sources of NR, to which, in our opinion, the scientific community pays insufficient attention. His homeland is the Karatau mountain range in Southern Kazakhstan. The content of NR in the roots of tau-sagyz changes with age. The roots of an annual plant usually contain 1-8% NR per dry mass. In 2-3-year-old plants, the content increases to 8-30%. Interestingly, when growing tau-sagyz under optimal conditions, the accumulation of NR in the roots can reach 40% of the dry weight. Unfortunately, the number of tau-sagyz in natural conditions

critically decreased in the 1940s due to intensive harvesting. More than 12 million roots with a total dry weight of about 900 tons were used for the needs of the military industry. This was enough to produce about 300 tons of NR [102-104]. Currently, tau-sagyz is rarely found in nature, and the restoration of its natural habitats is very slow. Tau-sagyz is less competitive than other plants in the same habitats, and intensive development of adjacent territories leads to an even greater reduction in the number of this rare species. To restore it, technologies based on the use of microbiological preparations, in particular fungi that form arbuscular mycorrhizae, can be used.

Fig. 9. Tau-saghyz (*Scorzonera tau-saghyz* Lipsch. et Bosse): A — tau-saghyz plants in natural conditions; B — roots of tau-saghyz plants from places of natural growth; C — milky juice of tau-saghyz. Photos courtesy of K.K. Boguspaev (Kazakhstan)

Arbuscular mycorrhiza (endomycorrhiza) is a mutually beneficial symbiosis of microscopic fungi of the *Glomeromycota* department with higher vascular plants that significantly increases the viability of the host. Endomycorrhiza increases the availability of nutrients (in particular, phosphorus and nitrogen) for the host plant, increases the intensity of photosynthesis, which, in turn, leads to a significant accumulation of the root and aboveground mass of the mycorrhizal plant [105-107]. Mycorrhization of tau-sagyz seedlings using fungi of the genera *Claroideoglomus* and *Rhizophagus* [104] revealed structures in the roots (unsepted mycelium, vesicles and arbuscules) characteristic of fungi forming arbuscular mycorrhizae. The control samples lacked these structures. Plants treated with mycorrhizal fungus inoculum grew noticeably better than non-mycorrhizal ones [107)]. The average height and number of leaves in mycorrhizal plants were 1.5-2.0 times higher than in non-mycorrhizal plants. The conducted studies indicate that arbuscular mycorrhizae play an essential role in the life of tau-sagyz, and optimized biotechnologies for growing this rare and endangered species and promising rubber plant can be developed on their basis.

Summing up the discussion of the problem of natural rubber plants, we note that the interest in plants that can function as sources of various materials is due to many reasons. NC is not only one of the most important polymers used by mankind, but also a renewable polymer. Unfortunately, the source of this polymer,

H. brasiliensis, is under the influence of negative biotic (SALB) [20, 21] and abiotic factors (economic development negatively affecting the natural habitat, and climate change). The development of alternative sources of NR is, of course, extremely important in the medium and long term, because it will not only ensure greater availability of this polymer, but also reduce the dependence of mankind on fossil fuels needed to produce synthetic analogues of NR. Rubber carriers can also be used to produce other important products, such as bioethanol from lignocellulose (*S. tau-saghyz, P. argentatum*) or inulin from *T. kok-saghyz*. The achievements of genomics, proteomics, metabolomics, and biotechnology will certainly help to make significant progress in understanding the processes of NR biosynthesis. This, in turn, will lead to the creation of new forms and genotypes of plants with a high rate of growth and development, the ability to super-synthesize NR, and will also allow the development of optimal technologies for their cultivation.

So, the ever-growing demand for natural rubber (NR) cannot be satisfied in the future at the expense of the rubber tree alone. Alternative crops are needed that can be grown on large areas in industrial volumes, and appropriate technologies for processing and obtaining final products. The economic feasibility of introducing new NR producing crops depends not only on increasing the productivity of the plant, but also on the complex processing of the entire plant to obtain additional products. The introduction of any new culture is an extremely difficult task. In the case of rubber carriers, simultaneous coordinated expansion of agricultural areas and processing capacities is required. In the long term, rubber from alternative crops, especially its thermostable derivatives such as epoxidized rubber, can supplement the market share currently occupied by various synthetic rubbers, with a significant reduction in the carbon footprint.

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THE FEATURES OF ROOT FORMATION OF SOME FODDER SEMI-SHRUB AND SHRUB HALOPHYTES IN THE FOOTHILL DESERT OF UZBEKISTAN

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Abstract

Distinguishing feature of halophytes as fodder plants are high nutritional value, stable balance of nutrients over seasons, especially during critical periods of pasturing during autumn and winter, and a high content of essential amino acids. Halophytic fodder dwarf semi-shrubs, *Kochia prostrata* (L.) Schrad. and *Salsola orientalis* S.G. Gmel., and shrubs, *Haloxylon aphyllum* (Minkw.) Iljin and *Aellenia subaphylla* (C.A. Mey) Aellen. perform high and sustainable fodder productivity under xerothermic conditions of the Central Asian deserts. In these conditions, shrub and semi-shrub halophytes can successfully complete a full life cycle due to structural, physiological and biological adaptations. These re a succulent type of the leaf photosynthetic apparatus (R.M. Ogburn et al., 2010), a multilayer epidermis, thickening of the cuticle (R.F. Sage et al., 2011) and the C-4 plants which are more efficient in transpiration compared to C_3 plants and lower water consumption (V.I. Pjankov et al., 1991; V.I. Pankov, 1993). Roots play a central role in the yield formation and now considered key drivers of the second "green revolution". Knowledge of the *Chenopodiaceae* shrubs' and semi-shrubs' root formation in the foothill desert conditions elucidates fundamental peculiarities of these halophytic plant biology and provides the correct placement of the crops in arid zones. We compared parameters of root formation in shrubby and semi-shrubby halophyte species to identify their ecological role in the conditions of the Central Asian foothill desert (Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 2015-2020) in plants of the 1st and 5th year of life. The halophytes of family *Chenopodiaceae* have acquired adaptive properties and increased production functions due to evolutionary developed powerful and deeply penetrating roots capable of the use of precipitation, condensation moisture and shallow ground water. Semi-shrubs *Kochia prostrata* (L.) Schrad., *Salsola orientalis* S.G. Gmel. and shrubs *Haloxylon aphyllum* (Minkw.) Iljin, *Aellenia subaphylla* (C.A. Mey) Aellen. are capable of rapid root growth and development. The roots of 1-year old plants penetrate into the soil to a depth of 235 cm in *H. apyllum*, 150 cm in *A. subaphylla*, 200 cm in *S. orientalis*, and 215-295 cm in *K. prostrata*. At the age of 5 years, the roots reached a depth of 1240 cm, 600 cm, 550 cm, and 580 cm, respectively. Therefore, the root length exceeds the height of the aerial part in the 1st year by 4-4.5 times, and at the age of 5 years by 6 times. The ability to high growth rates of the root system is an important condition for uninterrupted water absorption by the roots in conditions of moisture deficiency and drought. The depth of penetration of the root system of plants of different life forms (shrubs, semi-shrubs) is strongly influenced by the water-physical properties of the edaphic environment. In conditions of permanent soil moisture deficiency, the root system tends to constantly go deeper into the soil-soil environment, breaking through dense, cemented soil layers. In our opinion, for semi-shrubby, shrubby halophytes can not only uptake water by roots from deep soil but also move it to drier soils horizons where this water can be used by plants with a shallow root system. Therefore, the studied

halophytes can obviously provide a function of hydraulic lift.

Keywords: fodder halophytes, shrubs, semi-shrubs, *Salsola orientalis* S.G. Gmel., *Kochia prostrata* (L.) Schrad., *Aellenia subaphylla* (C.A. Mey) Aellen, *Haloxylon aphyllum* (Minkw.) Iljin, root system, morphology, hydraulic lift

The distinctive features of halophytes as forage plants are high nutritional value, stable balance of nutrients according to the seasons, especially during critical periods of grazing, in autumn and winter, a high content of essential amino acids [1]. The forage shrubs and semi-shrubs are important as protein sources in many regions. Thus, in Western Australia, the crude protein content in *Kochia brevifolia* R. Br. is 20%, in *Atriplex nummularia* L. and *Atriplex vesicaria* Heward ex Benth. 14-18% [2]. An important biological feature of shrubs such as *Haloxylon aphyllum* (Minkw.) Iljin, *Aellenia subaphylla* (C.A. Mey) Aellen and semi-shrubs (*Kochia prostrata*) is rapid growth and development in culture [3, 4].

During haloxerophilization, under the influence of the increasing aridization of the climate, shrubs and semi-shrubs of the *Chenopodioideae* family underwent the deepest adaptive restructuring of various traits and functions, including physiological and biochemical ones. Such a restructuring in xerothermal conditions is primarily the reduction of evaporating vegetative organs and the strengthening of the absorbing (suction) function of the root system [5, 6].

In improving the adaptive properties of shrubby and semi-shrub halophytes, which ensured their successful functioning and reproduction in the harsh xerothermal conditions of the Central Asian desert, root systems that penetrate deeply into the soil are obviously of great importance [7]. It is known that roots are very important for the consolidation and absorption of water and mineral resources, but not all researchers consider roots as an important organ taking an active part in the formation of phytomass (harvest) [8]. Traditionally, most researchers have focused on the study of the aboveground part of plants (stems, leaves, flowers, fruits, and seeds) and overlooked the root system [9]. Nevertheless, many researchers are currently beginning to understand that plant roots play a central role in crop formation. In the review published in 2010 in the Nature journal, V. Gewin [10] notes that the success of the first ”green revolution” is associated with the selection of dwarf short-stemmed wheat varieties, in which energy and metabolites are mainly spent on the formation of grains, rather than stems. According to forecasts [10], the key factor of the second “green revolution" is the root system, i.e., the improvement of its architectonics, ecological and physiological functions.

An in-depth understanding of issues related to the root system of plants is associated with solving practical problems in crop production, in particular, more efficient use of fertilizers and water and ensuring sustainable productivity under various biotic and abiotic stresses [11]. Salinization of land creates unfavorable conditions for agricultural production, leading to global annual losses of products in the amount of exceeding 12 billion US dollars [12]. In China, saline-alkaline soils account for 25% of agricultural land and are underutilized. One of the sustainable strategies for more effective involvement of saline lands in agricultural production is the breeding of halophytes that can survive and complete their life cycle in soil environments containing more than 200 mM NaCl [13]. Recently, studies conducted in Iran found that halophytic species of the genus *Suaeda* spp. contain nitrogen-fixing endophytic bacteria in the roots, which can make a significant contribution to providing plants with nitrogen [14].

The study of halophytes is additionally actualized die to climate change and the need to provide food to the growing population of the Earth [15, 16].

According to I.I. Sudnitsyn [17], the rate of water absorption by a plant is directly proportional to the depth of penetration and the density of the root

placement in the soil layer. Therefore, information about peculiarities of the root system formation in shrubs and semi-shrubs of the family *Chenopodiaceae* in the foothill desert conditions (e.g., the growth rate, the depth of penetration into the soil) is very important not only for the knowledge of these halophytic plant life forms but also for the correct placement of crops in arid zones.

This paper for the first time examines the role of the root system in the water supply and water balance maintenance in halophytic shrubs and semi-shrubs under the xerothermal conditions of the Central Asian desert.

The aim of the work is to compare the formation of the root system of shrub and semi-shrub species of halophytes and to reveal their ecological role in the conditions of the Central Asian desert.

Materials and methods. The study was conducted in the area of the foothill desert (Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 38.62624 N, 65.69219 E) in 2015-2020 in the introduction nursery of fodder shrubs and semi-shrubs, founded in 2015. The excavation area of root systems of plants of various life forms is located in the lower belt of the foothill desert at 354 m above sea level. The climatic conditions of the research area are characterized by high dryness and continental climate.

In experiments with the semi-shrub *Kochia prostrata*, three ecotypes were studied: rocky (seeds were collected in the Osh region of Kyrgyzstan), sandy (seeds were collected in the Kyzylkum deserts in Uzbekistan, Muyunkum in Kazakhstan and in the Caspian semi-desert) and solonetzic (the Achikulak Forest Research Experimental Station, Russia).

Phenological observations for each studied species were carried out on 75 plants in three repetitions.

The excavation of the root systems of shrubs *Haloxylon aphyllum* (Minkw.) Iljin, *Aellenia subaphylla* (C.A. Mey) Aellen. and semi-shrubs *Kochia prostrata* (L.) Schrad., *Salsola orientalis* S.G. Gmel. was carried out by the trench method [18]. The excavation of roots of shrubs and semi-shrubs at the age of 1 year was carried out in three plants of each species at different phases of development (seedlings, true leaves, branching, flowering, fruit formation). To excavate the roots systems of the *Haloxylon aphyllum*, *Aellenia subaphylla*, *Salsola orientalis*, *Kochia prostrata* at the age of 5 years, a plant was selected that outwardly corresponded to an average representative of each of the studied species.

Statistical processing of the obtained data was carried out in the Microsoft Excel 2010 program. The results are presented as means (*M*) and their standard errors (±SEM).

Results. In the zone of the foothill desert of Kashkadarya region, the growing season consists of mesothermal and xerothermal periods characterized by a certain temperature and humidification regime [5]. The mesothermal (cool and humid) period falls on November to April. At this time, an average of 224 mm of precipitation falls annually in the research area. The xerothermal (dry) period covers May to October. In summer, the soil dries up due to physical evaporation and transpiration of plants because of high temperatures, insolation and constantly blowing winds. The average annual air temperature is 14.8 °C, +47 °C the maximum, 27 °C the minimum. The temperature transitions through 0 °C predominately occurs at the end of February and the beginning of March. The average air temperature in February is 3.6 °C, in March 9.4 °C, and in April 15.7 °C. Relative humidity over the year is 30% on average, in summer 10% on average.

The soils where the root systems were excavated, as well as the entire lower belt of the foothill desert, are mainly light gray. A characteristic feature of the soil profile is its stratification where horizons of light loam, medium loam, heavy loam and sandy loam alternate.

The soils are largely salinized and can be attributed to saline soils. Only the upper 8-centimeter layer is not salinized, below the salinity is weak, 0.25-0.45%, and at a depth of 94-610 cm the content of water-soluble salts reaches 1.35-2.77%. The gypsum content in the soil of these sections is small and ranges from 0.5-35.8% along the horizons, humus concentration in the root layer is 1.86-2.02%, total nitrogen along the horizons is 0.006-0.12%, total phosphorus 0.02-0.19%. Potassium is present in sufficient quantities throughout the root-inhabited horizon (936 mg/kg in the upper layers, 30 mg/kg in the lower layers).

Salsola orientalis S.G. Gmel. (family *Chenopodiaceae*) is a perennial plant of 40-60 cm in high with a stem of 5-10 cm in high from which 3-6 skeletal axes depart [19]. The *Salsola orientalis* is a haloxerophytic semi-shrub characterized by high tolerance to drought and resistance to salt stress [6]. The ability of the *S. orientalis* to successfully perform a full life cycle under xerothermal arid conditions and high soil salinity is due to structural adaptations and succulent form of leaves [20], including multilayered epidermis, thickening of the cuticle]21] and C4-type photosynthesis to provide more efficient use of water for transpiration than in C3-plants [22, 23].

The data characterizing the growth of the root system of the *S. orientalis* in the first year of life are given in Table 1. At the end of April, at the 0.9-1.0 cm height of the aboveground part of the plant, the roots of the *S. orientalis* penetrate to a depth of 44±5.3 cm, at the end of May they deepen to 80±4.1 cm, at the end of the growing season (2.XII.2016) up to 200±11.2 cm.

1. Root growth and development of *Salsola orientalis* S.G. Gmel. Plants of the 1st year of life (n = 9, M±SEM; introduction nursery, foothill desert zone, Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 2016)

Date and phase of development	Depth of penetration of the root system, cm	Width of the horizontal spread of the root, cm	The ratio of the length of the roots to the height of the aerial part
24.IV. Seedlings	44.0±5.3	16.5±3.3	4.7
31.V. True leaves	80.0±4.1	95.0±2.7	4.1
5.VII. Branching	105.0±7.4	75.0±5.2	2.6
3.IX. Flowering	125.0±5.7	85.0±7.3	2.5
2.XII. Fruiting, the end of growing season	200.0±11.2	145.0±6.4	3.4

Interestingly, the depth of penetration of the roots of the *S. orientalis* is 4.1-4.7 times greater than the height of its aboveground part, and remained 2.5-3.5 times greater in the second half of the growing season. The root coefficient (the maximum depth of root penetration into the soil × the maximum diameter of its spread) [24] was 726-7600 in the first half of the growing season and 10625-29000 in the second half. In the second and subsequent years of life, the root system continues to develop. According to our observations, in May, the root system of the 5-year-old *S. orientalis* plants was powerful, penetrating the soil to a depth of 550 cm (Fig. 1).

The main root at a depth of 8 cm is divided into two large roots going down at a slight angle to each other. In turn, one of them at a depth of 12 cm, the other at a depth of 17 cm are divided into two, forming four rather large roots. One of them at a depth of 25 cm turns at an angle of 45 degrees to the side by 65 cm, gradually deepening into the ground. At a depth of 140 cm, one of the roots turns sharply to the side, horizontally by approx. 1 m in length, then goes down again, and at a depth of 330 cm, entering a dense fine-grained horizon, goes horizontally to the side. The other, the thinnest of the three roots, goes vertically down, branches strongly into small white tails. The third root at a depth of 340 cm, making a loop, goes slightly up and to the side by 30 cm, and then turns sharply down. The main root of the *S. orientalis* plant, having reached the dense horizon,

turns aside, divides into several roots that diverge along the sides. Some roots even rise up and branch into numerous thin roots, which, in turn, dividing into smaller ones, end in a loose medium-loamy moist horizon. The rapid growth and formation of a powerful root system of the *S. orientalis* are crucial in the rational use of water and mineral resources from the soil to provide high forage and seed productivity under arid conditions of the foothill desert.

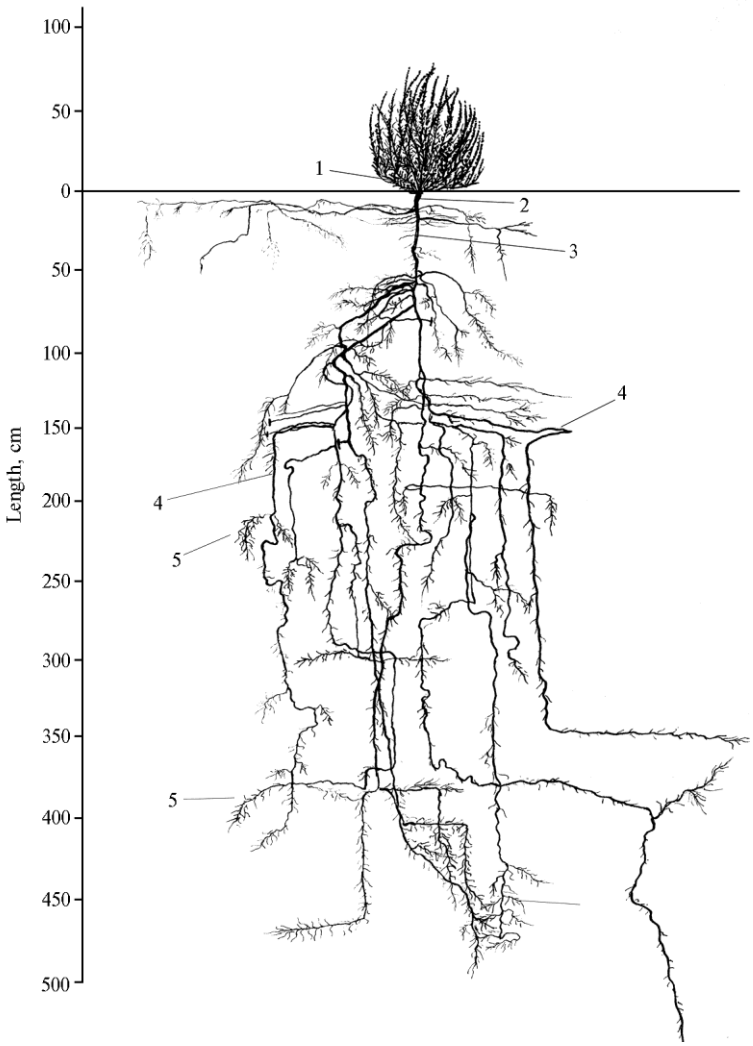

Fig. 1. Root system of *Salsola orientalis* S.G. Gmel. 5-year old plants on medium loamy gray soils: 1 — renewal buds, 2 — root neck, 3 — main root, 4 — lateral roots, 5 — root hairs (introductory nursery, foothill desert zone, Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 2020).

Kochia prostrata (L.) Schrad. (*Chenopodiaceae* family) is a perennial semi-shrub xerogalophyte, according to our observations, it has a height of 75-110 cm, forms 16-25 kg/ha of dry fodder mass which is 16-18% protein. It is intended for cultivation without irrigation to create long-term highly productive pastures in arid areas under low and medium soil salinity. In experiments conducted in the arid regions of the USA (Idaho and Utah), *K. prostrata* turned out to be the best in terms of productivity, nutritional value and digestibility of feeds obtained from it [25-27].

We compared the development of various ecotypes of *K. prostrata* plants in the 1st year of life (Table 2). It was found out that the depth of root penetration

varies significantly depending on the ecotype.

2. Growth and development of *Kochia prostrata* (L.) Schrad. of various ecotypes in the 1st year of life (n = 9, M±SEM; introduction nursery, foothill desert zone, Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 2016)

Ecotype	PH	CW	NS	DRP	HRS	NR1
Rocky (from Kyrgyzstan)	92,0±1,4	100,0±6,3	32,0±4,2	240,0±12,6	115,0±13,6	29,0±2,1
Sandy (from Kyzylkum)	67,0±0,8	65,0±5,2	23,0±3,0	237,0±18,7	130,0±15,8	25,0±1,8
Sandy (from Muyunkum)	73,0±2,7	36,0±2,5	18,0±3,6	270,0±10,5	100,0±19,6	19,0±2,3
Sandy (from Volgograd)	50,0±4,2	47,0±2,8	10,0±2,7	295,0±22,3	90,0±16,1	17,0±2,6
Solonetzic (from Achikulak)	54,0±1,8	67,0±0,4	25,0±3,4	215,0±17,3	135,0±10,5	21,0±1,6

N o t e. PH — plant height, cm; CW —crown width, cm; NS — the number of shoots; DRP — depth of root penetration, cm; HRS — horizontal root spreading, cm; NR1 — the number of roots of the 1st order.

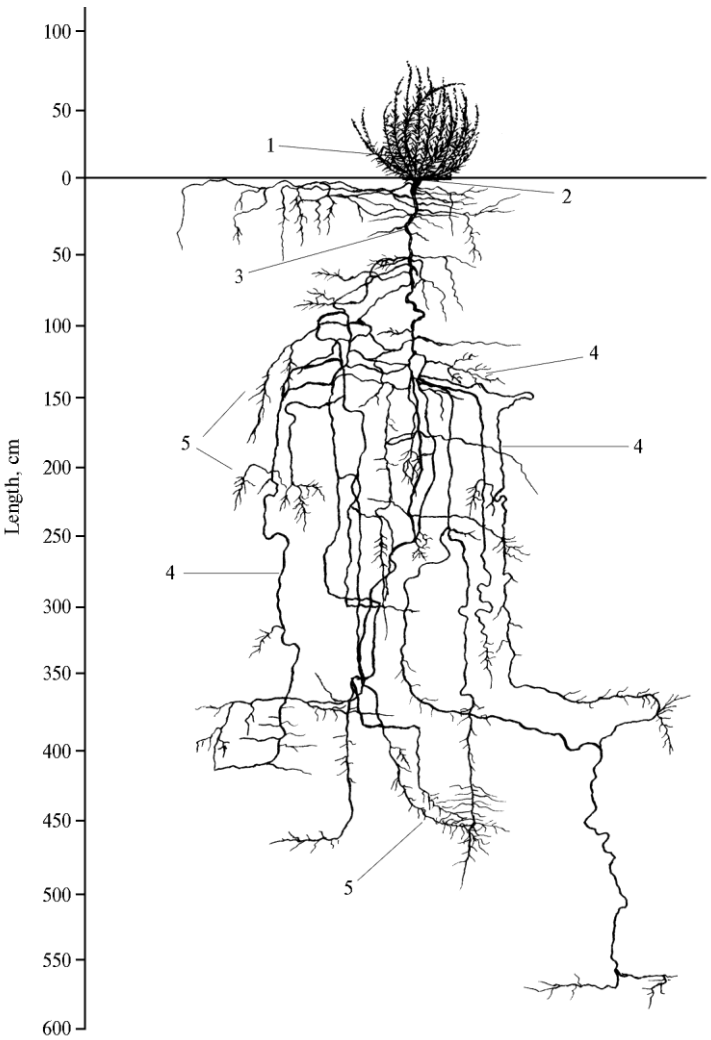

Fig. 2. Root system of *Kochia prostrata* (L.) Schrad. 5-year old plants on medium loamy gray soils:: 1 — renewal buds, 2 — root neck, 3 — main root, 4 — lateral roots, 5 — root hairs (introduction nursery, foothill desert zone, Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 2020).

There were differences in the structure of the root system between ecotypes. The *K. prostrate* rocky ecotype has a pronounced main root. In sandy ecotypes, root systems are quite powerful and deeply penetrating. However, at a depth of 80-100 cm, their main root in size and development becomes similar to the lateral roots of the 1st order. The root system of the *K. prostrate* solonetzic ecotype is the weakest of all.

In the 5-year-old *K. prostrate* (rocky ecotype) plants, the roots penetrate to a depth of 580 cm under the conditions of the Nishan steppe (Fig. 2). The main root is vertically directed downward. The diameter of the root neck is 4 cm. At a depth of 10 cm, a large root departs from the main root, going in a horizontal direction. It does not go deep into the soil and branches strongly. The main root deepens, while forming small bends and turns. At a depth of 50-75 cm, many small and several large lateral roots appear on the main root. Small roots in these layers end, and large ones go down. At a depth of 75 cm, the main root is noticeably thinned, at a depth of 120-125 cm, it branches strongly and forms many thin roots directed downward. Up to a depth of 300 cm, large lateral roots carry a large number of living thin roots. Most of the roots heading down at a depth of 400 cm penetrate into the dense forest horizon, here they are greatly thinned, breaking up into a large number of small ones, and in a layer of 450-470 cm from the surface, they end, forming a dense network of root hairs. Only one root of the *K. prostrate* plant passes through the sedimentary horizon and is buried in a loose medium loamy layer at a depth of 580 cm.

In the morphology of the root system of *K. prostrate*, growing in the conditions of the foothill desert (Nishan steppe), there are three clearly distinguishable tiers. The first tier is ephemeral roots located in a layer of 0-25-30 cm, the second tier is located at a depth of 120-150 cm in the zone of strong lateral branching of the roots. The third tier is the zone of the end of the roots where they are strongly branched and carry many sucking small roots.

Thus, the root system of the *K. prostrata* cultivated in the foothill desert can be characterized as powerful and deeply penetrating, capable of utilizing water and mineral resources from a large volume of soil and soil solution.

Aellenia subaphylla (S.A. Meu) Aellen (*Chenopodiaceae* family) is a perennial haloxerophytic shrub with stems covered with light gray bark [19]. The plants are strongly branched, with branches inclined mostly away from the stem, having a light green color, sometimes with a bluish tinge. The plant height can reach 1.5-2.0 m. The species is exceptionally polymorphic and found in various ecological conditions of the arid zone. A distinctive feature of the *Aellenia subaphylla* is its high drought resistance and ability to grow on saline soils. In the initial growth phases, the root system develops vigorously and deepens into the soil (Table 3).

3. Growth and development of *Aellenia subaphylla* (C.A. Mey) Aellen in the 1st year of life (n = 75, M±SEM; introduction nursery, foothill desert zone, Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 2016)

Date and stage of development	Seedling and plant height, cm	Depth of root penetration, cm	The number of roots of the 1st order a
24.IV. Seadlings; seed leaves	2.0±0.2	21.0±2.0	
30.V. Appearance of true leaves:			
two	2.5±0.5	30.4±1.8	5.6±1.4
four (5.VI.)	6.4±0.4	36.0±4.3	7.4±1.1
six (13.VI.)	8.0±1.2	43.0±2.8	11.8±2.0
eight (20.VI.)	9.6±1.6	46.0±3.3	12.8±1.5
5.VII. Branching begins	20.0±2.6	46.0±2.8	21.0±2.7
3.IX. Flowering begins	55.0±1.4	53.0±1.5	50.0±5.8
2.XII. Fruit formation	77.0±3.3	150.0±3.7	58.0±3.6

In the phase of cotyledon leaves, a plant height is 2.0±0.2 cm, the roots deepen into the soil by 21.0±2.0 cm, at a height of 2.5±0.5 cm by 30.4±1.8 cm, at 8.0±1.2 cm by 43.0±2.8 cm. At the end of the growing season in the 1st year of life (in the fruiting phase), the roots penetrate to a depth of 150.0±3.7 cm.

In May, we excavated the underground part of the *A. subaphylla* plant of the 5th year of vegetation. The main root, which was 6 cm thick, goes vertically down. At a depth of 12-15 cm, two powerful lateral roots, spreading apart, are

directed vertically downwards (Fig. 3). The main root at a depth of 37 cm is divided into two roots. The latter, in turn, were divided into smaller ones several more times as they went deeper into the soil. All the *A. subaphylla* roots of the 1st order, with the exception of some small ones, have very few lateral branches in the 0-140 cm layer, and only from a depth of 140 cm the number of lateral roots increases. In all strongly compacted horizons, for example at a depth of 170-180 and 220-230 cm, there are loose layers with a thickness of 5-15 cm. The roots, once in these layers, creep in a horizontal direction, moving away from the main root to the sides (at a distance of up to 2 m or more). They have many branches in the vertical direction, which, in turn, branch into many small roots. At a depth of 300-320 cm, there are many living and dead root hairs on the roots. At a depth of 500 cm in the soil, there are often empty or containing loose rocks cracks. The roots, falling into them, form bundles of thin roots that fill these spaces. Below 600 cm there is a dense, as if cemented gravel horizon. The roots of *A. subaphylla*, having reached this horizon, do not penetrate into it, but branching strongly, creep over it, forming a dense network of small roots.

Haloxylon aphyllum (Minkw.) Iljin] (*Chenopodiaceae* family) is a leafless shrub (or semi-tree) with a height of 3-4 m. The assimilating function belongs to annually falling twigs, succulent halophyte [19]. The ability of the *H. aphyllum* to successfully perform a full life cycle at high concentrations of salts in the soil is largely realized due to transformation of lamellar leaves into cylindrical photosynthetic organs, the layering of the epidermis and thickening of the cuticle. *H. aphyllum* has broad ecological resistance to soil salinization, it grows on both sandy and clay and gravelly soils with varying degrees of salinity. *H. aphyllum* withstands mineralization of groundwater up to 40 g/l. The root system is powerful, penetrating deeply into the soil. It occurs mainly in areas with close groundwater occurrence, but can also grow in automorphic conditions. The eaten parts of *H. aphyllum* plants in the autumn-winter period are annual shoots, last year's twigs, fruits. The shoots contain 10-12% protein (fruits are up to 20% protein), 2.2-2.7% fat, 21.2-38.6% ash substances, 39.3% nitrogen-free excretory substances, 14.9% fiber.

In the 1st year of life, the roots of the *H. aphyllum* plants at the beginning of the growing season (25.IV.) penetrate to a depth of 29-36 cm and are 4-5 times longer than the aboveground part. By the end of the growing season, they spread to a depth of up to 235 cm, and in the horizontal direction up to 160 cm. In the foothill desert (Nishan steppe) the *H. aphyllum* 5-year old plant forms a powerful root system which deeply penetrates into the soil (Fig. 4). The main root at a depth of 30-40 cm branches into three roots with a diameter of 8-10 cm, and at a depth of 50 cm one of the roots divides, in turn, into three more parts. The soil in the 0-75 cm layer is quite dense, then it becomes looser, and at a depth of 300-360 cm it compacts again. When they reach the compacted horizon, the roots branch out strongly and pass through cracks deep into the soil. At a depth of 400-500 cm, there is a slight increase in soil moisture. Here, the structure of the soil is layered. In these layers, the roots branch little, going deep into the soil. The root system as a whole tends vertically downwards. At a depth of 800 cm, a very dense layer with a thickness of 30 cm lies. In this layer, the roots thin out, flatten, passing through a dense layer, take a rounded shape. From a depth of 860 cm, a small-granulated layer begins, turning into a homogeneous sandy horizon. Here, the soil is moist, a lump forms when compressed. At a depth of 1130-1200 cm, the soil is sandy loam and very moist. Here the root branches strongly and forms a large number of living white roots. At a depth of 1240 cm, the soil becomes very moist, water droplets are exposed in the lumps when breaking. At this depth, salty water has been accumulating for some time.

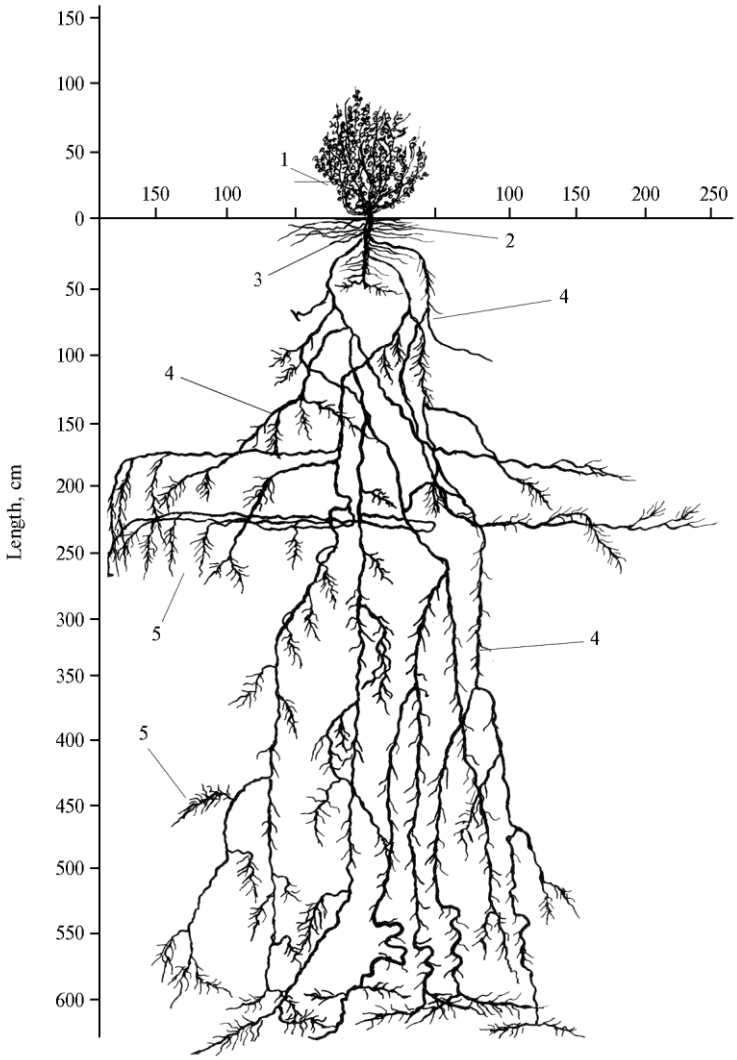

Fig. 3. Root system of *Aellenia subaphylla* (C.A. Mey) Aellen 5-year old plants on medium loamy gray soils:: 1 — renewal buds, 2 — root neck, 3 — main root, 4 — lateral roots, 5 — root hairs (introduction nursery, foothill desert zone, Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 2020).

Thus, at the age of 5 years, *H. aphyllum* plants form a powerful root system of a universal type, adapted to the use of atmospheric precipitation, condensation moisture and groundwater.

Analyzing the results obtained, it should be noted that plants of different life forms, in which we studied the peculiarities of the formation of root systems, have different types of adaptive strategies according to the Ramensky-Grime classification. *Haloxylon aphyllum*, *Aellenia subaphylla*, *Kochia prostrata*, being "violents" according to L.G. Ramensky [28] or "competitors" according to J. Grime [29], have a high competitive ability characterized by rapid growth, the ability to capture and hold territory for a long time, suppressing the opponent, and fully use the resources of the environment.

Salsola orientalis, according to the adaptive strategy, refers to tolerators. Stress-tolerators [28, 29] are species that exist not due to high energy of vital activity, but due to endurance under the influence of stressful environmental factors. Therefore, tolerant plants, depending on the growing conditions, are resistant to low water availability, soil salinity or other unfavorable environmental factors.

The ability to restrict moisture use for transpiration plays an essential role in the formation of the violents' properties of *Haloxylon aphyllum*, *Aellenia subaphylla*, *Kochia prostrata* and the tolweators' properties of *Salsola orientalis* in the xerothermal conditions of the Central Asian desert. According to our data (4) obtained in the Central Asian Karnabchul desert, in April, the average daily transpiration intensity in *Haloxylon aphyllum*, *Aellenia subaphylla*, *Salsola orientalis* was 301.6-492.0 mg/h. Under the same conditions, salt-loving *Aremisia halophile* Krasch., a typical representative of the desert flora consumes 2 times more water for transpiration, 957.7 mg/h..

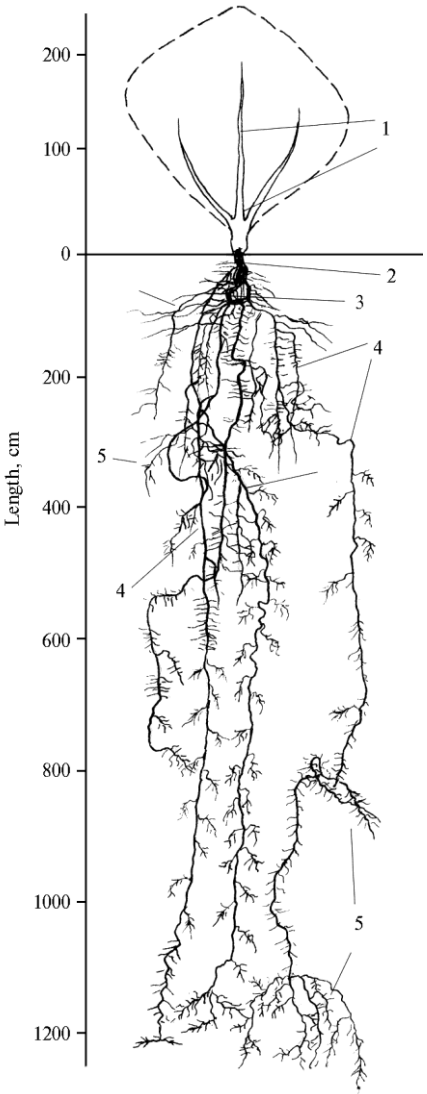

Fig. 4. Root system of *Haloxylon aphyllum* (Minkw.) Iljin 5-year old plants on medium loamy gray soils:: 1 — renewal buds, 2 — root neck, 3 — main root, 4 — lateral roots, 5 — root hairs (introduction nursery, foothill desert zone, Nishan steppe, Kashkadarya region, Republic of Uzbekistan, 2020).

Thus, our data and the results of other researchers confirm the position that forage shrubs (*Haloxylon aphyllum*, *Aellenia subaphylla*) and semi-shrub halophytes (*Kochia prostrata* (L.) Schrad., *Salsola orientalis*) are characterized by economical consumption of water for transpiration. During the long-term evolution of plants from true mesophytes to xerogalophytes, a profound adaptive

transformation of their morphology occurred, which was primarily expressed in the reduction of producing organs [30]. As a result, in the *Kochia prostrata* and *Aellenia subaphylla* species, the lamellar leaves turned into small pubescent leaves, and in the *Salsola orientalis* and *Haloxylon aphyllum* species, the leaves turned into cylindrical assimilating succulent fleshy leaves with a multilayer cuticle [31], which reduces the evaporation surface and the intensity of transpiration [20].

In the formation of various types of adaptive strategies during evolution under the xerothermal conditions of the Central Asian desert, along with the development of haloxerophilized properties of photosynthetic organs, the ability to form a rapidly growing and deeply penetrating root system played an important role in forage shrubs and semi-shrubs. The results obtained by us (see Fig. 1-4) show that under moisture deficiency, excessively high temperature and dry air, *Salsola orientalis*, *Kochia prostrata* (semi-shrubs), *Haloxylon aphyllum* and *Aellenia subaphylla* (shrubs) are able to maintain normal hydration of tissues [4] due to increased water absorption by roots [32]. It follows that the ability of plants to continuously absorb water is associated with the activation of the growth of their roots [10]. It turned out that the lack of soil moisture causes increased root growth, thereby increasing the possibility of water absorption [33]. Thus, the ability of the roots to continuously grow plays a decisive role in adapting to water scarcity, since thanks to this the plant receives water in the required amount [34].

The data obtained show that *Salsola orientalis*, *Kochia prostrata*, *Haloxylon aphyllum* and *Aellenia subaphylla* have a high growth rate of the root system which penetrates deeply into the soil. In the 1st year of life, the depth of the root system of these semi-shrubs and shrubs is more than 4-5 times higher than the height of their aboveground part. The fast-growing and deeply penetrating roots of semi-shrub and shrub halophytes ensures their successful functioning in the harsh conditions of the Central Asian desert and allows them to survive a long dry summer period. We found that the roots of semi-shrubs and shrubs during growth and development penetrate through very dense layers of soil, comparable in density to concrete. The scientific literature discusses the ability of plants to penetrate dense layers of soil. It is associated with the structural features of the root tip which provides overcoming the resistance of dense dry soils. It is assumed that one of the mechanisms of this may be the formation of root hairs that act as an anchor when the root moves through dry dense soil layers [35]. Water transport along the phloem to the root tip can play an important role in maintaining continuous root growth in dense dry soil layers. Its role was established using three-dimensional modeling of water distribution depending on the location of phloem endings [36].

V.G. Onipchenko [37] described the phenomenon of the so-called hydraulic lift, when a plant is able not only to lift water by its roots, but also to release it into drier soil horizons. The phenomenon of hydraulic lift is widespread in arid regions [38]. In desert conditions, plants with their roots penetrating deeply into the soil raise water into the surface layers, where plants with a shallow root system can use this water. Similar results were obtained in other studies in the forests of *Acer saccharum* Marshall where plants of the lower tiers received water due to a hydraulic lift provided by maple. It is shown that during the night an adult sugar maple tree can pump about 100 l of water from the lower soil horizons to the upper ones [39]. One tree of the umbrella acacia *Acacia tortilis* (Forssk.) Hayne in Africa raises from 70 to 235 l of water per night [40]. Currently, more than 90 plant species with this ability have been described [41, 42].

Hydraulic lifting is the passive movement of water from the roots into the

soil layers with a lower water potential, while other parts of the root system in the wetter soil layers, usually at depth, absorb water [43, 44]. Hydraulic redistribution ensures the passive movement of water between different parts of the soil through the root systems of plants, caused by gradients of water potential at the soil—plant interface. Hydraulic redistribution can have important consequences on a community scale, affecting net primary productivity, as well as the dynamics of water reserves and growth development. On a global scale, it can affect hydrological and biogeochemical cycles and, ultimately, the climate. The results obtained by comparing the features of the formation of the root system of shrubs and semi-shrubs give reason to assume that *Salsola orientalis*, *Kochia prostrata*, *Haloxylon aphyllum* and *Aellenia subaphylla* are capable of performing the function of a hydraulic lift due to the formation of a root system that penetrates deeply into the soil. The release of water in the soil due to a hydraulic lift ensures better absorption of mineral nutrition elements by plants from the upper dry soil horizons and increased activity of soil microorganisms [37].

The importance of the root systems of halophytes for their resistance to salinization has been revealed. The ability of plants to tolerate a saline environment is determined by a variety of physiological and biochemical processes that contribute to the retention and/or absorption of water, protect the functions of chloroplasts and maintain ion homeostasis. Halophytes synthesize osmotically active metabolites, specific proteins and certain enzymes that capture free radicals . Many halophytes accumulate methylated metabolites which play a crucial role as osmoprotectors and neutralize free radicals [45].

The variety of microorganisms associated with the roots of halophyte plants is enormous. This complex microbial community, which is called the second genome of a plant, is crucial for its stress resistance. Plants are able to form their own rhizospheric microbiome, as evidenced by the fact that different species of plants are hosts of certain microbial communities on the same soil [46].

Recent studies have shown that the use of rhizobacteria halophytes has a beneficial effect on the growth of agricultural plants and increases their yield. Five salt-resistant bacteria were isolated from the roots of the halophyte *Arthrocnemum indicum*. Under conditions of salt stress, inoculated peanut seedlings maintained ionic homeostasis, accumulated less reactive oxygen species, and showed enhanced growth compared to non-inoculated seedlings [47]. Inoculation with the rhizospheric bacterium *Azospirillum brasilense* NH, originally isolated from saline soil in northern Algeria, significantly increased the growth of durum wheat (T*riticum durum* var. *waha*) under saline soil conditions. In inoculated plants, the germination rate, stem height, ear length, dry weight of roots and shoots, chlorophyll a and b content, 1000 seed weight, the number of seeds per ear and seed weight were significantly higher than in non-inoculated plants [48].

Halotolerant bacteria are able to adapt to the increased salinity of the environment and maintain normal functioning thanks to effective osmoregulatory mechanisms. Rhizobacteria of halophytes stimulate the growth of plant roots at high salinity by the synthesis of indoleacetic acid, gibberellins, cytokinins, abscisic acid, solubilization of insoluble phosphate, synthesis of 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase), which reduces the ethylene content in plants during salt stress [49].

So, forage halophytic *Kochia prostrata*, *Salsola orientalis* (semi-shrubs), and *Haloxylon aphyllum*, *Aellenia subaphylla* (shrubs) when grown in xerothermal conditions, form powerful root systems that penetrate deep into the soil. In the first years of life, the roots deepen to 200-295 cm. On the light gray soils of the Central Asian foothill desert zone (Nishan steppe), at the plant age of 5 years, the

roots penetrate to the depth from 500-600 cm for *Salsola orientalis*, *Kochia prostrata*, and *Aellenia subaphylla* up to 1200 cm (that is, 2 times deeper) for *Haloxylon aphyllum*. These crops form root systems of a universal type adapted to the use of atmospheric precipitation, condensation moisture and shallow groundwater. Plants are characterized by rapid growth and development of root systems. In the 1st year of life roots are 4-4.5 times longer than the aboveground part, at the age of 5 years 6 times longer. This ensures the absorption of water in the amount necessary for the plant despite the moisture deficiency and drought. The depth of root penetration in plants of different life forms (i.e., shrubs, and semi-shrubs) is strongly influenced by the water-physical properties of the edaphic environment. With a constant lack of soil moisture, the root system continuously tends to deepen, breaking through dense, "cemented" soil layers. We believe that semi-shrub and shrub halophytes have the function of a so-called hydraulic lift when the plant is able not only to lift water by its roots, but also to release it into drier soil horizons. In the conditions of the Central Asian desert, forage semi-shrubs (*Kochia prostrata*, *Salsola orientalis*) and shrubs (*Haloxylon aphyllum*, *Aellenia subaphylla*) with fast-growing and deeply penetrating roots raise water into the surface soil layers where plants with a shallow root system can use it.

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ACCUMULATION OF PHOTOSYNTHETIC PIGMENTS AND SECONDARY METABOLITES IN LEAVES OF GALEGA (*Galega orientalis* Lam.) cv. GALE DEPENDING ON STAND AGE AND AGROTECHNOLOGIES DURING INTRODUCTION IN THE MIDDLE TAIGA OF WESTERN SIBERIA

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Abstract

Plant biomass production and accumulation of bioactive substances are determined by a complex of physiological and biochemical mechanisms, environmental factors and agrotechnologies. The use of *Galega orientalis* as a forage crop throughout the world is largely due to its unique environmental adaptability and a large yield potential. Despite the widespread use of forage *G. orientalis* around the world, research data on photosynthetic pigments, vitamin C and flavonoids in green mass of the plants under a new environment are scarce, and for the north of Russia, it is completely absent. Earlier, we were the first to describe the phenological, eco-morphological features and photosynthetic potential, the productivity of green mass and seeds of *G. orientalis* for the zone of the Middle taiga of Western Siberia. This paper systematizes our first data on the accumulation of photosynthetic pigments, vitamin C, and flavonoids in *G. orientalis* plants at the site of introduction. The study aimed to characterize the content of these compounds during adaptation to new environment, depending on cropping practices and the age of the herbage. Introductory studies were carried out on the cv. Gale (an experimental plot, the village of Barsovo, Khanty-Mansi Autonomous Okrug — Yugra, Surgut district, 61°15′00″ N, 73°25′00″ E. 2013-2015). Plants were grown using peas as a cover crop, in monoculture with pre-sowing treatment of seeds with the Baikal-EM1 microbiological preparation (OOO NPO EM-Center, Russia), and in monoculture without treatment. The effects of the cropping practices on the total chlorophylls (Chl a + Chl b) in the leaves appeared in the 2nd year plants. Upon seed pre-sowing treatment with the Baikal-EM1 preparation, in the 2nd and 3rd year plants, the level of total chlorophylls by plant development phases was 19-22 % and 16-18 % higher than in the control). In mixed sowing total chlorophylls decreased at the end of the 2nd year but exceeded the control (by 33 %) by the end of the 3rd year. In the control, the Chl a level in the leaves of the 1st, 2nd and 3rd year plants averaged 1.23±0.10, 1.29±0.12 and 1.32±0.14 mg/g dry weight over the growing season. Over the 2nd year of growth, the content of Chl a in the leaves increased by 15 % on average upon the Baikal-EM1 application compared to the control and remained within the control values (1.20±0.23 mg/g) ($p \le 0.05$) in the mixed stands with pea plants. For the microbiological preparation, the average Chl a/Chl b ratio significantly ($p \le 0.05$) decreased over 3 years, which may indicate an increase in the adaptive potential of plants, and for the mixed crops, it remained within the control values. The proportion of chlorophylls (Chl a + Chl b) localized in the light-harvesting complexes (LHC) varied from 20 to 90 % depending on the plant phenophase, stand age, and the agrotechnology. In the control and two treatments, the correlation coefficients between Chl a/Chl b and the proportion of chlorophylls (Chl a + Chl b) localized in the LHC were $r = -0.83$, $r = -0.93$, and $r = -0.65$, respectively. Treatments did not lead to a statistically significant change in the Chl/Car index. Nevertheless, after inoculation with the Baikal-EM1 biological and in mixed sowing with peas, the accumulation of carotenoids exceeded the control. For all treatments over the years, the accumulation of all pigments in the leaves

directly correlated with the hydrothermal coefficient (HTC). The content of Chl b and carotenoids turned out to be weaker associated with the temperature regime, while the first parameter directly correlated with precipitation during the season, and a negative correlation occurred for the second parameter. When inoculated with Baikal-EM1, the leaf level of vitamin C in the 1st and 2nd year plants increased compared to the control and was almost equal to the control in the 3rd year plants. In the 3rd year mixed sowing, the vitamin C content decreased compared to control. After application of the microbiological preparation and in the control, the content of flavonoids in the 3rd year plants switched to generative development sharply decreased, while in the sowing with the cover crop, where the virginal stage continued, it sharply increased (1.6 times compared to previous years). In general, our findings indicate that the biological Baikal-EM1 largely contributed to the adaptation of the 2nd and 3rd year plants of *G. orientalis* cv. Gale to a new environment.

Keywords: photosynthesis pigments, vitamin C, flavonoids, *Galega orientalis* Lam., cv. Gale, introduction, Baikal-EM1

In the countries of the European Union, especially in Central and Northern Europe, there has been a shortage of feed protein for many years, which is primarily associated with unfavorable climatic conditions, the short growing season and frequent droughts. The potential of perennial leguminous crops, a significant part of which are resistant to drought, is still not realized, although their protein yield is often 2 times higher than that of annual crops [1]. This is due to the attention to alternative perennial legumes that can provide more stable yields of green mass with high feed value.

As such, the eastern galega (*Galega orientalis Lam.*), a perennial forage legume plant (family *Fabaceae*) which has a complex of valuable properties, is increasingly being used. The eastern galega possesses winter hardiness, drought resistance, and high efficiency of using spring moisture reserves. Plants show early regrowth in spring and rapid growth, significant foliage (60-70%), and stability of seed production (up to 6 kg/ha or more). Longevity of crop use makes 10-15 years or more with high productivity (for 2 mowing, up to 60-70 t/ha of green mass, up to 10-15 t/ha of hay) and nutritional value (1 feed unit contains 150-270 g of digestible protein) [2, 3].

The possibilities of widespread use of eastern galega are largely due to its biological features, and in particular, its high yield potential and exceptional adaptability to various environmental conditions [4]. The natural territory of habitat of the eastern galega is the North Caucasus and the Transcaucasia (https://powo.science.kew.org/taxon/urn:lsid:ipni.org:names:495682-1) [5]. The Eurasian Caucasian region is considered the geographical center of origin of this species [6]. However, at present it has significantly expanded its range, including due to introduction [5, 7]. As a forage plant, eastern galega is cultivated in Ukraine, Belarus, Estonia, China, in Western Europe, e.g., in Austria, France, where the naturalization of the species is also noted [7], Baltic countries, the Czech Republic and Slovakia, Kazakhstan [5], Moldova [8], Canada where productivity was compared within the geographical coordinates of 45-56 N 52-120 W [9], and in Japan [10].

In Russia, studies on the introduction of eastern galega have been carried out in many regions, in the Central Chernozem Zone [11], the Volga region [12, 13], the Middle Urals [14], and Siberia [15, 16]. Based on the data obtained, regional technologies for growing eastern galega are being developed and optimized, considering the timing and norms of seeding, the effectiveness of cover crops, the use of microbiological preparations, the number of mowing, the impact on the soil, the duration of economic use of crops, seed productivity. The authors of these works mainly evaluated the photosynthetic potential, crop productivity, the content of dry matter, protein, and amino acids in the green mass during the growing season and depending on the cultivation method. However, there are other components in the green mass of galega that characterize its feed advantages

and quality, i.e., fiber, pectins, chlorophylls, secondary metabolites (vitamin C, flavonoids, carotenoids), as well as anti-nutritional substances, for example, tannins [17-19] which worsen protein digestibility and give plants a bitter taste, undesirable in feed cultures. The number and ratio of such components can also change in plant ontogenesis and under the influence of environmental conditions and cultivation technologies.

The physiological role of chlorophylls, carotenoids, vitamin C and flavonoids is diverse. These are strong natural antioxidants [20-22], whose protective properties are due to the ability to prevent or slow down oxidative damage to cells caused by physiological oxidants, including reactive oxygen species, nitrogen, and free radicals [23]. In addition, carotenoids play an important role in metabolism (vitamin A is a derivative of beta-carotene) [24]. Along with the antioxidant effect, the anti-inflammatory, hepatoprotective, antibacterial, antiviral, and anticancer activity of flavonoids is well known [22]. Chlorophylls and carotenoids are pigments involved in photosynthesis. They are part of the main pigment-protein complexes of the photosynthetic apparatus [25]. The photosynthetic apparatus is capable of restructuring, which ensures the successful growth and development of plants in continuously changing lighting conditions. The key components of the photosynthetic apparatus, the pigment-protein complexes are characterized by constancy of composition and structure, and adaptive transformations are carried out by changing their number and correlation in thylakoid membranes [25]. Ascorbic acid (AA) is a low-molecular-weight antioxidant, most common in plants where it is involved in a variety of metabolic processes, including reactions that determine resistance to stress and adaptive response to environmental influences [26]. The role of ascorbic acid in maintaining photosynthesis and protecting the photosynthetic apparatus from reactive oxygen species and photoinhibition is known [26, 27]. Ascorbic acid can be an electron donor that ensures the full functioning of the photosynthetic electron transport chain [28-30]. Flavonoids are secondary metabolites of plants with high biological activity they can directly or indirectly weaken or prevent cellular damage caused by free radicals [22, 31]. Flavonoids play an extremely important functional role in the interactions of plants and the environment. They participate in the regulation of auxin transport, creating its gradients. This leads to the formation of phenotypes with various morphoanatomical features, which can be of great importance in stress-induced morphogenic response of plants [22].

Despite the rather long history of the introduction of *G. orientalis* as a forage crop in different regions of the world and in Russia, there is little information about the accumulation of photosynthetic pigments, vitamin C and flavonoids in galega plants when adapting to new growing conditions [4, 17-19, 32, 33], and for the north of Russia there are none.

Earlier, we described for the first time the phenological, eco-morphological features and assessed the photosynthetic potential, productivity of green mass and seeds [34-36] in eastern galega and prospects for growing in the Middle taiga zone of Western Siberia [37-39]. In this paper, we systematize the data we obtained for the first time on the accumulation of photosynthetic pigments, vitamin C and flavonoids in the plants of the eastern galega at the site of introduction.

The aim of the study was to characterize the content of chlorophyls, carotenoids, vitamin C and flavonoids in the Eastern galega (*Galega orientalis* Lam.) plants when adapting to new environmental conditions of cultivation with different agrotechnical techniques (pre-sowing seed preparation, the use of peas as a cover crop) and depending on the age of the herbage.

Materials and methods. Introduction studies of *G. orientalis* were carried out in 2013-2015 at an experimental site in the village of Barsovo (Khanty-Mansi

Autonomous Okrug — Yugra, Surgut district, 61°15'00" N., 73°25'00" E.) on the variety Gala (in 1988, the variety was included in the State Register of Breeding achievements admitted to use). The seeds were purchased in 2013 (OOO AF Seeds of the Ob region, Novosibirsk, category RS1 — first reproduction).

The soil of the experimental site is sandy podzolic, cultivated, the mass fraction of organic matter is 5.63%, pH 5.21, the soil is 4.7 mmol/100 g absorbed bases, 3.85 mg/kg N-NH^4, 129 mg/kg N-NO_3, 396.1 mg/kg P_2O_5, and 66.5 mg/kg K_2O [36]. The growing season of 2013 was arid, the sum of the average daily temperatures was 1751 °C, precipitation was 252.7 mm, HTC (hydrothermal coefficient) = 1.4 (with an average annual value of HTC = 1.7). In the warm periods of 2014 and 2015, the sum of average daily temperatures was equal to 1546 and 1579 °C, respectively, with excessive accumulation of moisture — 356 and 458 mm, respectively (with a norm of 1648.6 °C and 287 mm), HTC = 2.3 in 2014, HTC = 2.9 in 2015 [36]. During the growing seasons, there were sharp fluctuations in the main meteorological parameters, generally unfavorable for the growth and development of the eastern galega. Monitoring of weather conditions at the site of introduction was carried out based on data from the Surgut weather station.

The influence of meteorological factors of the growing seasons were assessed in micro plot field tests. Sowings were performed in 2013 (for herbages of 1-3 years of life in 2013-2015), in 2014 (for herbages of 1-2 years of life in 2014-2015), and in 2015, taking into account in 2015 (for an herbage of the 1st year of life in 2015). The plants were grown in three variants. Control was a single-species sowing without seed treatment. The second option was a single-species sowing with seed pretreatment with a microbiopreparation Baikal-EM1 based on a complex of lactic acid, photosynthetic, nitrogen-fixing bacteria, saccharomycetes (NPO EM-Center LLC, Moscow Ulan-Ude, Russia). A 1:1000 dilution of the preparation was used for seed soaking during 30-60 min. The third treatment included a mixed sowing with peas as a cover culture without pre-sowing bacterial inoculation of galega seeds. The seeding rate was 2.8 million seeds per 1 ha for galega and 1 million seeds per 1 ha for peas. Weeding was not carried out. At the end of the growing season, the herbage of the eastern galega was not mowed. The biological repeatability in each variant was 4-fold, the placement of plots is randomized [40], 1.5 m^2 per plot, the total test area for each year is 18 m^2. Phenological observations were carried out as reported [41], the phases of ontogenesis were recorded, the formation of morphological structures was considered [42-46]. At each phenophase of development and at the end of vegetation [41], functionally mature leaves from 20 plants were selected for analysis and a combined sample was formed (the total number of samples was 372). The samples were air-dried and crushed.

To determine the amount of photosynthetic pigments — chlorophylls a and b (Chl a, Chl b) and carotenoids (Car), a 0.05-0.08 g portion of biomass was extracted with 96% ethyl alcohol with the addition of $CaCO_3$ and filtered to a colorless state. The optical density of the extract was determined at λ = 665 nm (chlorophyll a), λ = 649 nm (chlorophyll b) and λ = 470 nm (carotenoids) (SF-56, Lumex LLC, Russia), control was 96% ethyl alcohol [47]. The proportion of chlorophylls in light-collecting complexes (CCCs) was calculated as (Chl b + Chl 1.2 b)/(Chl a + Chl b), assuming that all Chl b is in the CC of photosystem II (PSII), and the ratio of Chl a/Chl b in total is approximately 1.2 [48, 49]. The ratios of Chl a/Chl b and (Chl a + Chl b)/Car were determined.

The amount of amino acids (AA) was determined by E.J. Hewitt and G.J. Dickes [50] in the modification of G.N. Chupakhina [51]. A sample of vegetable raw materials (0.3 g) was poured with 5% metaphosphoric acid, ground and extracted for 10 minutes at 4 °C and 20 minutes in a thermostat at 100 °C. The

extracts were transferred to an ice bath followed in 1 hour by a photometric measurement at λ = 520 nm (SF-56, Lumex LLC, Russia); control was 5% metaphosphoric acid.

The concetration of flavonoids was determined in accordance with recommendation [52] in a color reaction with aluminum chloride. A 0.25 g sample was extracted with 70% ethyl alcohol for 30 minutes with heating in a water bath. The optical density was determined at λ = 410 nm vs. the standard rutin solution (SF-56, Lumex LLC, Russia).

Statistical data processing (Microsoft Office Excel 2016 software package and the Statistica 6.0 program, StatSoft, Inc., USA) included calculation of arithmetic means (*M*) and standard errors of means (±SEM). The significance of the differences was assessed by the Student's *t*-test at p = 0.05. Pearson pair correlation analysis was used to assess the interrelationships of the studied parameters.

Results. Plant productivity and accumulation of biologically active substances are determined by complex physiological and biochemical interactions, environmental factors and agricultural technologies. The introduction of plants in northern latitudes is limited by unfavorable soil and weather conditions. In the Middle taiga of Western Siberia, this is a cold climate, sharp daily temperature fluctuations, frosts, an increase in daylight hours in the first half of the growing season, a short growing season, low fertility, and high soil acidity. Earlier, we showed that the yield of green mass in eastern galega of the Gale variety in the Surgut District (the Khanty-Mansi Autonomous Okrug, 61°15'00" N, 73°25'00" E) on average for 3 years was 243.0 c/ha in the control, 280.0 c/ha when using Baikal fertilizer-EM1, and 66.7 c/ha in mixed sowing with peas. The dry matter was 68.8, 76.4 and 19.9 c/ha, respectively {35].

Photosynthetic pigments. When growing eastern galega, the effect of the compared methods with respect to the number of chlorophylls in the leaves by the phases of plant development was statistically significant (p ≤ 0.05) from the 2nd year of life (Table 1). Thus, when inoculated with a microbiological preparation for the 2nd and 3rd years of life at germination phase, the number of green pigments was higherby 22 and 16%, , respectively, at the tillering phase by 26 and 19%, at stem branching by 19 and 18% vs. control. In mixed sowing with peas in plants of the 2nd and 3rd years of life, the amount of chlorophylls in the leaves increased statistically significantly (p ≤ 0.05) by 19.4% at germination phase, by 24.0% at tillering, by 18.7% at stem branching, but decreased at the end of the 2nd year of life by 53.0% vs. control. On the 3rd year, with the cover crop at tillering and stem branching, the total content of chlorophylls, on the contrary, first decreased (by 46 and 21%), and at the end of the growing season was 33% higher vs. control.

1. The content of photosynthetic pigments (mg/g of dry matter) **in the leaves of *Galega orientalis* Lam. cv. Gale depending on the age of herbage and agrotechnology during introduction** (Barsovo settlement, Khanty-Mansi Autonomous Okrug — Yugra, Surgut District)

PD	Chl a		Chl b		Chl a + Chl b		Car		Chl a + Chl b + Car	
	M±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %
The 1st year of life										
Monoculture (not treated seeds, control)										
Sown in 2013										
1	1.28±0.02	17.0	0.33±0.04	37.0	1.61±0.05	14.0	1.28±0.12	16.8	2.89±0.02	18.3
2	1.55±0.02	14.3	0.42±0.04	54.0	1.97±0.03	16.2	1.12±0.10	14.5	3.04±0.02	15.0
3	1.76±0.07	15.6	0.54±0.08	24.7	2.30±0.05	15.0	0.92±0.14	18.0	3.22±0.08	17.4
7	0.21±0.05	18.2	0.10±0.06	22.0	0.31±0.09	16.0	0.49±0.09	15.3	0.80±0.05	20.3
Sown in 2014										
1	1.30±0.03	24.1	0.18±0.12	32.4	1.48±0.04	22.3	1.18±0.10	17.4	2.66±0.09	19.4
2	1.62±0.09	19.0	0.42±0.10	50.1	2.04±0.03	18.7	1.09±0.14	19.2	3.13±0.07	17.0
3	1.88±0.02	17.3	0.46±0.05	48.0	2.34±0.10	15.6	0.83±0.10	16.4	3.17±0.05	15.8

Continued Table 1

7	0.20±0.03	15.0	0.55±0.07	32.0	0.73±0.07	27.3	0.42±0.09	15.0	1.15±0.06	16.4
					Sown in 2015					
1	1.34±0.05	16.3	1.29±0.05	27.6	2.63±0.06	19.2	1.22±0.08	14.8	3.85±0.07	15.0
2	1.53±0.07	21.0	1.47±0.03	25.3	3.00±0.04	20.0	1.18±0.10	19.2	4.18±0.08	14.8
3	1.75±0.05	12.0	1.57±0.09	52.0	3.32±0.06	24.5	0.85±0.13	19.0	4.17±0.10	22.3
7	0.20±0.08	12.7	0.11±0.07	47.3	0.30±0.07	19.3	0.33±0.09	14.3	0.63±0.09	17.5
					Phenophase average (2013-2015)					
1	1.30±0.02	12.0	0.61±0.35	54.8	1.91±0.36	33.0	1.23±0.03	18.0	3.29±0.29	15.2
2	1.53±0.06	16.0	0.77±0.35	41.0	2.34±0.33	24.6	1.13±0.03	14.0	3.47±0.36	17.8
3	1.80±0.04	14.3	0.86±0.36	39.2	2.65±0.33	21.8	0.87±0.03	15.0	3.52±0.33	16.0
7	0.20±0.03	13.7	0.25±0.15	40.0	0.86±0.15	31.0	0.41±0.05	19.0	0.86±0.15	30.8
					Average for the growing season (2013-2015)					
	1.22±0.18	52.5	0.62±0.15	84.0	1.84±0.29	54.0	0.91±0.09	37.0	2.75±0.36	25.3
					Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)					
					Sown in 2013					
1	1.37±0.07	15.4	0.21±0.03	41.3	1.58±0.10	22.1	1.20±0.02	17.3	2.78±0.07	15.3
2	1.42±0.12	14.0	0.38±0.05	52.0	1.80±.005	15.4	1.13±0.01	11.7	2.93±0.05	17.8
3	1.64±0.09	19.2	0.43±0.05	37.8	2.07±0.09	15.0	1.00±0.03	15.6	3.07±0.09	21.0
7	0.21±0.10	23.0	0.12±0.09	50.3	0.33±0.07	15.8	0.51±0.03	22.0	0.84±0.05	14.0
					Sown in 2014					
1	1.37±0.05	20.2	0.48±0.12	48.6	1.85±0.08	16.7	1.17±0.03	24.2	3.02±0.12	19.1
2	1.72±0.05	17.1	0.51±0.10	470	2.23±0.10	21.0	1.08±0.05	19.4	3.31±0.10	18.0
3	2.00±0.03	18.3	0.58±0.08	34.2	2.58±0.13	19.3	0.84±0.04	17.6	3.42±0.09	23.0
7	0.16±0.10	16.0	0.07±0.03	51.0	0.23±0.15	27.4	0.42±0.02	19.0	0.65±0.07	20.0
					Sown in 2015					
1	1.37±0.07	14.3	1.21±0.08	36.8	2.58±0.15	19.5	1.21±0.02	20.0	3.79±0.05	15.7
2	1.42±0.10	24.1	1.34±0.05	45.9	2.76±0.09	18.0	0.97±0.05	18.3	3.73±0.07	14.2
3	1.72±0.12	18.0	1.75±0.05	37.4	3.47±0.06	20.3	0.80±0.09	24.5	4.27±0.21	20.0
7	0.19±0.05	16.0	0.10±0.09	28.4	0.29±0.10	17.8	0.42±0.01	30.0	0.67±0.08	23.7
					Phenophase average (2013-2015)					
1	1.37±0.09	13.2	0.63±0.29	31.0	1.99±0.30	26.1	1.19±0.01	33.0	3.20±0.30	16.5
2	1.50±0.10	12.7	0.74±0.30	25.8	2.26±0.35	24.6	1.06±0.05	24.6	3.32±0.23	12.0
3	1.79±0.11	11.3	0.92±0.42	35.0	2.71±0.41	26.2	0.88±0.06	21.8	3.59±0.36	17.2
7	0.19±0.02	15.0	0.09±0.01	43.0	0.72±0.06	14.5	0.45±0.03	21.0	0.72±0.60	14.5
					Average for the growing season (2013-2015)					
	1.22±0.19	54.0	0.60±0.12	31.0	1.84±0.30	57.9	0.89±0.09	33.0	2.71±0.37	27.0
					Mixed culture with pea					
					Sown in 2013					
1	1.25±0.03	16.2	0.48±0.08	42.4	1.73±0.12	20.0	1.25±0.05	17.8	2.98±0.09	20.0
2	1.70±0.01	15.0	0.57±0.13	38.0	2.27±0.09	19.2	1.17±0.08	14.5	3.44±0.15	31.3
3	1.70±0.05	17.3	0.64±0.19	27.4	1.77±0.08	15.6	0.98±0.08	16.0	2.75±0.10	27.8
7	0.71±0.03	14.5	0.36±0.05	30.0	1.07±0.12	17.3	0.61±0.07	21.8	1.68±0.08	21.0
					Sown in 2014					
1	1.25±0.04	23.0	0.53±0.07	49.0	1.78±0.10	18.0	1.22±0.05	13.0	3.0±0.16	15.3
2	1.53±0.09	20.0	0.18±0.05	41.3	1.71±0.09	21.3	0.93±0.04	15.0	2.64±0.21	18.9
3	1.92±0.12	16.7	0.34±0.07	36.5	2.26±0.19	18.3	0.83±.09	18.4	3.09±0.09	20.0
7	0.21±0.06	15.0	0.14±.12	31.3	0.35±0.23	20.0	0.36±.03	20.0	0.71±0.10	16.8
					Sown in 2015					
1	1.28±0.14	22.0	1.32±0.06	28.4	2.60±0.07	14.6	1.24±0.13	18.7	3.84±0.07	22.0
2	1.64±0.09	14.8	1.64±0.05	28.0	3.28±0.09	18.0	1.17±0.05	14.8	4.45±0.06	19.3
3	1.83±0.15	18.2	1.98±0.09	37.6	3.81±0.10	17.5	0.97±0.05	15.2	4.78±0.03	24.8
7	0.21±0.08*	19.0	0.14±.10	37.0	0.35±0.16	20.1	0.28±0.09	16.0	0.63±.05	16.0
					Phenophase average (2013-2015)					
1	1.26±0.01	14.0	0.78±0.27	28.4	2.04±0.28	23.9	1.24±0.01	12.0	3.27±0.28	14.9
2	1.62±0.05	15.7	0.80±0.43	42.0	2.42±0.46	32.8	1.09±0.08	13.0	3.51±0.53	25.8
3	1.82±0.06	16.3	0.99±0.50	37.6	2.61±0.61	40.8	0.93±0.05	19.0	3.54±0.63	30.7
7	0.38±0.17	16.0	0.21±0.07	26.0	1.00±0.34	58.1	0.42±0.1	41.0	1.01±0.34	38.0
					Average for the growing season (2013-2015)					
	1.27±0.17	46.7	0.69±0.18	39.0	1.92±0.3	54.3	0.92±0.09	37.0	2.83±0.38	46.0
					The 2nd year of life					
					Monoculture (not treated seeds, control)					
					Sown in 2013					
1	1.32±0.06	14.0	0.27±0.04	17.0	1.59±0.16	18.0	1.12±0.18	12.5	2.71±0.05	20.0
2	1.43±0.06	16.3	0.31±0.09	21.0	1.74±0.22	16.3	1.10±0.05	14.8	1.93±0.03	23.7
3	1.57±0.03	15.2	0.35±0.12	14.5	1.92±0.19	14.7	0.83±0.05	25.0	2.75±0.07	28.1
7	0.62±0.08	14.8	0.34±0.09	16.0	0.96±0.07	16.0	0.66±0.09	19.1	1.62±0.07	28.0
					Sown in 2014					
1	1.42±0.04	24.0	0.30±0.07	22.0	1.72±0.07	25.7	0.19±0.07	10.0	1.91±0.05	14.6
2	1.52±0.05	16.3	0.34±0.05	16.3	1.86±0.10	22.1	0.79±0.12	24.6	2.65±0.09	23.0
3	1.74±0.07	14.0	0.34±0.05	20.0	1.92±0.11	20.8	0.68±0.15	27.5	2.60±0.08	18.4
7	0.25±0.01	16.0	0.15±0.08	19.7	0.40±0.08	23.5	0.30±0.10	13.0	0.70±0.05	29.0

Continued Table 1

					Phenophase average (2014-2015)					
1	1.37±0.04	15.0	0.29±0.02	10.0	2.25±0.19	21.3	0.94±0.27	18.0	2.31±0.40	24.5
2	1.48±0.03	14.0	0.33±0.11	17.0	2.30±0.16	17.2	0.64±0.07	15.0	2.29±0.36	22.0
3	1.64±0.05	16.0	0.36±0.01	15.6	2.69±0.05	14.2	0.75±0.06	16.0	2.68±0.08	13.9
7	0.42±0.11	13.0	0.27±0.05	30.0	1.17±0.21	43.0	0.49±0.07	30.0	1.16±0.46	56.0
					Average for the growing season (2014-2015)					
	1.23±0.18	42.0	0.30±0.02	23.0	1.50±0.19	36.2	0.71±0.12	47.0	2.10±0.25	34.0
					Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)					
					Sown in 2013					
1	1.40±0.12	17.3	0.43±0.02	24.0	1.83±0.07	14.0	1.00±0.05	20.0	2.83±0.03	12.3
2	1.63±0.10	19.2	0.47±0.03	22.0	2.10±0.07	16.0	0.87±0.08	17.8	2.97±0.03	17.8
3	1.90±0.10	23.4	0.52±0.05	18.4	2.42±0.09	16.3	0.82±0.13	14.5	3.24±0.09	16.0
7	0.57±0.09	14.5	0.29±0.08	15.3	0.86±0.12	22.0	0.73±0.10	18.0	1.59±0.05	19.0
					Sown in 2014					
1	1.38±0.50	18.0	0.43±0.02	17.0	1.84±0.05	15.4	1.14±0.09	16.4	2.95±0.03	25.4
2	1.85±0.13	12.8	0.48±0.07	21.0	2.33±0.07	14.0	0.97±0.14	22.0	3.30±0.02	15.6
3	2.30±0.08	14.0	0.52±0.05	23.2	2.82±0.05	27.0	0.72±0.10	13.8	3.54±0.07	16.0
7	0.48±0.02	16.3	0.29±0.03	15.7	0.77±.010	18.4	0.50±0.08	14.0	1.27±0.05	20.0
					Phenophase average (2014-2015)					
1	1.39±0.02	19.0	0.43±0.01	18.3	2.89±0.03*	17.0	1.04±0.05	11.0	2.89±0.06	12.8
2	1.80±0.07*	18.0	0.48±0.01	16.1	3.10±0.08*	16.2	0.92±0.02	14.0	3.14±0.17	17.4
3	2.14±0.12*	12.0	0.53±0.02	17.4	3.12±0.07*	14.9	0.80±0.05	13.0	3.29±0.15	16.2
7	0.53±0.02	18.0	0.29±0.01	20.3	1.42±0.08*	12.6	0.66±0.06	19.0	1.43±0.16	15.8
					Average for the growing season (2014-2015)					
	1.44±0.23*	44,3	0,43±0,03	22,0	1,90±0,26	38.9	0.84±0.07	24.0	2.71±0.29	34.0
					Mixed culture with pea					
					Sown in 2013					
1	1.27±0.05	15.0	0.32±0.10	17.0	1.59±0.05	16.8	1.20±0.12	19.2	2.79±0.02	11.0
2	1.78±0.07	17.0	0.10±0.05	16.2	1.88±0.07	17.2	1.15±0.08	20.0	3.03±0.02	16.0
3	1.53±0.05	14.5	0.08±0.03	15.4	1.61±0.03	21.3	0.81±0.06	27.3	2.42±0.02	13.7
7	0.19±0.03	12.3	0.09±0.03	14.5	0.28±0.03	17.0	0.35±0.04	15.0	0.63±0.05	14.2
					Sown in 2014					
1	1.33±0.04	14.0	0.62±0.08	14.0	1.95±0.03	14.3	0.93±0.05	19.0	2.88±0.08	19.2
2	1.78±0.05	13.5	1.28±0.12	17.3	2.76±0.07	15.0	0.76±0.05	18.2	3.52±0.07	18.0
3	1.53±0.08	15.0	1.35±0.07	15.2	3.08±0.05	19.0	0.52±0.08	16.3	3.60±0.05	20.0
7	0.19±0.12	15.7	0.11±0.05	20.3	0.28±0.04	16.4	0.18±0.06	14.0	0.46±0.03	13.7
					Phenophase average (2014-2015)					
1	1.30±0.03	14.0	0.51±0.07*	19.0	2.79±0.05*	18.2	1.10±0.08	14.0	2.84±0.06	12.4
2	1.78±0.01*	19.0	0.77±0.30*	16.8	3.04±0.12*	15.2	0.96±0.09	20.0	3.28±0.25	10.6
3	1.51±0.01	14.0	0.93±0.29*	15.0	3.31±0.26*	20.8	0.70±0.07	19.0	3.01±0.59	27.7
7	0.19±0.01*	19.0	0.11±0.01*	12.0	0.55±0.05*	19.5	0.28±0.04	26.0	0.55±0.09	22.0
					Average for the growing season (2014-2015)					
	1.20±0.23	52,0	0,53±0,18*	44,0	1,70±0,36	60,3	0,74±0,13	50,0	2,41±0,43	50,1
					The 3dyear of life					
					Monoculture (not treated seeds, control)					
					Sown in 2013					
1	1.40±0.04	18.2	0.40±0.05	17.3	1.78±0.08	15.8	1.40±0.05	17.3	2.93±0.07	25.0
2	1.60±0.05	17.4	0.43±0.08	15.0	1.99±0.12	18.1	0.83±0.08	15.6	2.92±0.04	30.0
3	1.70±0.03	15.3	0.45±0.08	18.0	2.13±0.09	12.9	0.93±0.06	20.0	2.99±0.07	24.8
4	2.50±0.08	12.8	0.32±0.03	22.0	2.82±0.17	12.3	1.35±0.10	14.3	4.17±0.03	29.0
5	1.90±0.05	14.3	0.23±0.09	27.0	2.10±0.10	14.3	0.92±0.12	12.8	3.02±0.05	25.0
6	0.52±0.07	19.0	0.16±0.05	19.8	0.68±0.09	15.4	0.73±0.08	14.0	1.41±0.02	30.4
7	0.50±0.12	19.2	0.27±0.07	23.0	0.73±0.14	18.2	0.63±0.08	16.0	1.36±0.07	27.8
					Average for the growing season (2015)					
	1.45±0.27	50.0	0.32±0.04	34.0	1.75±0.29	44.6	0.94±0.09	26.0	2.68±0.38	37.0
					Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)					
					Sown in 2013					
1	1.43±0.04	16.0	0.69±0.03	14.5	2.13±0.08*	19.8	1.56±0.07	16.0	3.68±0.07	29.0
2	1.57±0.07	15.4	0.72±0.05	16.1	2.45±0.03*	12.9	1.10±0.05	22.3	3.39±0.09	24.5
3	1.91±0.05	20.0	0.75±0.04	17.0	2.63±0.02*	12.4	1.25±0.09	17.4	3.91±0.05	28.3
4	2.30±0.03	17.2	0.48±0.10	17.3	2.74±0.03*	12.9	1.42±0.07	16.5	4.20±0.06	27.1
5	1.42±0.05*	14.9	0.35±0.04	14.6	1.69±0.10*	18.7	0.83±0.05	14.3	2.60±0.08	30.0
6	0.70±0.02*	16.1	0.27±0.08	18.2	0.92±0.01*	13.0	0.74±0.08	16.0	1.67±0.09	32.1
7	0.60±0.05	17.0	0.55±0.04	18.0	0.74±0.03*	18.4	0.54±0.10	18.2	1.32±0.05	18.6
					Average for the growing season (2015)					
	1.42±0.23	43.0	0.54±0.07*	35.0	1.90±0.30	41.6	1.06±0.14	35.0	2.97±0.43	38.0
					Mixed culture with pea					
					Sown in 2013					
1	1.40±0.07	20.0	0.55±0.05	18.0	1.92±0.02	12.8	1.35±0.09	15.7	3.29±0.09	19.0
2	0.80±0.10*	14.7	0.34±0.05	16.1	1.07±0.02*	13.9	0.56±0.05	16.8	1.65±0.10	25.7
3	0.50±0.08*	16.2	0.18±0.08*	15.8	0.63±0.03*	12.9	0.81±0.13	18.0	1.48±0.09	29.0
7	0.42±0.04	16.0	0.35±0.09	16.0	1.05±0.02*	13.5	0.86±0.10	17.2	1.93±0.15	32.4

Continued Table 1

Average for the growing season (2015)									
0.86±0.19*	45.0	0.36±0.06	43.0	1.20±0.27	44.8	0.89±0.16	37.0	2.09±0.24	39.0
Average over the years of study									
Monoculture (not treated seeds, control)									
1.28±0.12	47.9	045±0.07	36.5	1.78±0.16	47.2	0.86±0.06	37.0	2.54±0.2	41.2
Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)									
1.33±0.12	46.5	0.53±0.07	32.0	1.9±0.29	46.9	0.92±0.06	32.0	2.78±0.2	38.9
Mixed culture with pea									
1.18±0.12	49.0	0.58±0.10	45.0	1.72±0.19	56.6	0.84±0.07	40.0	2.57±0.25	46.6
Average for the 1st year of life									
1.23±0.10	49.5	0.64±0.09	52.3	1.88±0.18	55.9	0.91±0.05	35.0	2.76±0.21	44.6
Average for the 2nd year of life									
1.29±0.12	45.5	0.42±0.07**	48.0	1.68±0.15	44.7	0.76±0.06	39.0	2.41±0.19	38.9
Average for the 3d year of life									
1.32±0.14	48.4	0.40±0.04	43.0	1.69±0.17	43.0	0.98±0.07	32.0	2.66±0.19	38.2

N o t e. PD — phase of devlopment; 1 — seedlings (regrowth for the 2nd and the 3d years of life), 2 — tillering, 3 — stem branching, 4 — budding, 5 — flowering, 6 — fruiting, 7 —the end of vegetation; Chl a, Chl b, Car — chlorophylls and carotenoids.

* Differences vs. control are statistically significant at $p \leq 0.05$.

** Differences vs. the value in the previous year are statistically significant at $p \leq 0.05$.

Quantitative and qualitative changes in the pigment complex reflect the state of the photosynthetic apparatus and physiological status of plants [53, 54]. With quantitative changes in the pigment apparatus of leaves (the content of Chl a, Chl b, Chl a + Chl b, Chl a/Chl b, the content of carotenoids and the Chl/Car ratio) in response to environmental conditions, light is the main factor, but other conditions, the temperature and humidity have a certain influence [55]. When adapting to new environmental conditions, quantitative changes may occur in the pigment complex [56] and LHC [57]. If the light flux collected by the plant does not limit photosynthesis, the amount of LHC decreases and the ratio Chl a/Chl b increases [7]. At high latitudes, the percentage of blue-violet rays absorbed by carotenoids increases in the spectrum of scattered radiation, and a proportion of carotenoids increase in the profile of photosynthetic pigments. This indicates an increase in their protective role with the advance to the north [53].

The content of Chl a. In our tests, the leaf content of Chl a (see Table. 1) in the galega plants of the year of sowing, for the 2nd and 3rd years of life, averaged 1.23±0.10; 1.29±0.12 and 1.32±0.14 mg/g of dry weight (control values). With the use of microbiological fertilizer, the values for the 2nd year of life significantly ($p \leq 0.05$) increased vs. control (by 18% at tillering and by 24% at stem branching). In mixed sowing, for the 2nd year of life, the content of Chl a was maximum at tillering (1.78±0.01 mg/g) with a significant ($p \leq 0.05$) excess over the control value by 17% and a decrease to 1.51 mg/g at stem branching and to 0.19 mg/g by the end of the growing season. On average, in the 2nd year, when using Baikal-EM1 fertilizer, the content of Chl a in leaves ($p \leq 0.05$) increased by 15% compared to the control, while in mixed sowing with peas, it remained within the control values (1.20±0.23 mg/g).

In the 3rd year of vegetation, a gradual increase in the Chl a content in the leaves occurred in control and bacterial inoculation, starting from the regrowth phase to budding (from 1.40 to 2.50 mg/g of dry matter). At the end of the growing season, the Chl a content decreased to 0.50-0.60 mg/g of dry matter. Under inoculation, the Chl a content was significantly lower than in the control (by 25%) during the flowering period and higher than the control (by 26%) at fruiting phase. In the mixed sowing, the content of Chl a in leaves reached its maximum during the regrowth period and at the end of the growing season.

The Chl a/Chl b ratio. The Chl a/Chl b values (Table. 2) in the galega leaves ranged from 2.78 to 4.41 depending on the age of the plants. The analyzed

parameter for the 2nd year of life significantly increased (by 37%) vs. that of plants in the 1st year of life. Upon reaching the generative age of plants (for the 3rd year of life), it significantly decreased to 3.44.

2. The ratio of photosynthetic pigments and the proportion of chlorophylls in light-harvesting complexes (LHC) **in the leaves of *Galega orientalis* Lam. cv. Gale depending on the age of herbage and agrotechnology during introduction** (Barsovo settlement, Khanty-Mansi Autonomous Okrug — Yugra, Surgut District)

PD	Chl a/Chl b		(Chl a + Chl b)/Car		Pproportion of Chl a + Chl b in LHC, %	
	M±SEM	*Cv*, %	*M*±SEM	*Cv*, %	*M*±SEM	*Cv*, %
			The 1st year of life			
			Monoculture (not treated seeds, control)			
			Sown in 2013			
1	3.88±0.02	17.0	1.28±0.07	22.4	52.0±4.12	45.0
2	3.69±0.02	12.8	1.78±0.05	18.3	57.3±5.00	37.4
3	3.26±0.05	19.4	2.50±0.05	18.0	60.2±6.18	40.3
7	2.10±0.03	21.0	0.63±0.02	17.4	71.7±3.48	42.0
			Sown in 2014			
1	7.22±0.08	12.6	1.25±0.03	14.0	27.8±6.52	58.2
2	3.86±0.03	14.0	1.87±0.10	19.0	60.0±5.30	32.4
3	4.09±0.03	13.0	2.82±0.04	18.2	53.6±5.00	29.5
7	2.00±0.06	12.7	0.71±0.02	22.0	96.4±7.24	40.3
			Sown in 2015			
1	1.04±0.07	15.0	2.16±0.06	19.3	75.3±6.32	48.2
2	1.04±0.03	13.4	2.54±0.04	13.4	60.0±8.00	36.0
3	1.11±0.04	19.5	3.91±0.07	12.4	70.2±5.41	34.3
7	1.82±0.02	20.0	0.94±0.12	13.7	81.3±3.87	52.0
			Phenophase average (2013-2015)			
1	4.05±1.79	12.7	1.56±0.29	33.0	50.2±6.00	47.2
2	2.86±0.91	19.0	2.06±0.24	20.0	60.7±5.09	32.4
3	2.82±0.89	19.3	3.08±0.43	24.0	60.3±4.70	36.0
7	1.97±0.08	18.0	0.76±0.09	21.0	70.2±7.12	38.1
			Average for the growing season (2013-2015)			
	2,96±0,52	61,0	1,87±0,28	51,0	74,8±11,6	28,7
			Monoculture (seeds pre-treated with microbiological agent Baikal-EMI)			
			Sown in 2013			
1	6.52±0.04	21.0	1.32±0.09	14.0	29.4±7.10	40.0
2	3.74±0.07	11.4	1.59±0.08	19.3	48.3±5.00	48.0
3	3.85±0.08	18.3	2.07±0.06	22.4	50.4±6.41	35.7
7	1.75±0.03	22.0	0.65±0.04	20.0	80.1±7.00	29.3
			Sown in 2014			
1	2.85±0.21	14.7	1.58±0.12	24.1	57.2±6.42	50.0
2	3.37±0.19	21.2	2.06±0.15	20.6	60.0±6.00	34.1
3	3.45±0.24	24.0	3.07±0.22	15.7	62.7±8.34	29.0
7	2.28±0.18	19.3	0.55±0.09	17.0	67.0±5.42	35.7
			Sown in 2015			
1	1.13±0.14	20.5	2.13±0.04	14.3	62.0±6.70	32.4
2	1.06±0.22	15.7	2.84±0.07	12.8	70.0±9.10	35.0
3	0.98±0.15	11.8	4.34±0.09	18.0	69.7±4.35	31.0
7	1.97±0.20	28.0	0.76±0.13	21.4	76.0±7.12	28.7
			Phenophase average (2013-2015)			
1	3.50±1.59	49.0	1.68±0.29	25.0	50.3±5.43	36.4
2	2.72±0.84	53.0	2.16±0.36	29.0	60.8±9.12	30.5
3	2.76±0.89	56.2	3.16±0.66	36.0	60.0±8.00	29.0
7	2.03±0.18	16.7	0.65±0.06	16.0	70.4±5.21	32.7
			Average for the growing season (2013-2015)			
	2.76±0.46	57.0	1.91±0.32	58.0	68.4±7.83	24.6
			Mixed culture with pea			
			Sown in 2013			
1	2.60±0.18	14.3	1.38±0.09	14.1	61.0±8.00	21.5
2	2.98±0.24	18.2	1.94±0.09	10.8	75.4±4.68	29.4
3	1.77±0.21	12.4	1.81±0.05	14.0	80.0±7.39	32.3
7	1.97±0.17	19.0	1.75±0.14	12.3	74.4±8.22	24.5
			Sown in 2014			
1	2.36±0.08	19.4	1.46±0.08	14.5	66.3±6.00	21.3
2	8.50±0.08	15.6	1.84±0.15	16.0	63.0±8.00	27.8
3	5.65±0.12	23.0	2.73±0.18	13.5	55.0±7.84	30.0
7	1.50±0.15	21.0	0.97±0.12	20.0	88.0±9.37	29.5
			Sown in 2015			
1	0.97±0.09	16.4	2.10±0.24	22.1	45.2±5.46	19.7
2	1.00±0.12	17.3	2.80±0.09	18.4	70.4±7.00	32.4
3	0.92±0.12	15.2	3.93±0.02	15.3	73.2±6.21	25.3

Continued Table 2

7	1.50±0.14	16.3	1.25±0.28	27.0	88.8±4.78	29.2
			Phenophase average (2013-2015)			
1	1.98±0.51	45.2	1.65±0.23	24.0	60.2±3.42	30.0
2	4.16±2.24	52.0	2.19±0.34	21.0	70.6±7.55	24.8
3	2.78±1.46	34.0	2.82±0.61	38.0	70.7±5.00	26.0
7	1.66±0.16	16.4	1.32±0.23*	30.0	80.50±.31	21.3
			Average for the growing season (2013-2015)			
	2.64±0.65	48.2	2.00±0.24	41.2	75.30±8.5	28.7
			The 2nd year of life			
			Monoculture (not treated seeds, control)			
			Sown in 2013			
1	4.8±0.07	18.8	1.42±0.04	21.0	37.1±4.45	32.0
2	4.6±0.06	12.6	1.58±0.04	14.5	39.6±7.00	30.0
3	4.5±0.12	14.0	2.31±0.08	20.0	40.8±5.60	24.8
7	1.8±0.09	12.3	1.45±0.05	15.8	78.2±4.89	26.2
			Sown in 2014			
1	4.7±0.03	12.0	1.05±0.13	17.3	66.4±8.00	27.3
2	4.4±0.05	18.8	2.35±0.09	22.3	75.8±9.17	32.0
3	4.7±0.03	16.7	3.1±0.10	18.3	74.6±5.00	19.8
7	1.7±0.08	19.3	1.33±0.18	15.6	33.2±4.04	25.3
			Phenophase average (2014-2015)			
1	4.73±0.09	13.0	1.29±0.08	13.0	50.1±8.15	27.0
2	4.5±0.1	13.0	1.93±0.22	23.0	50.6±9.03	25.0
3	4.6±0.1	13.0	2.74±0.19	14.0	60.4±7.14	30.0
7	1.75±0.05	14.0	1.4±0.03	14.0	70.5±5.08	28.3
			Average for the growing season (2014-2015)			
	3.9±0.47	34.0	1.82±0.07	38.0	56.1±7.3	30.0
			Monoculture (seeds pre-treated with microbiological agent Baikal-EMI)			
			Sown in 2013			
1	3.26±0.09	14.4	1.83±0.07	19.3	52.2±6.31	29.0
2	3.16±0.08	18.0	2.44±0.12	13.7	49.3±8.00	27.6
3	3.65±0.10	22.3	2.95±0.08	22.0	47.4±5.78	28.4
7	1.97±0.12	15.7	1.18±0.15	18.0	74.6±9.10	39.5
			Sown in 2014			
1	3.21±0.07	17.0	1.59±0.07	16.4	52.7±10.2	32.4
2	3.85±0.07	14.6	2.40±0.06	21.0	45.9±7.06	24.3
3	4.42±004	18.0	3.92±0.08	14.7	41.4±5.00	28.5
7	1.66±0.03	15.4	1.54±0.04	17.0	83.0±6.33	34.2
			Phenophase average (2014-2015)			
1	3.25±0.03	15.0	1.68±0.08*	19.0	50.0±4.89	24.6
2	3.66±0.19	17.0	2.42±0.02*	16.0	60.5±8.00	28.0
3	4.04±0.38	14.0	3.44±0.29*	17.0	70.6±5.30	38.4
7	1.82±0.16	12.0	1.37±0.12	17.0	80.40±4.01	36.2
			Average for the growing season (2014-2015)			
	3.18±0.33	25.0	2.23±0.32	40.0	55.40±5.27	42.0
			Mixed culture with pea			
			Sown in 2013			
1	3.97±0.08	19.3	1.33±0.12	18.2	44.50±6.00	30.0
2	17.80±0.10	12.4	1.63±0.21	15.7	37.30±7.42	32.7
3	19.13±0.07	18.5	1.99±0.09	18.0	11.80±5.65	30.0
7	2.10±0.05	14.0	0.80±0.25	22.0	71.30±4.85	28.4
			Sown in 2014			
1	2.15±0.08	18.3	2.10±0.30	19.3	70.60±9.36	25.6
2	1.16±0.09	20.0	3.63±0.15	14.8	56.20±8.00	32.4
3	1.28±0.08	22.0	5.92±0.24	41.0	96.10±7.15	30.1
7	1.55±0.05	30.4	1.56±0.17	21.0	89.00±5.10	36.3
			Phenophase average (2014-2015)			
1	3.05±0.48	32.0	1.74±0.19*	24.0	40.70±8.04	28.7
2	9.48±0.60	28.7	2.59±0.56*	37.0	50.20±11.02	38.0
3	10.21±0.54*	25.6	3.65±1.03*	40.0	40.40±7.30	29.0
7	1.83±0.28	22.0	1.18±0.24*	66.0	60.30±5.80	35.4
			C Average for the growing season (2014-2015)			
	6.14±2.71*	45.0	2.73±0.58*	22.7	61.88±12.70	27.4
			The 3dyear of life			
			Monoculture (not treated seeds, control)			
			Sown in 2013			
1	3.50±0.03	17.4	1.59±0.04	22.0	50.20±8.34	35.1
2	3.72±0.05	17.4	2.18±0.09	19.3	50.40±6.00	28.4
3	3.78±0.03	21.0	2.50±0.03	19.8	50.30±4.78	26.3
4	7.82±0.18	15.2	2.09±0.04	18.3	30.40±9.01	38.4
5	8.26±0.09	14.5	2.32±0.04	14.0	20.40±5.06	40.2
6	2.60±0.12	18.0	0.98±0.05	15.6	50.00±7.12	21.8
7	2.50±0.09	13.3	1.11±0.04	18.4	60.20±5.00	33.5

Continued Table 2

			Average for the growing season (2015)			
	4,60±0,91	52,0	1,83±0,23	19,0	44,30±5,28	22,0
	Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)					
			Sown in 2013			
1	2.07±0.06*	15.0	1.36±0.09	23.2	70.04±8.36	27.8
2	2.18±0.07*	25.0	2.08±0.09	25.0	70.12±7.01	40.3
3	2.55±0.12*	22.3	2.30±0.05	22.1	60.1±9.12	30.3
4	4.79±0.05*	16.8	1.96±0.03	19.0	40.4±8.00	40.2
5	4.06±0.05*	15.7	2.13±0.04	19.0	40.0±6.32	39.0
6	2.59±0.08*	18.0	1.31±0.07*	19.2	60.0±5.00	35.4
7	1.50±0.13*	12.3	1.85±0.05*	13.4	90.3±7.31	28.6
			Average for the growing season (2015)			
	2.82±0.44*	42.0	1.82±0.13	19.0	61.40±4.78	30.1
			Mixed culture with pea			
			Sown in 2013			
1	2.55±0.09*	20.0	1.44±0.13	23.0	60.4±5.10	32.5
2	2.35±0.07	23.0	2.04±0.07	18.7	60.2±6.23	40.0
3	2.78±0.09*	18.1	0.84±0.08*	23.4	70.0±4.57	42.5
7	2.40±0.10*	21.0	1.19±0.05	25.0	80.3±6.00	30.7
			Average for the growing season (2015)			
	2.52±0.09*	38.0	1.34±0.26*	37.0	67.5±4.79	26.7
			Average over the years of study			
			Monoculture (not treated seeds, control)			
	3.65±0.37	53.2	1.85±0.16	37.0	61.3±6.13	38.4
	Monoculture (seeds pre-treated with microbiological agent Baikal-EM1)					
	2.90±0.25*	44.0	1.99±0.17	45.0	62.7±4.20	40.0
			Mixed culture with pea			
	3.79±0.98*	47.0	2.02±0.23	56.0	69.50±6.00	29.8
			Average for the 1st year of life			
	2.78±0.31	66.0	1.93±0.15	49.0	73.00±5.30	37.5
			Average for the 2nd year of life			
	4.41±0.92**	58.0	2.14±0.23	53.0	57.8±5.00**	49.2
			Average for the 3d year of life			
	3.44±0.44**	54.0	1.73±0.12*	29.0	56.1±4.05	41.3

Note. PD — phase of devlopment; 1 — seedlings (regrowth for the 2nd and the 3d years of life), 2 — tillering, 3 — stem branching, 4 — budding, 5 — flowering, 6 — fruiting, 7 —the end of vegetation; Chl a, Chl b, Car — chlorophylls and carotenoids.

* Differences vs. control are statistically significant at $p \leq 0.05$.

** Differences vs. the value in the previous year are statistically significant at $p \leq 0.05$.

In the 2nd and 3rd years of life, in mixed culture with peas, statistically significant differences in the value of Chl a/Chl b in leaves depend on the phases of galega plant development (see Table 2). In the 2nd year, the Chl a/Chl b value significantly decreased (by 36%) during regrowth and increased 2-fold at tillering and stem branching vs. control (4.73±0.09, 4.50±0.10, and 4.60±0.10, respectively). In the 3rd year of life, a statistically significant decrease in the Chl a/Chl b value occurred during the regrowth phase (by 27%), at tillering (by 37%), and at stem branching (by 26%) vs. 3.50±0.07, 3.72±0.09, and 3.78±0.08 in the control, respectively. With pre-sowing seed inoculation with Baikal-EM1, the Chl a/Chl b values changed statistically significantly only in the 3rd year of life (for all phases of development, on average, the values y were 33-51% lower compared to control).

On average over 3 years, the Chl a/Chl b values in mixed crops remained within the control range and amounted to 3.79±0.98. When using a microbiological preparation, the Chl a/Chl b significantly decreased by 21%, to 2.90±0.25 vs. 3.65±0.37 in control. A decrease in the Chl a/Chl b values may indicate an increase in the adaptive potential of plants under stress and their stability [57-59].

In plants of the Russian European north-east taiga, antenna (light-collecting) chlorophylls were reported to account for 55-65% of the total green pigments [53]. In our tests, the proportion of the leaf LHC chlorophylls varied from 20 to 90% depending on the phenological phase, the age of the herbage and the agrotechnology (control, inoculation, mixed sowing) (see Table. 2). There was a strong negative correlation between the value of Chl a/Chl b and the proportion of chlorophylls (Chl a + Chl b) in the LHC. In general, the lower the Chl a/Chl b (x)

value, the higher the proportion of the LHC chlorophylls (r = –0.83; R^2 = 0.666, y = –7,698x + 84,994). The correlation in the control (r = –0.80; R^2 = 0.694, y = –6.2859x + 79.81) was lower than when using Baikal-EM1 (r = –0.93; R2 = 0.856, y = –12.971x + 98.602), but higher than in binary crops (r = –0.65; R^2 = 0.429, y = –2.3476x + 76.206). Correlations between the sum of leaf green pigments and the Chl b content were the same. For (Chl a + Chl b) to Chl b proportion in control, pre-sowing treatment with a microbiological preparation, and binary sowing), accounted for r = 0.57, r = 0.55, and r = 0.89 ($p \leq 0.05$).

The content of carotenoids. A sufficiently high accumulation of carotenoids in the galega leaves is quite expected (see Table 1). It is known that in the spectrum of scattered radiation at high latitudes, the percentage of blue-violet rays absorbed by carotenoids increases. Carotenoids can additionally perform a light-harvesting function during white nights [53]. Thanks to carotenoids, plants can use light energy in the blue region of the spectrum [54]. In addition, they protect chlorophyll and other components of photosystems from light overexcitation [54]. We consider the accumulation of carotenoids noted in our experiments as an adaptive response of the photosynthetic apparatus to the conditions of high geographical latitudes [60, 61].

On average, in our tests, the Chl/Car value in the year of sowing was 1.93±0.16, in the 2nd year it increased to 2.44±0.36, but statistically significantly increased (by 19%) only in the 3rd year of plant life (see Table 2). Chl/Car values in the range of 2.0-3.9 correspond to a high content of carotenoids vs. the content of green pigments [53]. The Chl/Car = 3 was reported for plants of the Circumpolar Urals, among which the proportion of Arctic and Arctic-Alpine species is high [53]. This indicates a raising role of carotenoids with the advance to the north.

In all years of observations, in the control, inoculated and mixed sowing, the Chl/Car ratio decreased to minimum values in the autumn period (0.76-1.85) compared to the spring-summer time (2.30-3.65), when intensive linear growth occurs (see Table 2).

The agrotechnologies we compared did not lead to a statistically significant change in the Chl/Car ratio. Nevertheless, when inoculated with Baikal-EM1 and grown together with peas, there was an excess in accumulation of carotenoids in the galega leaves vs. control (see Table 2).

In general, in our tests, there was a wide variation in the Chl/Car ratio, which, in our opinion, can be used in the selection of crops based on productivity and adaptability to the conditions of the Middle taiga of Western Siberia.

Hydrothermal conditions and pigment content. On average, over the years of the study, the accumulation of pigments in the leaves of eastern galega directly correlated with LHC (x) (r = 0.90, R^2 = 0.839, y = 0.804x + 0.5586) for all treatments. The pigment content decreased with an increase in the sum of active temperatures during the growing season (Fig. 1). The Chl a content in the leaves inversely depended on the average daily air temperature. The content of Chl b and carotenoids was less associated with the temperature regime of the region (see Fig. 1).

Eastern galega, like all legumes, is demanding of the amount of moisture, which is consistent with a high correlation between the content of all photosynthetic pigments in plant leaves and the amount of precipitation during the growing season (r = 0.80, $p \leq 0.05$) (see Fig. 1). The content of Chl b directly correlated with the amount of precipitation during the growing season (r = 0.71), whereas for carotenoids, there was an inverse relationship (r = –0.72) ($p \leq 0.05$ for all correlation coefficients obtained).

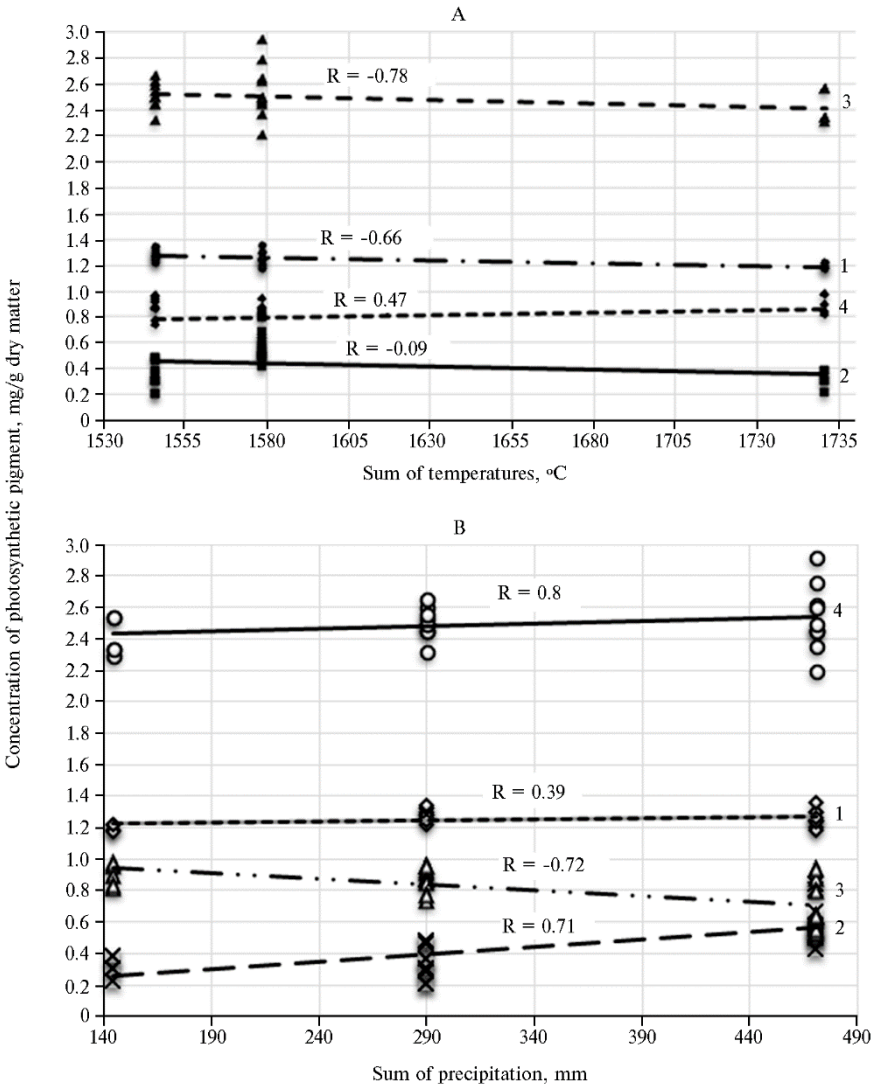

Fig. 1. Accumulation of photosynthetic pigments in the leaves of *Galega orientalis* Lam. cv. Gale depending on the sum of active temperatures ≥ 10 °C (A) **and the sum of precititation** (B)**:** 1 — Chl a, 2 — CHl b, 3 — Car, 4 — total pigments (Barsovo settlement, Khanty-Mansi Autonomous Okrug — Yugra, Surgut District, 61°15′00″ N, 73°25′00″ E, 2013-2015).

The content of vitamin C. Although most mammals are able to synthesize ascorbic acid (AA), its amount may not be sufficient for full growth and ensuring high productivity of animals or under stress, and therefore additives containing AA are used to enrich feed [62-65]. According to reports, the feed mass of the eastern galega contains from 136.2 to 522.1 mg of AA per 100 g of dry matter, at the beginning of the growing season this value may be 800-900 mg% [66]. Earlier we showed that the plant mass of *Galega orientalis* Lam. is a source of ascorbic acid after plants enter the generative phase of development with a predominant (96%) localization of vitamin in leaves [67], which is expected given the role of ascorbic acid in photosynthesis [27]. In our tests, the concentration of AA during the observation period increased from 37 mg% in plants of the 1st year of life to 60 mg% in the 3rd year of life [67]. In the leaf mass of the 3-year-old plants, the content of ascorbic acid (60 mg%) exceeded 1.6 times the same parameter for the 1st and 2nd years of vegetation (37 and 39 mg%, respectively).

When inoculated with the Baikal-EM1 preparation, in the year of sowing, the accumulation of AA in the plant mass was 20% higher (41 mg%, $p \leq 0.05$), in the 2nd year 26% lower (31.0 mg%, $p \leq 0.05$) than the control, in the 3rd year, it was at the control level (61-62 mg%). In mixed sowing with peas, in the 3rd year of herbage life, a significant ($p \leq 0.05$) decrease to 56.0 mg% was noted, which is 6 mg% less than in the control.

We have not revealed a relationship between the AA accumulation and water availability (data are not shown). With a decrease in the average daily air temperature (x), the vitamin C content in the green mass increased ($r = -0,69$; $R^2 = 0.47$, $y = -8,0838x + 133,73$). A strong negative correlation occurred between the AA content in the leaves and the the specific leaf surface (r from –0.83 to –0.88) [67].

The content of flavonoids. According to V.I. Filatov et al. [68], during the introduction of eastern galega in Eastern Siberia, the amount of flavonoids was 0.40% at branching, 0.35% at budding, 0.27% at flowering, and 0.25% to dry matter at fruiting. In our tests, the average content of flavonoids in the aboveground biomass of galega varied from 0.7 to 3.2% over the years of research for all treatments. When inoculated with Baikal-EM1, the maximum amount of flavonoids in aboveground biomass was degected in the 1st year of vegetation (2.4% with 1.9% in the control). In the 2nd year of life, both during inoculation with a microbiological preparation and in the control, the content of flavonoids increased by another 0.3%, in the 3rd year it decreased sharply (to 0.7%), but it did not differ significantly from the control values. In plants under the cover of peas in the 1st and 2nd year of life, the content of flavonoids in the aboveground biomass was 2.1-2.4%, in the 3rd year it increased sharply (to 3.2%), significantly exceeding the indicators in the other two variants of the experiment.

In general, in our tests, the content of flavonoids in the leaves of galega plants was higher than in the stems, and varied from 0.3 to 2.8% (0.2-0.5%in the stems).

In the 1st year of vegetation, we did not detect significant differences in the content of flavonoids in the leaves according to the experimental variants (the values were 1.6-1.9%). In the 2nd year, in control and inoculation with Baikal-EM1, the analyzed parameter increased by 0.5%, in mixed sowing it remained the same as in the 1st year of life (1.7%). In the 3rd year, the content of flavonoids in the leaves decreased sharply in the control (up to 0.05%, i.e., 3-fold compared to the 1st year and 5-fold compared to the 2nd year) and when using a microbiological preparation (6- and 8-fold, respectively). In crops with peas, it sharply increased and exceeded the value for the previous years by 1.6 times (2.8% vs. 1.8 and 1.7%, respectively). We associate a sharp decrease in the content of flavonoids in the 3rd year of life in the control and when using a microbiological preparation with the transition of plants to generative development and entry into the phases of flowering and fruiting (unlike binary sowing, where the virginal stage continued). The use of a microbiopreparation contributed to a more intensive growth of vegetative organs in the pregenerative period, the formation of a larger number of peduncles and fruit formation. It should be noted that studies on different plant species have described both similar [69, 70] and inverse [71] patterns.

One of the factors that was associated with the content of flavonoids during intensive vegetative growth is the amount of precipitation (x) ($r = 0.79$, $R^2 = 0.63$; $y = 0.0046x + 0.5037$).

In eastern galega, we also found a close inverse correlation between the content of vitamin C, on the one hand, and the accumulation of flavonoids and carotenoids, on the other (for all ages of the herbage and experience variants) (Fig. 2).

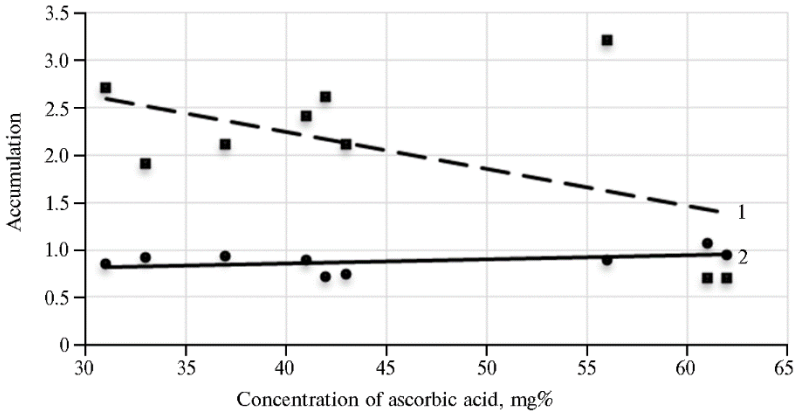

Fig. 2. Accumulation of flawonoids (%, 1) **and carotenoids** (mg/g dray mattem, 2) **in the leaves of *Galega orientalis* Lam. cv. Gale depending on the ascorbic acid concentration** (Barsovo settlement, Khanty-Mansi Autonomous Okrug — Yugra, Surgut District, 61°15′00″ N, 73°25′00″ E, 2013-2015).

The obtained results allow us to conclude that the eastern galega of the Gale variety successfully adapts to the natural and climatic factors of the Middle taiga zone of Western Siberia and is promising as a fodder crop. The temperature and moisture availability at the point of introduction were sufficient for the operation of the photosynthetic apparatus formed by the plants of the eastern galega in the light conditions of the region (intensity and spectral composition of solar radiation, daylight duration) during the growing season. As a result, the productivity of the herbage was 23-35 t/ha. To ensure high and stable yields, the highest protein content and high nutritional value of feed, it is advisable to improve the elements of crop cultivation technologies, including through the selection of microbiopreparations, growth regulators [72], effective cover crops [4]. As an additional reserve, optimization of harvesting techniques through fractionation of its elements (leaves and stems) [1].

A detailed study of biochemical composition of eastern galega which also contains substances classified as anti-nutritional, e.g., trypsin inhibitors, lectins [2], coumarins, saponins, tannins, alkaloids [19], and of physiological and biochemical mechanisms of their accumulation in the plant is important both in matters of feeding and in view of future breeding of the crop. For example, coumarin-based preparations are already used in clinical practice, and many coumarins and their derivatives are considered as potential medicines [73], but sweet clover contains coumarin which in hay, under the action of mold fungi, turns into dicumarol (3,3-methylene-bis-4-oxycoumarin), preventing blood clotting, as a result of which painful bleeding may occur in cattle [74]. Tannins and saponins in high concentrations are considered anti-nutritive substances, but tannins serve as a preservative in feed, and saponins have an immunomodulatory effect [75]. Saponins can promote intestinal health in chickens (76). Alkaloids, tannins and saponins was reported to influence the nutritional behavior of cattle and sheep [77].

The influence of fertilizers and the accumulation of micro- and macroelements, heavy metals in the biomass of galega is also subject to assessment [78, 79]. Other promising areas are the study of the root system, allowing galega plants to use nutrients better, the elucidation of the influence of galega as a precursor on the yield of agricultural plants, and the determination of its suitability in the system of extensive organic farming [3, 4].

Finally, the ecological aspect of the galega introduction is extremely important. Legumes are one of the leaders in the harmful effects of plant invasion [7, 80]. In Central Russia, legumes occupy the fifth place among alien species. The aggressiveness of legumes is associated with their mass use as fodder grasses

and “green fertilizers”. *G. orientalis* is one of the most aggressive invasive species of legumes [80]. During invasions, changes occur at the ecosystem level, so even the complete removal of insiders does not return the community to its original status [80].

Thus, during the introduction of eastern galega cv. Gale in the North of Russia (61 15'00" N, 73 25'00" E), the effect of three studied agrotechniques (t.e., monoculture, monoculture with pre-sowing treatment of seeds with microbial preparation Baikal-EM1, and mixed culture with peas) on the Chl a + Chl b in the leaves appeared since the 2nd year of plant life. For the 2nd and 3rd years of life, this value, as influenced by a microbiological preparation, was higher than in the control (by 19-22% and 16-18% over the development phases). In mixed sowing it decreased at the end of the 2nd year, but by the end of the 3rd year it exceeded the control values by 33%. In the control, the content of Chl a in the leaves in the year of sowing, for the 2nd and 3rd years of life was 1.23±0.10, 1.29±0.12 and 1.32±0.14 mg/g of dry weight. On average, in the 2nd year, when using Baikal-EM1 fertilizer, the content of Chl a in the leaves increased by 15% compared to the control. In mixed sowing with peas, it remained within the control values (1.20±0.23 mg/g). Over 3 years, when using a microbiological preparation, the value of Chl a/Chl b in leaves significantly decreased ($p \leq 0.05$), which may indicate an increase in the adaptive potential of plants. In mixed crops it remained within the control values. The proportion of Chl a + Chl b localized in the light-harvesing complexes (LHC) varied from 20 to 90% depending on the phenological phase, the age of the herbage and the treatment. In the control, under inoculation with a microbial preparation and in mixed spwings, the correlation between Chl a/Chl b and the proportion of chlorophylls Chl a + Chl b localized in the LHC was characterized by $r = 0.83$, $r = 0.93$ and $r = 0.65$ ($p \leq 0.05$), respectively. The used agrotechniques did not significantly change the Chl/Car values. Nevertheless, during inoculation with Baikal-EM1 and in mixed sowing with peas, the accumulation of carotenoids in the leaves of eastern galega exceeded that in the control. On average, over the years of the study, for all variants of the experiment, the accumulation of all pigments in the leaves directly correlated with the LHC. The content of Chl b and carotenoids was less associated with the temperature regime of the region, while the first parameter directly correlated with the amount of precipitation for the season and the second parameter showed a negative correlation. When inoculated with Baikal-EM1, the content of ascorbic acid in the leaves in the 1st and 2nd year of plant life increased cpared to control, by the 3rd year, it was almost equal to the control values, in mixed sowing for the 3rd year it decreased vs. the control. The content of flavonoids in the leaves of 3-year-old plents with the microbiological preparation and in the control (when the plants switched to generative development) decreased sharply, while the mixed sowing, where the virginal stage continued, it sharply increased (1.6 times compared to previous years). In general, the data obtained indicate that the use of the microbiological preparation Baikal-EM1 largely contributed to the galega plant adaptation to new environmental conditions during the 2nd and 3rd years of life.

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COMPARATIVE ASSESSMENT OF SPRING SOFT WHEAT LINES (*Triticum aestivum* L.) IN THE STEPPE ZONE OF THE NORTH KAZAKHSTAN REGION

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Abstract

Spring soft wheat (*Triticum aestivum* L.) is one of the most highly demanded crops in Kazakhstan. In 2020, the gross harvest of spring soft wheat reached in recent years the highest outcome of 18.0 million tons. The most important resource for increasing the yield of spring soft wheat is the adaptability and implementation of the variety according to a complex of economically valuable traits. New varieties must be flexible under different environmental conditions. In the presented work, we, for the first time, have identified lines of spring soft wheat well adapted to the conditions of the North Kazakhstan region, distinguished by productivity, a set of economically valuable parameters, environmental stability and plasticity. The aims of the work were i) a comparative assessment of the lines of spring soft wheat of different ripeness groups to the highest extent adapted to the conditions of the steppe zone of Northern Kazakhstan and ii) the assessment of economically valuable traits and their interrelationship with grain yield. The trial was performed using an extended set of spring soft wheat lines of various ripeness from research centers of Kazakhstan (fallow soil, the North Kazakhstan Agricultural Experimental Station LLP, Republic of Kazakhstan, 2018-2020). A total of 28 lines were studied, including 20 middle-early and 8 mid-season lines. Two cultivars registered in North Kazakhstan region served as the standards, the middle-early cv. Astana and the mid-season cv. Omskaya 35. The duration of inter phase and vegetation periods, yield and the main elements of yield structure were studied. The length of growing season was 79 days for the mid-early lines and 80 days for the mid-ripening lines. A shorter growing season was characteristic for the mid-early lines Lutescens 1125 SP 2/09 (73 days), Lutescens 528 (74 days), Lutescens 630 SP 2/08 (74 days), Lutescens 742 SP 2/19 (74 days), Lutescens 715 SP 2/04 (75 days), Lutescens 687 SP 2/04(75 days), Lutescens 1148 SP 2/09 (76 days) vs. the standard cv. Astana (79 days). In the mid-season group, the Liniya 12/93-01(82 days), Liniya 33/93-01-15 (82 days), Lutescens 2194 (82 days), Lutescens 1919 (85 days) stood out for the optimal length of growing season vs. the standard cv. Omskaya 35 (80 days). In terms of crop yield in the mid-early ripeness group, the following lines were distinguished: Lutescens 588 SP 2/05 (2.3 t/ha), Erythrospermum 738 2/09 (2.3 t/ha), Lutescens 857 SP 2/05 (2.4 t/ha), Lutescens 821 SP 1/08 (2.4 t/ha), Lutescens 715 SP 2/04 (2.4 t/ha) vs. cv. Astana (2.0 t/ha). In the mid-season group, Lutescens 371/06 (2.4 t/ha), Line 12/93-01-10 (2.4 t/ha), Lutescens 1919 (2.5 t/ha), Line 55/94-01 (2.6 t/ha), and Line 33/93-01-15 (2.8 t/ha) were superior to cv. Omskaya 35 (1.8 t/ha). In the studied mid-early lines, the main elements of the yield structure were the number of productive stems (154-244 stems/m^2), the grain number per ear (21-28 grains), and the 1000-grain weight of 36.6-43.4 g. In the mid-season group, the number of productive stems was 170-252 stems/m^2, the number of grains per ear was 23-30 grains, and the 1000-grain weight of was 34.2-45.2 g. The yield of mid-early lines showed correlation with the

grain number per ear (r = 0.35-0.86, p = 0.36-1.29) and tight correlation with the number of productive stems (r = 0.68-0.83, p = 0.82-1.18). The yield of mid-season lines correlated with the number of productive stems (r = 0.74-0.86, p = 0.95-1.29) and the grain number per ear (r = 0.31-0.71, p = 0.32-0.88). The correlation between yield of the studied lines and the 1000-grain weight was medium (r = 0.37-0.54, p = 0.38-0.60) and, in a dry year, weakly negative (r = –0.16, p = 0, 16). Therefore, for the North Kazakhstan steppe zone, we propose to involve the mid-early lines Lutescens 715 SP2/04, Lutescens 821 SP2/08, Lutescens 588 SP2/05, Erythrospermum 738 2/09 and mid-season Line 33/93-01-15, Line 55/94-01, Lutescens 371/06, Lutescens 1919, Line 12/93-01-10 in breeding for drought resistance and adaptive potential.

Keywords: spring soft wheat, mid-early lines, mid-ripe lines, growing season length, grain productivity, yield structure elements

Based on the predicted needs of humanity associated with population growth, changing diets and an increase in the need for biofuels, world crop production should double by 2050. However, with the current increase in wheat yields of 0.9% per year, global grain production will increase only by 38%, and therefore the emphasis should be on increasing the yield of varieties [1].

Sustainable crop production [2)] is not possible without the use of high-yielding varieties [3]. Genetic improvement of varieties on the basis of productivity should be up to 1.16-1.31% per year [4]. In recent decades, the world has recorded fewer genetic advances than required [5, 6].

Common wheat (*Triticum aestivum* L.) is a natural allohexaploid that carries the genetic material of several species of the genus *Triticum* L. and *Aegilops* L. due to natural hybridization followed by amphidiploidization [7)]. In the northern regions of Kazakhstan, 15.0 million hectares are occupied by crops of spring soft wheat. The average yield in this zone, depending on climatic conditions, ranges from 0.9 to 1.4 t/ha over the years (https://primeminister.kz/ru/news/uborochnye-raboty-v-kazahstane-zaversheny-na-934 -msh-rk-2281145).

Varieties cultivated in the conditions of the steppe zone of Northern Kazakhstan are characterized by high grain quality. Nevertheless, to obtain a high yield, it is important to study the productive and adaptive potential of a variety, its biological characteristics [8].

Under relatively equal environmental conditions and the same productivity, the yield structure of different varieties is different. Some varieties have a high productive bushiness, others have a high absolute grain mass, and still others have an increased ear grain mass [9]. In obtaining high and stable yields, biological (elements of the crop structure) and technological traits (grain volumetric weight and protein content) are considered as leading ones, though the role of agricultural practices that affect the manifestation of these traits is also importanta [10, 11]. The entire agrotechnical complex must strictly correspond to the characteristics of varieties in specific environmental conditions.

The most important property of any variety is its adaptability, that is, the ability of the genotype to withstand the action of environmental factors that reduce productivity and yield, which is very important for the agroecological zoning of the variety [12].

In the conditions of Northern Kazakhstan, moisture availability becomes a limiting factor in the growth and development of spring soft wheat plants. In addition, high temperatures and low air humidity have a negative effect, especially during critical phases of growth and development (booting—heading). In the spring and early summer periods (June), crops are often damaged by dust storms, frosts, diseases, and pests (bread fleas).

To improve the drought resistance of spring soft wheat, it is necessary to select parental forms that have biochemical and physiological mechanisms that can mitigate the effects of abiotic stress at the grain filling stage [13, 14].

In this work, for the first time, we selected lines of spring soft wheat which

are well adapted to the conditions of the North Kazakhstan region, and distinguished by productivity, a set of economically valuable parameters, environmental stability and plasticity.

The purpose of the work is to compare the lines of spring soft wheat of various ripeness groups, maximally adapted to the steppe zone of Northern Kazakhstan for economically significant traits and to evaluate their relationship with grain yield.

Materials and methods. The experiments were laid with the fallow predecessor (the LLP North Kazakhstan Agricultural Experimental Station, Shagaly village, North Kazakhstan region, 2018-2020). We performed ecological testing of an extended set of lines of spring soft wheat of various ripeness groups from various scientific centers of Kazakhstan, 28 lines in total, including 20 mid-early and 8 mid-ripening lines. Two varieties registered in the North Kazakhstan region were used as standards, Astana for mid-early and Omskaya 35 for mid-season lines.

The total plot area was 25 m^2, plants were collected from a 20 m^2 area, The experiment design provided 4-fold repetition. Plots were randomly distributed. Sowing was carried out at the optimal time for the zone (May 20-25), the seeding rate was 3.0 million germinating seeds per 1 ha, the seeds were sown with a selective seeder SSN-7 (Omsk Experimental Plant, Russia)

The soil of the test plots is ordinary calcareous heavy loamy chernozem, pH 8.1. The soil layer of 0-40 cm was 4.5% humus, 16.6 mg/kg nitrate nitrogen, 10 mg/kg mobile phosphorus, abd 630 mg/kg potassium.

Immediately before sowing, soil samples were taken at a depth of 0-40 cm. The humus content was determined by the I.V. Tyurin's method modified by V.N. Simakov [15], the pH of the aqueous extract was measured potentiometrically [16], the content of nitrate nitrogen by the disulfophenyl Grandval-Lage method [17], the content of mobile phosphorus and exchangeable potassium by the method of B.P. Machigin [18].

The yield structure was analyzed in plants from the trial plots in 4 replicates for each sample [19]. Each harvested sheaf was analyzed by the number of plants, the main shoots and productive stems. The elements of the yield structure (the number of grains per ear and the 1000-grain weight) were determined in 25 plants in 4 replications. Harvesting was carried out at full grain ripeness with a Sampo-500 combine (Sampo Rosenlew, Finland). For each variety and line, grain quality parameter were adjusted to 14% moisture and 100% purity.

To assess the meteorological conditions during the years of the experiments, the amount of precipitation and the temperature regime were compared with the long-term average data (http://www.pogodaiklimat.ru). The experimental data were analyzed using the AgStat program (https://www.agstat.com/). Based on the results of the analysis of variance, the least significant difference (LSD_{05}), the means (*M*) and standard errors of the means (±SEM), coefficients of variation (*Cv*) and correlations (*r*) were calculated [20].

Results. In 2018, the reserves of productive moisture in a meter-deep soil layer before sowing amounted to 149.1 mm. The beginning of the summer months was cold, so the sowing was carried out on May 28, which was 3-5 days later than the optimal time for the zone. The average air temperature in May was 9.5 °C, in June it rose to 17.1 °C. Mass seedling emergence and setting of tillering elements occurred. In July, the average air temperature was 20.3 °C, which had a positive effect on the setting of generative organs and ear elements. The average air temperature in August, 16.6 °C, favorably influenced the ear productivity. During the growing season of 2018, 291.7 mm of precipitation fell, or 285% of the long-term norm. The culture was well supplied with moisture, the hydrothermal coefficient

(HTC) ranged from 0.6 to 0.9, with the average annual value of 0.8. It should be noted that the high humidity in August and the air temperature of 16.6 °C lengthened the phase of grain ripening.

In 2019, the reserves of productive moisture in a meter-deep soil layer were optimal and amounted to 128.0 mm. The beginning of May was characterized by hot and dry weather with strong winds. The maximum air temperature in May reached 29.0-31.6 °C. The amount of precipitation in May was low, 12.8 mm, or 46% of the long-term norm. Precipitation was unevenly distributed over decades, which occurs with frequent droughts in recent decades [21]. Rains fell only in the second and third decades of May, and their amount was 58-73% of the norm. June 2019 was cool (the average daily air temperature was 3 °C below the long-term average of 18.6 °C), precipitation amounted to 56.8 mm (129% of the norm). The warm period came late, at the end of June the sum of positive temperatures amounted to 917 °C with a long-term average of 1069 °C. The lack of heat affected the duration of the period from sowing to seedling emergence which was 14-16 days vs. 10-12 days according to long-term data. However, precipitation in the third decade of June had a positive effect on the passage of the tillering phase, in this year the highest tillering coefficient was the highest and accounted for 2.3. July 2019 was dry, the average monthly air temperature was 20.9 °C, 23 mm of precipitation fell vs. a norm of 71 mm (32%). The July maximum of precipitation, typical for the region, was not observed. During the ripening period in August, it was warm, the average daily air temperature was 18.1 °C, or 0.9 °C higher than the long-term average, 43.3 mm of precipitation fell, or 92% of the norm of 47.0 mm. In general, according to meteorological data, August corresponded to the average annual norm, the grain number per ear and the 1000-grain weight were formed under favorable conditions.

In 2020, May in the north of the region was abnormally hot and windy. The maximum air temperature was 33.5-35.6 °C, the sum of positive temperatures at the end of the month exceeded the long-term average by 267 °C. The second ten-day period of May was the hottest, the average daily air temperature was 20 °C vs. the norm of 13 °C. The amount of precipitation (28.1 mm) corresponded to the average annual norm. Despite the prevailing atypical conditions of the sowing period, mass seedlings emergence occurred. There was a 70-day period from sowing to seedling emergence. June 2020 was characterized by very contrasting meteorological conditions. The first two decades were extremely dry with 1.1 mm and 1.8 mm precipitation, or 8 and 16% of the norm. Precipitation in the III decade (33 mm) significantly leveled the situation. In general, 35.9 mm (82%) fell during the month. Excess heat in June reached 211 °C. The created meteorological conditions accelerated the tillering phase. In July 2020, precipitation was extremely uneven. Their amount was 75.6 mm (106%), and the distribution was as follows: the main amount fell in the first decade of July (66.6 mm), in the II and III decades precipitation was extremely low, 0.2 mm and 8.8 mm (1 and 34%). The sum of positive temperatures in July was 1938 °C, which was 268 °C higher than the long-term average values. The created weather conditions had a positive effect on the setting and formation of the ear elements. August 2020 was also dry and hot. The average daily air temperature was 19.8 °C, or 2.6 °C above the norm. Together with elevated temperatures, the precipitation of 2.6 mm (43%) accelerated the onset of the wax ripeness phase of wheat. In 2020, the grain ripening period decreased by 10 days compared to long-term observation. In general, in terms of agrometeorological conditions for the crop growth and development, 2020 was characterized by an early summer and August drought and a pronounced July maximum of precipitation. The provision of crops in 2020 with moisture during

the critical period (stem elongation—heading) had a positive effect on the formation of the crop and grain quality.

In conditions of limited water resources, wheat breeding is being updated for traits that increase the efficiency of moisture use [22]. According to A. Nawaz et al. [23], the most detrimental effect on the setting and maturation of grain is drought during the reproductive phase and at grain filling. In the steppe zone of Northern Kazakhstan, early summer drought often occurs, therefore, varieties with an extended interphase period from seedlings to heading and shortened period of heading-grain ripening are more adapted to local conditions.

The average yield of soft wheat in Kazakhstan is due not only to natural and climatic factors, but also to the imperfection of agricultural technologies for the cultivation of new varieties [24].

According to V.A. Krupnov [25], in the dry first half of the growing season, the optimal yield is formed by medium or late-ripening varieties. According to our data, mid-early varieties can also be assigned to this group, since there was no difference in the length of the growing season between them (79 days) and mid-ripening (80 days) lines. In addition, there was no difference in the duration of the interphase period between germination and heading in lines of different maturity groups (Table 1).

1. Interphase periods in vegetation of spring soft wheat (*Triticum aestivum* L.) varieties and lines in the steppe zone of Northern Kazakhstan (North Kazakhstan region, Akkayyn district, 2018-2020)

Parameter	Interphase period, days		
	seedlings—heading	heading—ripening	seedlings— ripening
	Mid-early (n = 21)		
M±SEM	45.0±4.00	34.0±5.50	79.0±1.52
Lim	41-49	29-40	78-81
R	8	11	3
Cv, %	7.3	13.1	1.8
	Mid-ripening (n = 9)		
M±SEM	46.0±4.16	34.0±4.50	80.0±1.52
Lim	43-51	30-39	79-82
R	8	9	3
Cv, %	7.3	10.7	1.6

Not. Lim — limit, R — range, *Cv* — the coefficient of variation.

The data we obtained show the advantages of mid-early lines and varieties created at the scientific centers of Kazakhstan. Due to the slow development from germination to earing, they are more resistant to spring-early summer drought. It is known that varieties with early earing under conditions of high temperature and lack of water increase the yield index [26, 27]. Improvement of agronomic phenotypes is carried out on the basis of the analysis of the genetic variability of the breeding material [28].

In general, it should be noted that the range of variation and the coefficient of variation in the length of interphase and vegetation periods over the years largely depended on meteorological conditions and genetic backgrounds of the lines. In mid-early and mid-ripening lines, the length of seedlings-heading period was characterized by Cv = 7.3%. For the heading-ripening period, an average trait variability accounted for 13.1 and 10.7%, respectively. Over the growing season, the variability was insignificant and accounted for 1.8 and 1.6%.

The correlation relationship between the length of the growing season and the yield in lines of different types of ripeness was expressed differently. Excessive elongation of the seedling-heading period in mid-early lines had a negative relationship with productivity (r valued from –0.05 to –0.27, p = 0.05-0.27), since it reduced the period of grain formation and filling

In 2018, mid-season lines showed a weak negative relationship between

the length of the growing season and yield (r = –0.05, p = 0.05). This is due to the negative influence of high humidity and low temperature during the formation and filling of grain. In 2019 and 2020, the meteorological conditions in the same periods were favorable, which was confirmed by a clearly pronounced correlation of the average strength (r = 0.64-0.68, p = 0.75-0.82) with the yield.

Consequently, the duration of interphase periods and the growing season in the studied lines is genetically determined, but their variability is largely determined by meteorological conditions. According to P.L. Goncharova et al. [29], the influence of a variety genetic background on the length of the growing season in arid conditions is 69.8%. The correlations observed by us between the yield and the length of the growing season in lines of different types of ripeness can be associated with the features of the redistribution of assimilates, genetic systems of photoperiodic reactions, vernalization, signaling, which affect the formation of grain productivity of plants and the area of cultivation of the variety [30-32].

It has been proven that high yields and grain quality are achieved with optimal performance for various elements of the crop structure [33]. Thus, the number of productive stems depends on environmental conditions and genotypic characteristics (with the heritability of the trait at the level of 0.51-0.72) [34]. In cultivars with a longer germination-tillering interphase, an increase in the number of productive stems is observed [35). According to E.V. Ionova [36], plants of the mid-season type have an extended tillering period, delayed wilting, a flattened plant shape and a good root system; they are characterized by a decrease in assimilation during hot daytime hours. Such plants accelerate development, striving to complete the cycle faster, which sharply reduces their productivity. Plants of mid-early varieties lose turgor, wither, but retain the viability of lateral shoots. When precipitation falls, their rapid growth resumes, the second half of the growing season is reduced. Plants of mid-season varieties suffer from drought, the upper leaves turn yellow, the lower and side shoots die off, only the main ear remains. In our studies, the tillering coefficient averaged 1.2 for mid-season lines and 1.3 for mid-early lines (Table 2).

In wheat breeding, relationships of three components, the number of productive stalks per 1 m^2, the number of grains per ear, and the 1000-grain weight are often studied, which are largely correlated with yield [37). In our experiments, mid-early and mid-season wheat lines mainly differed in the number of productive stems per unit area. Thus, in mid-season lines this figure was 219 pcs/m^2, which is 15 pcs/m^2 (or 7%) more than in mid-early lines (see Table 2). In the mid-early group, the range of variation and the coefficient of variation (R = 90, Cv = 8.8%) was higher than in the mid-season group (R = 62, Cv = 7.8%), which is due to less fluctuation in the values of the limits over the years

In general, the yield of grain crops depends on a number of factors, including the ability of plants to synthesize and redistribute assimilates, form elements of the crop structure, as well as the timing of the development and maturation phases [38]. Various traits, including the number of grains per ear, yield potential, timing of flowering and grain filling, are considered as a complex indicator that explains 76% of the variation in grain yield (r = 0.70, p = 0.86), which can be used in programs for selection of lines of spring soft wheat for high productivity during droughts [39]. Yield potential can be increased through more efficient fruiting [40], i.e., selection for high ear fertility, which is estimated as the ratio of the number of caryopses to the number of flowers per ear, to achieve high and stable yields [41]. Isogenic mutant lines showed a significant increase in the weight of 1000 grains (by 6.6%), width (by 2.8%) and length of the grain (by 2.1%) in hexaploid wheat, which led to an increase in the mass of grain per ear [42].

2. Element of crop structure of spring soft wheat (*Triticum aestivum* L.) varieties and lines of various ripening groups in the steppe zone of Northern Kazakhstan over the years of observation (North Kazakhstan region, Akkayyn district, 2018-2020)

Variety, line	The productive stem number per m^2				Productive tillering				The grain number per ear.				1000-grain weigh, g			
	2018	2019	2020	*M*±SEM	2018	2019	2020	*M*±SEM	2018	2019	2020	*M*±SEM	2018	2019	2020	*M*±SEM
Mid-early (n = 21)																
Astana (standard)	217	192	260	223.0±29.78	1.0	1.0	1.2	1.1±0.10	14	30	30	24.6±8.00	33.5	43.7	34.2	37.1±4.93
Lutescens 932 SP 2/04	215	231	196	214.0±15.17	1.1	1.8	1.4	1.4±0.30	14	29	27	23.3±7.05	35.2	36.6	43.1	38.3±3.65
Erythrospermum 738 2/09	179	211	221	203.6±19.0	0.9	1.9	1.3	1.4±0.43	22	31	30	27.6±4.27	37.7	36.6	39.2	38.8±1.13
Lutescens 817 SP 2/09	227	263	241	243.6±15.71	1.0	1.6	1.2	1.3±0.26	13	28	27	22.6±7.26	35.6	42.5	39.4	39.1±2.99
Lutescens 753 SP 2/09	172	185	201	186.0±12.57	1.0	1.8	1.2	1.4±0.36	18	28	25	23.6±4.44	37.0	42.5	40.2	39.9±2.39
Lutescens 1125 SP 2/09	214	225	183	207.3±18.86	1.1	1.5	1.3	1.3±0.17	11	33	27	23.6±9.84	36.9	45.1	42.5	41.5±3.62
Lutescens 736 SP 2/04	204	201	237	214.0±17.29	1.1	1.5	1.2	1.3±0.18	19	26	28	24.3±4.09	39.4	41.8	43.8	41.7±1.90
Lutescens No. 528	203	201	165	189.6±18.52	1.1	1.8	1.3	1.5±0.31	13	30	30	24.3±8.50	35.7	46.5	40.1	40.8±4.70
Lutescens 1148 SP 2/09	209	253	163	208.3±38.97	1.0	1.6	1.3	1.3±0.25	12	33	32	25.6±10.25	29.1	42.7	38.0	36.6±5.98
Lutescens 588 SP 2/05	189	204	216	203.0±11.71	1.2	1.7	1.6	1.5±0.22	13	28	35	25.3±9.73	44.3	45.2	40.8	43.4±2.01
Lutescens 857 SP 2/05	239	234	191	221.3±22.85	1.0	1.7	1.1	1.3±0.32	18	28	30	25.3±5.56	36.2	45.7	40.2	40.7±4.13
Lutescens 1206 SP 2/19	176	217	192	195.0±17.89	1.2	2.3	1.3	1.6±0.52	21	29	29	26.3±4.00	36.8	38.0	38.0	37.6±0.60
Lutescens 1143 SP 2/09	216	212	192	206.6±11.13	1.1	2.0	1.1	1.4±0.45	13	27	28	22.6±7.26	31.5	48.2	41.6	40.4±7.28
Lutescens 783 SP 2/07	197	181	219	199.0±16.52	1.2	1.8	1.1	1.4±0.32	11	25	28	21.3±7.85	35.8	47.8	40.4	41.3±5.24
Lutescens 687 SP 2/04	195	181	168	181.3±11.69	1.1	1.4	1.0	1.2±0.18	14	31	29	24.6±8.04	37.8	37.2	42.2	39.1±2.36
Lutescens 821 SP 2/08	186	284	199	223.0±46.09	1.1	1.7	1.5	1.4±0.26	15	31	30	25.3±7.76	33.8	44.1	40.0	39.3±4.49
Lutescens 742 SP 2/19	170	208	199	192.3±17.19	1.0	1.4	1.5	1.3±0.22	15	22	30	22.3±6.50	40.2	36.6	40.0	38.9±1.75
Lutescens 822 SP 2/0927	184	222	201	202.3±16.48	1.1	1.5	1.2	1.3±0.18	13	27	29	23.0±7.54	36.9	42.2	38.2	39.1±2.39
Lutescens 1068 SP 2/09	212	205	189	202.3±10.21	1.2	1.5	1.3	1.3±0.13	15	27	26	22.6±5.76	34.4	45.8	45.7	40.2±5.67
Lutescens 715 SP 2/04	180	273	181	211.3±46.25	1.3	2.1	1.4	1.6±0.37	18	30	28	25.3±5.56	39.3	46.2	42.2	42.6±3.00
Lutescens 630 SP 2/08	154	156	152	154.0±1.73	1.1	1.5	1.1	1.2±0.20	16	31	25	24.0±6.53	39.9	43.3	43.6	42.3±1.77
M±SEM	197.0±21.07	216.1±31.6	198.3±26.6	203.8±18.05	1.1±0.09	1.6±0.27	1.2±0.15	1.3±0.10	15.1±3.06	28.7±2.61	28.7±2.27	24.2±1.49	36.5±3.19	42.7±3.70	40.6±2.48	39.9±1.88
Lim	154-239	156-284	152-260	154-244	0.9-1.3	1.0-2.3	1.0-1.6	1.1-1.6	11-22	22-33	25-35	21-28	29.1-44.3	36.6-48.2	34.2-45.7	36.6-43.4
R	85	128	108	90	0.4	1.3	0.6	0.5	11	11	10	7	15.2	11.6	11.5	9.2
Cv, %	10.6	14.5	13.3	8.8	8.5	16.3	11.8	9.0	20.0	9.2	9.0	6.2	8.7	8.1	6.0	4.6

Continued Table 2

Mid-ripening (n = 9)																
Omskaya 35 (standard)	192	237	232	220.3±21.36	1.0	1.0	1.1	1.0±0.05	17	26	27	23.3±4.76	38.8	37.0	34.2	36.7±2.00
Line 55/94-01	213	228	221	220.6±6.50	1.0	1.5	1.5	1.0±0.25	23	35	33	30.3±5.56	33.4	42.6	40.0	38.7±4.10
Line 12/93-01-10	227	196	264	229.0±29.48	1.1	1.0	1.6	1.2±0.27	12	32	29	24.3±9.34	43.3	48.4	41.0	44.2±3.27
Lutescens 2174	300	140	215	218.3±69.32	1.3	1.3	1.5	1.4±0.10	20	26	30	25.3±4.35	36.2	47.0	37.7	40.3±5.06
Lutescens 371/06	225	188	220	211.0±17.38	1.1	1.6	1.4	1.4±0.21	24	31	31	28.6±3.50	39.3	40.6	43.2	41.0±1.71
Lutescens 1919	172	212	300	228.0±56.70	1.0	1.7	1.1	1.3±0.32	16	29	33	26.0±7.69	40.5	40.3	38.3	39.7±1.05
Lutescens 43/01	159	174	237	190.0±35.84	1.2	1.5	1.3	1.3±0.13	18	26	29	24.3±4.92	34.6	46.3	45.2	42.0±5.59
Line 33/93-01-15	201	310	245	252.0±47.48	1.2	2.1	1.5	1.6±0.39	17	31	35	27.6±8.18	32.1	42.3	36.1	36.8±4.45
Lutescens 248/01	232	188	175	198.3±25.86	1.3	1.4	1.5	1.4±0.08	17	28	34	26.3±7.46	34.5	45.3	39.1	39.6±4.69
M±SEM	213.4±39.77	208.1±46.50	234.3±33.59	218.6±19.48	1.1±0.11	1.4±0.32	1.3±0.17	1.2±0.11	18.2±3.52	29.3±3.03	31.2±2.57	26.2±2.18	36.9±3.56	43.3±3.56	39.4±3.27	39.9±2.31
Lim	159-300	140-310	175-300	170-252	1.0-1.3	1.0-2.1	1.1-1.6	1.0-1.6	12-24	26-35	27-35	23-30	32.1-43.3	37.0-48.4	34.2-45.2	36.7-44.2
R	141	170	125	62	0.3	1.1	0.5	0.6	12	9	8	7	11.2	11.4	11.0	7.5
Cv, %	18.1	21.7	13.9	7.8	10.2	22.2	12.5	14.4	19.0	10.2	8.1	8.7	9.5	8.1	8.0	5.7

N o t e. Lim — limit, R — range, *Cv* — the coefficient of variation.

Successful breeding to increase the productivity and adaptability of varieties is based on a detailed analysis of the heritability of yield traits and the influence of the genotype-environment interaction on their manifestation [43], the use of molecular markers to better understand the genetic basis and the relationship of economically significant traits [44].

During the years of our research, the average value of the number of grains per ear in the mid-early group was 24 pieces, in the mid-ripening group 26 pieces. The graininess of the ear in both groups of maturity showed low variation (*Cv* accounted for 6.2 and 8.7%, R = 7). Extreme high limit values were higher for mid-season lines (30 pcs). According to Yu.S. Krasnova [45], between the number of grains per ear and the mass of grain per ear or the yield, there is a positive relationship of medium strength. A higher yield of mid-season forms was due to their good grain content, 33 pcs for Line 55/94-01, 33 pcs for Lutescens 1919, 34 pcs for Lutescens 248/01, 35 pcs for Lines 33/93-01-15 (see Table 2).

The 1000-grain weight compared to the grain number per ear, despite the rather wide range of variation (R = 36.6-43.4 g in mid-early varieties, R = 36.7-44.2 g in mid-season varieties), turned out to be a more stable trait, the *Cv* is 4.6 and 5.7%, respectively, which indicates the efficiency of its selection under local conditions (see Table 2). A decrease in the 1000-grain weight can occur with an increase in the productivity index [46].

In the mid-early group, the lines Lutescens 783 SP 2/07 (41.3 g), Lutescens 1125 SP 2/09 (41.5 g), Lutescens 736 SP 2/04 (41.7 g), Lutescens 1068 SP 2/09 (42.0 g), Lutescens 630 SP 2/08 (42.3 g), Lutescens 715 SP 2/04 (42.6 g), Lutescens 588 SP 2/05 (43.4 g) were characterized by a high 1000-grain weight. In the mid-season group, in terms of this trait, the lines Lutescens 2174 (40.3 g), Lutescens 371/06 (41.0 g), Lutescens 43/01 (42.0 g), Line 12/93-01-10 (44, 2 d) stood out with an average value of 39.9 g. These lines are of interest for selection for the 1000-grain weight.

V.S. Valekzhanin and N.I. Korobeinikov noted [47] that the grain number per ear has a higher variability than the 1000-grain weight. However, in our studies in the steppe zone of Northern Kazakhstan, these traits are more stable with low variation.

Ear productivity (the number of grains per unit mass of the spike rod) provides an opportunity to increase yields in regions with its high potential [48, 49], while low moisture availability during flowering reduces grain yield by 46.7%, an increased air temperature by 33.6% [50]. The results of the experiment showed that the lines we studied were characterized by an average degree of grain yield variability (Cv = 11.4-13.4%). In terms of average yield, mid-early lines (2.1 t/ha) were inferior to mid-season lines (2.3 t/ha). Within the middle early group, the lines Lutescens 817 SP 2/09 (2.2 t/ha), Erythrospermum 738 2/09 (2.3 t/ha), Lutescens 857 SP 2/05 (2.4 t/ha), Lutescens 715 SP 2/04 (2.4 t/ha), Lutescens 821 SP 2/08 (2.4 t/ha) should noted when compared to the Astana standard value of 2.0 t/ha. In the mid-season group, a high yield was formed by the lines Lutescens 12/93-01-10 (2.4 t/ha), Line 1919 (2.5 t/ha), Line 55/94-01 (2.6 t/ha), Line 33/93-01-15 (2.8 t/ha) vs. the value of the Omskaya 35 standard (1.8 t/ha).

In the studied lines, we evaluated the relationship of each element of the crop structure with the yield by years. In mid-early forms, the yield in all years of research had a significantly positive relationship with the graininess of the ear (r = 0.35-0.86, p = 0.36-1.29). In favorable years 2018 and 2019, there was a close relationship between yield and the number of productive stems (r = 0.68-0.83, p = 0.82-1.18). In the same 2018 and 2019 years favorable during the period of grain formation and filling, the relationship between the 1000-grain weight and the yield turned out to be medium (r = 0.37-0.54, p = 0.38-0.60), and under the

August drought in 2020, the relationship was weakly negative (r = –0.16, p = 0.16).

When creating high-yielding varieties, it is proposed to increase the productivity of the main ear and secondary shoots, improve the architectonics of the ear, and select according to the grain filling rate, yield index, and grain size [51-54]. In the group of mid-early lines studied by us, selection for a combination of coarse-grainedness (absolute weight 41.5-43.4 g) with a high grain content of the ear (up to 25-28 pcs) seems to be successful, which was well expressed in the lines Lutescens 588 SP 2/05, Lutescens 715 SP 2/04, Lutescens 687 SP 2/04.

In the mid-season lines studied by us, the most pronounced relationship was between the yield and the number of productive stems (r = 0.74-0.86, p = 0.95-1.29) and grain number per ear (r = 0.31-0.71, p = 0.32-0.88). The relationship with the 1000-grain weight was weak in 2018 and 2020 (r = 0.01-0.24, p = 0.01-0.24) and weak negative in 2019 (r = –0.08, p = 0.08) under unfavorable conditions during the grain formation and filling. Our data are consistent with the results of A.T. Babkenov et al. [55] who reported a weak correlation between yield and the 1000-grain weight (r from 0.03 to –0.33). Hence it follows that when selecting mid-ripening forms in the steppe zone of Northern Kazakhstan, special attention should be paid to the grain number per ear (up to 26-30 pcs) and the number of productive stems (234-300 pcs/m^2). From this point of view, Line 33/95-01-05, Lutescens 371/06, Line 55/94-01 had the best performance.

3. Yields of spring soft wheat (*Triticum aestivum* L.) varieties and lines of various ripening groups in the steppe zone of Northern Kazakhstan over the years of observation (North Kazakhstan region, Akkayyn district)

Variety, line	Yield, t/ha							
	2018		2019		2020		average	
	t/ha	DS, %	t/ha	DS, %	t/ha	DS, %	t/ha	DS, %
Mid-early (n = 21)								
Astana (standard)	0.9		2.5		2.6		2.0	
Lutescens 932 SP 2/04	1.1	122	2.4	96	2.3	90	1.9	95
Erythrospermum 738 2/09	1.6	178	2.6	104	2.6	0	2.3	115
Lutescens 817 SP 2/09	1.0	111	3.1	124	2.6	0	2.2	100
Lutescens 753 SP 2/09	1.4	156	2.1	84	2.3	90	1.9	95
Lutescens 1125 SP 2/09	0.9	0	2.8	112	2.1	90	1.9	95
Lutescens 736 SP 2/04	1.5	167	2.1	84	2.9	112	2.2	100
Lutescens No. 528	0.9	0	2.8	112	2.0	77	1.9	95
Lutescens 1148 SP 2/09	0.8	90	3.5	140	2.0	77	2.1	105
Lutescens 588 SP 2/05	1.1	122	2.6	104	3.1	119	2.3	115
Lutescens 857 SP 2/05	1.6	178	3.0	120	2.7	104	2.4	120
Lutescens 1206 SP 2/19	1.4	156	3.1	124	2.1	80	2.2	100
Lutescens 1143 SP 2/09	0.9	0	3.6	144	2.2	85	2.2	100
Lutescens 783 SP 2/07	0.8	90	3.3	132	2.5	96	2.2	100
Lutescens 687 SP 2/04	1.0	122	2.1	84	2.1	80	1.7	85
Lutescens 821 SP 2/08	1.0	122	3.8	152	2.4	92	2.4	120
Lutescens 742 SP 2/19	1.0	122	1.6	64	2.2	85	1.6	80
Lutescens 822 SP 2/09	0.9	0	2.5	0	2.2	85	1.9	95
Lutescens 1068 SP 2/09	1.1	122	2.6	104	2.2	85	2.0	0
Lutescens 715 SP 2/04	1.3	144	3.8	152	2.1	80	2.4	120
Lutescens 630 SP 2/08	0.9	9	2.6	104	1.6	61	1.7	85
M±SEM	1.1±0.25		2.7±0.59		2.3±0.34		2.0±0.24	
Lim	0.8-1.6		1.6-3.8		1.6-3.1		1.6-2.4	
R	0.8		2.2		1.5		0.8	
Cv, %	23.0		20.7		20.7		11.7	
LSD_{05}	1.87		2.14		1.10		0.20	
Mid-ripening (n = 9)								
Omskaya 35 (standard)	1.1		2.2		2.1		1.8	
Line 55/94-01	1.6	145	3.2	145	2.9	138	2.6	144
Line 12/93-01-10	1.2	109	2.9	131	3.2	152	2.4	133
Lutescens 2174	2.2	200	1.7	77	2.8	133	2.2	122
Lutescens 371/06	2.1	190	2.3	104	2.8	133	2.4	133
Lutescens 1919	1.1	0	2.5	114	3.8	180	2.5	139
Lutescens 43/01	1.2	109	2.2	0	3.1	148	2.2	122
Line 33/93-01-15	1.1	0	4.1	186	3.1	148	2.8	156

Continued Table 3

Lutescens 248/01	1.3	18.2	2.4	9.1	2.4	14.3	2.0	122
M±SEM	1.4±0.43		2.6±0.70		2.9±0.48		2.3±0.30	
Lim	1.1-22		1.7-4.1		2.1-3.8		1.8-2.8	
R	1.1		2.4		1.7		1.0	
Cv, %	28.7		25.4		15.7		14.1	
LSD_{05}	2.21		2.04		1.30		0.30	

N o t e. DS — deviation from standard, Lim — limit, R — range, *Cv* — the coefficient of variation.и.

Fifteen lines out of 30 studied of different types of ripeness turned out to be ecologically plastic in yield (Table 3). In our studies, out of 20 lines of the mid-early group, four were distinguished, the Erythrospermum 738 2/09 (2.3 t/ha), Lutescens 588 SP 2/05 (2.3 t/ha), Lutescens 857 SP 2/05 (2.4 t/ha), Lutescens 821 SP 2/08 (2.4 t/ha), significantly (LSD_{05} 0.2 t/ha) superior in yield to the standard variety Astana by 0.3-0.4 t/ha. These lines were characterized by a higher grain number per ear in combination with a larger the 1000-grain weight. Of the eight lines of the mid-season group, the yield of five is Line 12/93-01-10 (2.4 t/ha), Lutescens 371/06 (2.4 t/ha), Lutescens 1919 (2.5 t/ha), Line 55/94-01 (2.6 t/ha), Line 33/93-01-05 (2.8 t/ha) was significantly (LSD_{05} 0.3 t/ha) higher than the Omskaya 35 standard by 0.6-1.0 t/ha.

Thus, in the mid-early lines of spring soft wheat under the conditions of the North Kazakhstan region, the seedlings—heading interphase period corresponds in duration to that of mid-ripening lines. The vegetation period is reduced due to the accelerated passage of the heading—grain ripening phase. Erythrospermum 738 2/09 (2.3 t/ha), Lutescens 588 SP 2/05 (2.3 t/ha), Lutescens 857 SP 2/05 (2.4 t/ha), Lutescens 821 SP 2/08 (2.4 t/ha) from the mid-early group and Line 12/93-01-10 (2.4 t/ha), Lutescens 371/06 (2.4 t/ha), Lutescens 1919 (2.5 t/ha), Line 55/94-01 (2.6 t/ha), Line 33/93-01-05 (2.8 t/ha) from the mid-ripening group stand out for a higher yield. In mid-ripening lines, a correlation was found between the yield and the number of productive stems (r = 0.74-0.86, p = 0.95-1.29) and the grain number per ear content (r = 0.31-0.71, p = 0.32-0.88). The mid-early lines showed a significant positive relationship between the yield and the grain number per ear (r = 0.35-0.86, p = 0.36-1.29) and a close correlation with the productive stem number (r = 0.68-0.83, p = 0.82-1.18). The relationship between yield and the 1000-grain weight is moderate positive (r = 0.37-0.54, p = 0.38-0.60), and in a dry year it is weakly negative (r = –0.16, p = 0.16). For the steppe zone of the North Kazakhstan region, as a starting material in breeding for drought resistance and increasing adaptive potential, we propose to use the mid-early lines Lutescens 715 SP 2/04, Lutescens 821 SP 2/08, Lutescens 588 SP 2/05, Erythrospermum 738 2/09 and mid-ripening Line 33/93-01-15, Line 55/94-01, Lutescens 371/06, Lutescens 1919, and Line 12/93-01-10.

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A NOVEL INTEGRATIVE APPROACH TO STUDY THE DYNAMICS OF AN INCREASE IN COMMON SPRING WHEAT ADAPTIVITY AND HOMEOSTATICITY (on the example of breeding programs in the Northern Trans-Ural)

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Abstract

Though there are a number of evolutionary theories of living nature, no approach is available to quantify changes occurring during long-term breeding programs. By N.I. Vavilov, selection is evolution directed by the man's will. Here, we suggest and used a novel method for studying shifts in statistical genetic parameters which have occurred in sets of varieties of soft spring wheat (*Triticum aestivum* L.) over an approximately 80-year period. During 8 years (in 2005-2012), 23 varieties of soft spring wheat zoned in the period from the 1930s were investigated in the conditions of the northern forest-steppe of Western Siberia (experimental field of the Research Institute of Agriculture of the Northern Trans-Urals, Tyumen, 57°09'N, 65°32'E). All of them were successfully cultivated in the Northern Trans-Urals in various years. The effects of genotype by environment interaction changing the crop ranks by year of testing were measured. The average yield of the varieties zoned in the 1940s was 20.2 c/ha (a reference point). These varieties showed a pronounced plasticity and homeostaticity of grain production. The regression lines for yields vs. ecological years (from bad to favorable conditions) were flat with a 31°-39° inclination. Milturum 321, the first zoned variety for the region is stable for grain yields (S^2d_i = 3.5). During 1950-1970s, Saratov varieties and the late-maturing variety of Siberian selection Milturum 553 have been zoned in the Northern Trans-Urals. The average yield of the group is 23.4 c/ha. The regression lines were above the lines of the first group and had similar inclination. Saratov varieties showed yield homeostaticity similarly to the varieties of the first group but lodging at yields above 20-25 c/ha. In 1970-1990s, the varieties resistant to lodging became widespread. Their yields in testing averaged 29.1 c/ha (+44 % to the reference point), the regression lines inclination reached 39°-47° indicating a decrease in yield homeostaticity. These varieties more strongly responded to a better or adverse environments compared to the varieties of the first and second groups. Strela and Tyumenskaja 80 varieties of local selection are quite stable in terms of yields (S^2d_i = 4.8-6.1). Currently used medium-ripe intensive varieties capable of producing grain yields of 34.3 c/ha on average (+70.0 % to the reference point) strongly responded to changes in environments, which followed from the inclination of the regression lines (50°-54°, b_i = 1.21-1.40). Plasticity and crop homeostaticity are characteristic of the Chernyava 13 variety showing a flat regression line (29°, b_i = 0.56). The most stable crop performance was characteristic of the varieties Lutescens 70 and Icar (S^2d_i = 8.7 and S^2d_i = 8.6, respectively). Modern zoned early-ripening varieties are less productive than the varieties of the previous group (x = 31.1 c/ha), with flat regression lines (37°-38°). The Tulunskaya 12 and Novosibirskaya 15 varieties are unstable in terms of yields (S^2d_i = 26.6 and S^2d_i = 29.0, respectively). The Novosibirskaya 29 variety is more productive (33.3 c/ha) and similar to the medium-ripe varieties from the previous group in terms of plasticity and stability. The assessment of a genotype response to environments affecting crop plasticity and stability (and homeostaticity) evaluates different characteristics of crop adaptability. So this allows us to investigate varieties under changing environments, to assess the effectiveness of their use in the Northern Trans-Urals environment, and to optimize breeding programs. High-yielding varieties with a well-pronounced adaptability should be involved in breeding.

Keywords: variety, yield, genotype by environment interaction, limiting factors, plasticity,

Currently, there are about 40 breeding centers in Russia, each of which has a long history of creating zoned varieties and preparing variety changes, leading to an increase in gross grain yields in cultivation zones. According to N.I. Vavilov, selection is evolution controlled by the human will [1]. For the processes occurring in nature, various evolutionary theories have been proposed and discussed, e.g., Lamarckism [2], Darwinism [3], synthetic [4] and epigenetic [5] theories. However, for the quantitative description of the changes observed in any national breeding center during the implementation of long-term breeding programs, no single methodological approach has been proposed.

N.I. Vavilov [1] emphasized the importance of adapting a species and variety to specific environmental conditions and noted that the behavior of varieties and species is not the same both in different agro-climatic zones and in one zone depending on the conditions of the year. The increase in wheat yield is associated with the ability of varieties to compensate for the effects of limiting environmental factors that reduce productivity [6-8], that is, with the degree of severity of the adaptive properties of varieties [9-11].

Ecological testing is an effective way to assess both adaptability [12, 13] and cultivar plasticity (and yield homeostaticity) [14]. The extent of the adaptability of varieties is provided by the ability to withstand the action of limiting environmental factors [15-19] due to genetic and physiological systems of adaptability (GPSA) [20]. The plasticity of a variety and the maintenance of its yield over the years depend on the number of GPSAs in the genome [9, 21-23].

The influence of environmental factors on varieties is accompanied by microevolutionary processes (artificial and natural selection) [24, 25], shifts in the intensity of recombinogenesis [26], the appearance of provocative backgrounds in different years [27-30], which determine the specific selection effects [31-33]. As a result, high-yielding varieties appear that are highly adapted to average local weather and climatic conditions for years, with homeostatic yields over a number of years, which is one of the main reasons for the long-term industrial cultivation of the variety [34]. The adaptability characteristics of a variety include its ecological plasticity [21, 35], which reflects the degree of responsiveness (yield increase) to the weakening of the inhibitory effect of the limiting environmental factor [22, 23]. The adaptability of the variety is due to its stability [17, 36, 37[, which allows combination of high yields with their minimum decrease under adverse conditions [38-41]. Statistical methods are used to assess the adaptability and stability of varieties [42-44). Methods have been developed for quantitative assessment of the functional plasticity of the genome in varieties and genotype-environment interaction through variances [22, 45], through the multidirectional effects of genotypic factors and the vector of environmental influence on the manifestation of productivity traits (in two-dimensional coordinate systems-traits), the contributions to the yield of each of seven genetic-physiological systems — HPS [46]. Indicators of plasticity and yield stability of a variety are interconnected [38, 47] and are integral component properties of adaptability [38]. Yield homeostasis (in breeding terms, variety plasticity) is an important characteristic of varieties [9, 48, 49].

Plants during the growing season are affected by different (in terms of the number and intensity of exposure) abiotic and biotic factors. Together with GPSA, they determine the features of the genotype–environment interaction (GEI) [18, 34]. The nature of the effects of such an interaction is complex [50-52]. Varietal differences in terms of genotype–environment interaction are quite significant [51, 53], which characterizes their modification capabilities [14, 54], expressed through

the effects of GEI [34, 55]. The evaluation of the latter gives an idea of the plasticity and stability of varieties [56-58]. A change in environmental conditions leads to a change in the limiting environmental factors (lim-factors) that determine yields [9, 59]. The change in productivity ranks in a set of varieties (in different years at one ecological point or in one year, but at different points) is due to the variability of GEI effects that determine yield [60]. Management of the effects of GEI (agrotechnological or genetic breeding activities) is a significant reserve for increasing yields [20, 61], which is confirmed by studies conducted in the Northern Trans-Urals [62]. Here, in the formation of the yield of spring wheat, varietal characteristics account for 25.2-29.0%, and in the old varieties cultivated here for GEI this indicator is 14.8%, for the variety factor 11.5%, and in modern varieties the first indicator increases to 19.3%, the second one decreases to 5% [62]. Increasing environmental sustainability [15, 40, 63] is an important factor in "bringing" adaptive systems to ensure yields, which is the real contribution of breeding to increasing crop production [64, 65] and increasing wheat yields [66-70].

In our work, we propose an approach that seems to us optimal for quantitative assessment of the results of wheat breeding in the historical aspect (on the example of the Tyumen breeding center).

The purpose of our study is to describe the dynamics of changes in the plasticity and adaptability of varieties created and released in the Northern Trans-Urals, from the 1930s to the present.

Materials and methods. The main set of 23 varieties of soft spring wheat (*Triticum aestivum* L.) include 19 varieties of different years of breeding at the Tyumen breeding center and 4 varieties from other breeding regions which have been registered for use in the Northern Trans-Urals. After equalizing sowing and reproduction, the varieties were grouped by the years of cultivation and for 8 years (2005-2012) and studied in the conditions of the northern forest-steppe of Western Siberia (experimental field of the Research Institute of Agriculture of the Northern Trans-Urals, Tyumen, 57 09' N, 65 32' E). The soil of the plot is dark gray forest, the predecessor is black fertilized fallow ($N_{30}P_{45}K_{30}$ kg a.i./ha), plot area is 10 m^2, repetition is 4-fold, plot placement is randomized, the seeding rate is 650 viable seeds per 1 m^2. Seeds were obtained at the Siberian Research Institute of Plant Growing and Breeding (Krasnoobsk settlement, Novosibirsk Province). Sowing was carried out at the optimal time for the region using a seeder SKS-6-10 (Russia). Yield records and observations were carried out according to the standard method ("Methodology of the State Variety Testing of Agricultural Crops", Moscow, 1989). Vegetation conditions for the years of testing differed in temperature and precipitation.

For statistical processing of the experimental data, analysis of variance [62, 71] was used, LSD_{05} and correlation coefficients were calculated. The response effect index (RE) of varieties on environmental conditions was calculated according to our proposed formula [72], plasticity (b_i values), stability (S^2d_i values) and environmental index (Ii) were calculated according to S.A. Eberhart and W.F. Russell [22] using the R.A. Urazalieva et al. [73], homeostaticity was determined by V.V. Khangildin [74].

Results. During the observation period, 2007 and 2008 were dry years, despite significant precipitation during the growing season (349 and 294 mm at a rate of 243 mm, by months the precipitation had a shower character). HTC values for the season were 1.50 and 1.84 (wet). However, the I-II decades of each month were dry (HTC of 0.30-0.56 and 0.15-0.67). The year 2009 turned out to be dry, with a deficit of precipitation during the growing season of 62 mm (–26%) at close to average long-term values of the hydrothermal coefficient (HTC = 0.96, dry).

July (HTC = 0.13) and the second half of August (HTC = 0.26) were very dry. The year 2012 was especially dry, when only 98 mm of precipitation fell during the growing season (44% of the norm of 243 mm). Under elevated active temperatures (> 10 °C), their sum amounted to 2210 °C with precipitation of 315 mm (or +20% to the norm), while on average HTC = 0.44 (dry). July turned out to be very dry (HTC = 0.11-0.13). During these years, the ear was formed small, and the grain was low-grade. 2005, 2010 and 2011 were average in terms of climatic conditions, when precipitation during the growing season was less than the norm (213, 210 and 225 mm), and the sums of active temperatures were slightly above the norm (+168 °C, +190 °C, +68 °C). The HTC values for the years were 1.04, 1.06 and 1.18 (weakly humid). Quite wet (349 mm of precipitation) and cool (–382 °C) was 2006 (HTC = 2.21, humid). In general, it should be noted that the spring-early summer type of drought (Siberian type) manifests itself in all years.

Contrasting conditions, while observing the principle of a single difference, allowed us to give an objective assessment of all the studied varieties.

According to the "Catalogue of varieties of agricultural crops created by scientists of Siberia and included in the State Register of the Russian Federation (zoned) in 1929-2008" (Novosibirsk, 2009, v. 4, issue 1), in 1924, the State Variety Testing Network under the People's Commissariat of the RSFSR has been established. On the territory of the Northern Trans-Urals (Tyumen region) since 1929, 29 varieties of soft spring wheat have been cultivated (with 10-15-20-year periods, successively covering six variety changes) ("State variety book of the inspection of variety testing in the Tyumen region", 2001; "Catalog of zoned varieties of agricultural crops in Siberia, 1997 and 2009").

In the first breeding varieties, zoned in the Northern Trans-Urals (group I) and cultivated from the 1930s to the 1950s, the grain yield averaged 20.2 c/ha (we took it as the base for further assessments) (Table 1). The potential yield of these varieties did not exceed 32.4-34.1 q/ha. They reacted strongly to dry conditions, which turned out to be more characteristic of the Cesium 111 variety. In wet and average years, these varieties lodging strongly.

1. Grain yield (c/ha) **of soft spring wheat (*Triticum aestivum* L.) varieties grouped by the periods of cultivation in Western Siberia over the years of observation** (trial fields of the Research Institute of Agriculture of Northern Trans-Urals, Tyumen, 57°09' N, 65°32' E)

Variety	2005	2006	2007	2008	2009	2010	2011	2012	Average
Group I (1930-1950)									
Lutescense 956	21.8	17.3	17.6	16.5	29.7	32.4	17.3	15.2	21.0
Cezium 111	18.2	9.6	10.4	16.2	22.0	34.1	19.4	14.7	18.1
Milturum 321	24.4	22.0	12.8	15.3	27.4	33.6	23.2	13.7	21.6
x	21.5	16.3	13.6	16.0	26.4	33.4	20.0	14.5	20.2
Group II (1951-1970)									
Lutescense 758	26.2	18.3	18.2	16.0	28.5	37.7	26.3	17.7	23.8
Milturum 553	29.7	25.9	14.4	15.1	29.5	43.3	16.5	13.5	23.5
Saratovskaya 29	28.1	19.1	18.7	17.9	31.2	37.3	26.7	19.9	24.9
Skala	22.4	29.7	20.4	20.2	29.7	40.5	36.8	18.1	27.2
x	26.6	23.3	17.9	17.3	29.7	39.7	26.6	17.3	24.9
Group III (1971-1990)									
Strela	28.8	27.3	21.1	24.6	34.5	45.8	34.3	20.0	29.6
Novosibirskaya 67	19.3	16.1	13.3	17.1	31.7	35.3	31.2	14.5	22.3
Rang	33.3	37.1	28.8	19.4	36.6	39.1	35.6	19.2	31.2
Tyumenskaya 80	34.5	35.5	30.5	22.7	41.2	49.4	44.6	23.1	35.2
x	29.0	29.0	23.4	21.0	36.0	42.4	36.4	19.2	29.6
Group IV (1991-2012)									
Omskaya 20	35.3	42.3	23.3	21.7	38.3	48.9	37.9	18.7	33.3
Lutescense 70	32.9	35.9	22.8	23.7	42.4	55.1	41.3	28.2	35.3
.Il'inskaya	34.5	40.5	29.9	22.7	38.4	58.0	37.6	22.8	35.6
AVIADa	35.3	27.9	28.8	19.5	41.5	50.5	50.0	19.6	34.2
Chernyava 13	34.3	34.6	26.0	30.1	41.7	34.9	44.0	28.1	34.2

Continued Table 1

Ikar	32.5	28.8	16.8	22.5	40.3	50.0	45.3	20.1	32.0
SKENT 3	28.8	33.7	22.1	18.2	36.6	44.9	49.7	21.6	32.0
Riks	47.0	38.7	18.0	28.2	38.3	57.6	46.3	30.1	38.0
x	35.1	35.3	23.5	23.3	39.7	50.0	44.0	23.7	34.3
				Group V (1991-2012)					
Tulunskaya 12	29.3	38.9	17.1	22.7	31.7	37.6	42.3	17.7	29.7
Novosibirskaya 15	27.9	39.3	23.5	29.1	39.2	38.8	31.1	15.1	30.5
Iren'	35.5	30.7	27.3	23.7	32.5	39.1	40.5	19.7	31.1
Novosibirskaya 29	33.6	35.3	25.8	21.9	45.0	46.3	42.3	16.5	33.3
x	31.6	36.1	23.4	24.4	37.1	40.5	39.1	17.3	31.2
Average	30.7	30.3	21.3	21.2	35.7	43.1	35.9	19.6	29.7
LSD_{05}	1.8	1.9	1.5	1.6	2.2	2.5	2.2	1.5	1.9

Note. Groups I-IV — mid-ripening varieties originated from Tyumen Breeding Center (except for Omskaya 20 variety); group V — early-ripening varieties originated from other regions; 10 m^2 plots, 4-fold repetition.

In the 1950s-1970s, varieties of the semi-intensive type were cultivated, of which the Milturum 553 variety is late-ripening, Skala is medium-early. The average yield for this group was 24.9 c/ha, which is 20% higher than that of the varieties of the previous group (see Table 1).

With the intensification of the farming system (1971-1990), associated with the development of the Tyumen energy complex of the USSR, intensive mid-ripening varieties Strela, Novosibirskaya 67, Rang, Tyumenskaya 80 (group III) became widespread in the region. Their average yield in our test was 29.6 c/ha which is 46% higher than in group I. The best yield values (45.8, 35.3, 39.1, and 49.4 c/ha, respectively) were recorded in 2010. In all years of study, the yield of Novosibirskaya 67 variety was lower than that of other varieties in the group. This is especially evident in dry conditions (2007, 2008 and 2012), which significantly affect productivity [23, 33, 37]. Varieties Novosibirskaya 67 (var. *albidum*) and Tyumenskaya 80 (var. *lutescens*) were characterized by a strong germination of grain in the ear during the pre-harvest period in humid conditions (in 2007, 2010 and 2011, from 46, 54 and up to 86%, respectively). Varieties Rang and Tyumenskaya 80 showed resistance to lodging. In the Rang variety, in cool, wet years (2005, 2006), the growing season was extended. Due to the revealed too strong reaction of the varieties of this group to the pronounced lim-factors of the environment (see Table 1), the physical indicators and technological properties of the grain are reduced.

In the 1990s, the use of mineral fertilizers decreased by more than 3 times, from 87 to 22-24 kg a.i. per ha). From 2005 to the present, over the Tyumen region, mineral fertilizers have been applied in an amount of 32 to 35 kg a.i. per ha which is clearly not enough. Despite this, due to the introduction of new, more productive varieties (mainly of local selection, group IV), the yield in that period increased from 17-18 to 22-24 c/ha. In our tests, the average yield in the group over the years of study was the highest, 34.3 c/ha (+70% to the value in group I). As in other programs [25, 66-70], the increase in this group was due to selection work.

The mid-early group in our study was represented by four varieties of foreign selection cultivated in the region. Their productivity potential turned out to be somewhat lower than that of varieties from group IV, and the reaction to drought conditions, which were most severe in 2012, was more pronounced (see Table 1).

The distribution of the studied genotypes by ranks (by years) and the sum of ranks (Table 2) reflects both the similarity of agroclimatic conditions in the years of research and the pronounced differences that manifested themselves in the dry years of 2007, 2008, and 2012. It should be noted that drought (especially under the conditions of ongoing climate aridization) is considered as the main

abiotic stress and a risk factor for yield losses in wheat cultivation [6, 17, 33].

2. Rank distribution of soft spring wheat (*Triticum aestivum* L.) varieties grouped by periods of cultivation in Western Siberia over the years of observation on grain yields (trial fields of the Research Institute of Agriculture of Northern Trans-Urals, Tyumen, 57°09' N, 65°32' E)

Variety	2005	2006	2007	2008	2009	2010	2011	2012	Sum	Ranking
Group I (1930-1950)										
Lutescense 956	16	21	16	16	14	22	21	17	143	19
Cezium 111	18	23	22	17	18	20	20	19	157	21
Milturum 321	14	18	21	19	17	21	19	20	149	20
Group II (1951-1970)										
Lutescense 758	13	20	14	18	16	15	18	15	129	16
Milturum 553	9	17	19	20	15	11	22	21	184	17
Saratovskaya 29	11	19	13	14	13	17	17	9	113	15
Skala	15	13	12	10	14	12	12	14	102	14
Group III (1971-1990)										
Strela	10	16	11	4	11	9	14	8	83	12
Novosibirskaya 67	17	22	20	15	13	18	15	19	139	18
Rang	7	6	3	12	10	13	13	12	76	10
Tyumenskaya 80	5	8	1	6	5	6	5	4	40	2
Group IV (1991-2012)										
Omskaya 20	4	1	8	9	9	7	10	13	61	6
Lutescense 70	8	7	9	5	2	3	8	2	44	3
.Il'inskaya	5	2	2	6	8	1	11	5	40	2
AVIADa	3	15	3	11	4	4	1	11	52	4
Chernyava 13	6	10	5	1	3	19	6	3	53	5
Ikar	9	14	18	7	6	5	4	7	70	8
SKENT 3	10	11	10	13	10	10	2	6	72	9
Riks	1	5	15	3	9	2	3	1	39	1
Group V (1991-2012)										
Tulunskaya 12	10	4	17	6	13	16	7	15	88	13
Novosibirskaya 15	12	3	7	2	7	14	16	18	79	11
Iren'	2	12	4	5	12	13	9	10	67	7
Novosibirskaya 29	6	9	6	8	1	8	7	16	61	6

Note. Groups I-IV — mid-ripening varieties originated from Tyumen Breeding Center (except for Omskaya 20 variety); group V — early-ripening varieties originated from other regions.

In 2007 and 2008, the first growing season was dry, which is typical for the region. In 2012, the entire growing season was characterized by a lack of moisture, when only 93 mm of precipitation fell at a rate of 243 mm. The most favorable year was 2010. Despite the apparent climatic differences, the general trend in the rank distribution of varieties over the years has a fairly significant similarity, with the exception of some genotypes. Thus, in the dry year of 2012, the Saratovskaya 29 variety showed a higher ranking mark compared to previous years, while the Omskaya 20 variety, on the contrary, decreased. It was shown that varieties of the early ripening group react sharply to climatic changes. Varieties of groups I, II and Novosibirskaya 67 occupied low places in the rank distribution in all the years of observations. A smaller sum of ranks and high places in the ranking indicate a pronounced plasticity of genotypes, which is typical for most varieties of groups III and IV, as well as for early ripe varieties Iren and Novosibirskaya 29. This is due to a rather strong share (up to 20%) of the influence of GEI on their yield formation which has been previously reported [46] and discussed in a number of other studies [50-52, 55, 60].

The rank correlation of genotypes by years of research (Table 3) was significant ($R_{05} \geq 0.413$). It should be taken into account that if $r = 1$, GEI = 0. This indicates rather high differences in GPSA, determining productivity traits [2, 16-19]) which affect yield formation under conditions of the ecological zone. An analysis of the correlation coefficients over the years of observation shows that in dry years, the formation of yields is controlled by other sets of gene products. This is due to less pronounced associations (r = 0.447-0.480) with the best years in

terms of productivity. The same is true for favorable years, when the correlation coefficients increased (up to $r = 0.716$). Given this circumstance, a breeding strategy should be planned aimed at creating productive varieties of spring wheat with a pronounced plasticity of yield formation.

3. Rank correlation of soft spring wheat (*Triticum aestivum* L.) varieties grouped by periods of cultivation in Western Siberia over the years of observation on grain yields (trial fields of the Research Institute of Agriculture of Northern Trans-Urals, Tyumen, 57°09' N, 65°32' E)

Year	2005	2006	2007	2008	2009	2010	2011	2012	Year
2005		0.6550*	0.6808*	0.5810*	0.6293*	0.7034*	0.6821*	0.6262*	2005
2006	0.6550*		0.6043*	0.7044*	0.5685*	0.5740*	0.5340*	0.4469*	2006
2007	0.6808*	0.6043*		0.5696*	0.6773*	0.4805*	0.5026*	0.5130*	2007
2008	0.5810*	0.7044*	0.5696*		0.6920*	0.4765*	0.6301*	0.6585*	2008
2009	0.6293*	0.5685*	0.6773*	0.6920*		0.6158*	0.7160*	0.5941*	2009
2010	0.7034*	0.5740*	0.4805*	0.4765*	0.6158*		0.6383*	0.6127*	2010
2011	0.6821*	0.5340*	0.5026*	0.6301*	0.7160*	0.6383*		0.7042*	2011
2012	0.6262*	0.4469*	0.5130*	0.6585*	0.5941*	0.6127*	0.7042*		2012

* Reliably higher than the significance level ($R_{05} \geq 0.413$).

4. Effects of response (RE) **to environment conditions in soft spring wheat (*Triticum aestivum* L.) varieties grouped by periods of cultivation in Western Siberia over the years of observation** (trial fields of the Research Institute of Agriculture of Northern Trans-Urals, Tyumen, 57°09' N, 65°32' E)

Variety	2005	2006	2007	2008	2009	2010	2011	2012
Group I (1930-1950)								
Lutescense 956	–0.12	–4.25	5.04	3.95	2.77	–1.96	–9.87	4.42
Cezium 111	–0.81	–9.01	0.75	6.56	–2.02	2.65	–4.86	6.73
Milturum 321	2.36	–0.17	-0.38	2.13	–0.15	–1.38	–4.59	2.20
Group II (1951-1970)								
Lutescense 758	2.88	–6.05	2.84	0.65	–1.23	0.54	–3.67	4.02
Milturum 553	5.28	1.85	–0.66	0.05	0.12	6.44	–13.17	0.12
Saratovskaya 29	2.31	–6.32	2.27	1.48	0.40	–0.93	–4.34	5.15
Skala	–5.75	1.37	1.61	1.42	–3.46	–0.09	3.40	0.99
Group III (1971-1990)								
Strela	1.21	5.38	6.07	–3.32	–0.30	–5.43	–1.74	–1.85
Novosibirskaya 67	–1.68	–2.81	–0.02	3.49	–0.99	2.88	–1.43	0.56
Rang	–3.94	–6.77	2.22	3.23	3.45	–0.38	2.71	2.30
Tyumenskaya 80	–1.63	–0.26	3.73	–4.06	0.06	0.83	3.22	–1.99
Group IV (1991-2012)								
Omskaya 20	1.07	8.44	–1.57	–3.16	–0.94	2.23	–1.58	–4.49
Lutescense 70	–3.32	0.05	–4.06	–3.15	1.17	6.53	–0.17	3.02
.Il'inskaya	–1.98	4.39	2.67	–4.41	–4.09	9.08	–4.13	–2.64
AVIADa	0.19	–6.84	3.05	–6.24	1.38	2.95	9.64	4.17
Chernyava 13	–0.84	–0.47	0.22	4.33	1.55	–12.68	3.61	4.00
Ikar	–0.47	–3.80	–6.81	–1.10	2.32	4.59	7.08	–1.83
SKENT 3	–4.08	1.19	–1.42	–5.31	–1.29	–0.42	11.57	–0.24
Riks	8.04	0.12	–11.59	–1.38	–5.66	6.21	2.10	2.19
Group V (1991-2012)								
Tulunskaya 12	–1.29	8.68	–4.13	1.48	–3.90	–5.43	6.46	–1.85
Novosibirskaya 15	–3.53	8.24	1.43	7.04	2.76	–5.07	–5.58	–5.29
Iren'	3.45	–0.98	4.61	1.02	–4.56	–3.39	3.20	–1.84
Novosibirskaya 29	–0.67	1.40	0.89	–3.00	5.72	–0.41	2.78	–6.73
Ii	+0.93	+0.56	–8.43	–8.44	+5.94	+13.37	+6.18	–10.11

Note. Groups I-IV — mid-ripening varieties originated from Tyumen Breeding Center (except for Omskaya 20 variety); group V — early-ripening varieties originated from other regions. Ii — index of environment conditions.

The response of varieties to climatic conditions is well reflected in the index of environmental conditions Ii (Table 4), which was high in the favorable year 2010 (+13.37) and good in 2009 and 2011 (+5.94 and +6.18). In 2007, 2008 and 2012 this index took negative values. Determination of yields by lim-factors is well reflected in the effects of the response (RE) of varieties to environmental conditions [72], which have a pronounced year-to-year ranking and are largely

genotypically determined. Extensive (group I) and semi-intensive (group II) varieties due to lodging showed negative reaction effects in wet years and well-pronounced positive ones in dry years (see Table 4).

In intensive varieties from group III, the response effects on the yield formation of are less contrasting. At the same time, in the Rang variety, in years when cool temperatures are combined with sufficient moisture during the grain formation phase, the growing season is extended, which leads to the production of low-grade grain. During these years, lodging in the Strela variety and grain germination in the spike in the Novosibirskaya 67 and Tyumenskaya 80 varieties reduced the yield to the level of that of the Rang variety in dry conditions (RE = –1.85 to –1.99 and –3.32 to –4.06).

In modern intensive varieties, the reaction effects were more pronounced and more contrasting over the years. This is explained by the fact that in the proportion of genotypic variability that determines the formation of their yield (24.3%), four-fifths (19.3%) falls on the genotype-environment interaction [46]. In this group, cv. Chernyava 13 responded less than others to dry conditions (2007, 2008, 2012), showing positive RE values in these years (see Table 4). In favorable years (2010), a strongly pronounced negative effect was observed (RE = –12.68) due to lodging, which also manifests itself in 2005 and 2006, good climatic conditions (RE = –0.84, RE = –0.47). The remaining varieties of this group responded with negative RE values to dry conditions, which is typical for this ecotype.

Early maturing varieties from group V showed negative RE of varying degree under favorable conditions, which is due to the low potential of these varieties, and negative RE values in dry years (for example, in 2012) which was determined by the biological features of varieties in group V.

The response effects revealed over a rather long period of time (by years) demonstrate their good manifestation in most varieties in IV and some varieties in V groups. This indicates a pronounced adaptability of such varieties to the conditions of the Northern Trans-Urals and serves as a model characteristic for newly created varieties of soft spring wheat. In addition to the above, we present an assessment of the entire set of studied varieties in terms of the ecological variability of their yield in terms of its maximum (max) and minimum (min) values, the scatter range R, stability S^2d_i, plasticity b_i, and homeostaticity Hom [22, 74].

5. Ecological yield variability in soft spring wheat (*Triticum aestivum* L.) varieties grouped by periods of cultivation in Western Siberia over the years of observation (trial fields of the Research Institute of Agriculture of Northern Trans-Urals, Tyumen, 57°09' N, 65°32' E, 2005-2012)

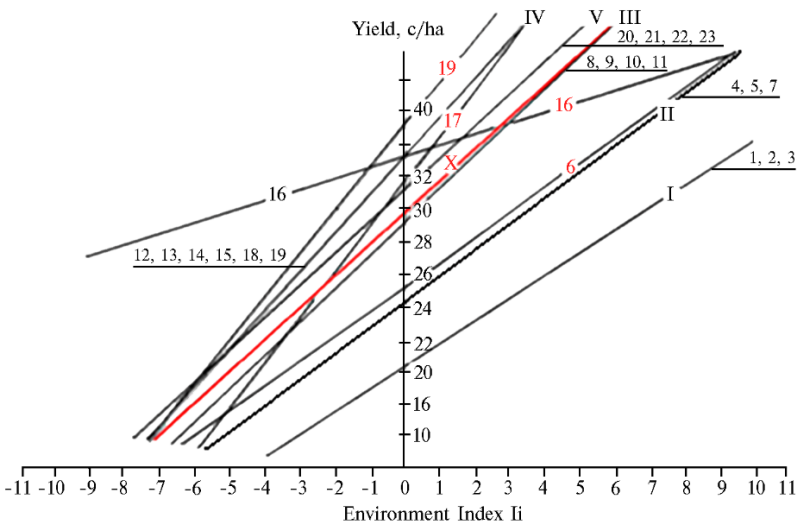
Variety	x, c/ha	Lim, c/ha		R, c/ha	S^2d_i	b_i	Hom
		min	max				
Group I (1930-1950 годы)							
Cezium 111	18.1	9.6	34.1	24.8	22.2	0.71	0.26
Milturum 321	21.6	12.8	33.6	20.8	3.4	0.83	0.94
Lutescense 956	21.0	15.3	32.4	17.1	16.0	0.61	0.42
x	20.2					0.72	
Group II (1951-1970)							
Lutescense 758	23.8	16.0	37.7	21.7	9.6	0.81	0.61
Milturum 553	23.5	13.5	43.3	29.8	35.4	1.03	0.42
Saratovskaya 29	24.9	17.9	37.3	19.4	19.9	0.76	0.74
Skala	27.2	18.1	40.5	22.4	8.9	0.92	0.93
x	24.9					0.86	
Group III (1971-1990)							
Strela	29.6	20.0	45.8	25.8	4.8	0.97	1.50
Novosibirskaya 67	22.3	13.3	35.3	22.0	11.7	0.96	0.51
Rang	31.2	20.9	46.3	26.3	14.2	0.83	0.98
Tyumenskaya 80	35.2	22.7	49.4	26.7	6.1	1.11	1.90
x	29.6					0.97	

Continued Table 5

			Group IV (1991-2012)				
Omskaya 20	33.3	18.7	48.9	30.2	13.0	1.21	1.16
Lutescense 70	35.3	23.7	55.1	31.4	8.7	1.24	1.60
Il'inskaya	35.6	22.7	58.0	35.3	19.7	1.24	0.93
AVIADa	34.2	19.5	50.5	31.0	22.3	1.35	0.96
Chernyava 13	34.2	26.0	44.0	18.0	16.6	0.56	1.08
Ikar	32.0	16.8	50.0	33.2	8.6	1.40	1.31
SKENT 3	32.0	18.2	49.7	31.5	22.6	1.23	0.81
Riks	38.0	18.0	57.6	39.6	33.0	1.32	0.95
x	34.3					1.28	
			Group V (1991-2012)				
Tulunskaya 12	29.7	17.1	42.3	25.2	26.6	0.98	0.65
Novosibirskaya 15	30.5	15.1	39.2	24.1	29.0	0.79	0.65
Iren'	31.1	19.7	40.5	20.8	10.3	0.78	1.14
Novosibirskaya 29	33.3	16.5	46.3	29.8	9.1	1.26	1.40
x	31.2					0.95	
Average	29.7						
LSD_{05}	2.2						

N o t e. Groups I-IV — mid-ripening varieties originated from Tyumen Breeding Center (except for Omskaya 20 variety); group V — early-ripening varieties originated from other regions; max means maximum, min means minimum values, R means a scatter range, S^2d_i is a stability parameter, b_i assese plasticity, and Hom means homeostaticity.

With a lower yield (20.2 c/ha) in extensive varieties (group I) compared to other genotypes, its minimum values (9.6-15.3 c/ha) occur in dry years and maximum (32.4-34.1 c/ha) in years with high moisture supply (at R = 17.1-24.5 c/ha) (Table 5). Among them, the most stable in terms of yield was the old Siberian variety Milturum 321 (S^2d_i = 3.4), the source material for which was selected at the beginning of the 20th century in the Trans-Urals by N.L. Skalozubov, the first agronomist of the Tobolsk province. Low yield stability was noted in the Lutescens 956 variety and especially in the Cesium 111 variety, S^2d_i = 16.0 and S^2d_i = 22.2, respectively). The pronounced plasticity of the latter (b_i = 0.61 and b_i = 0.71) should be noted (see Table 5), which is well reflected by regression lines with a gentle slope (31°-35°) (Fig.).



Plasticity of soft spring wheat (*Triticum aestivum* L.) varieties grouped by periods of cultivation in Western Siberia over the years of observation: group I (1930-1950), group II (1951-1970), group III (1971-1990), group IV (1991-2012) — mid-season varieties from the Tyumen breeding center; group V (1991-2012) — early-ripening varieties from other regions. Group I: 1 — Cezium 111, 2 — Milturum 321, 3 — Lutescense 956; group II: 4 — Lutescense 758, 5 — Milturum 553, 6 — Saratovskaya 29, 7 — Skala; group III: 8 — Strela, 9 — Novosibirskaya 67, 10 — Rang, 11 — Tyumenskaya 80; group IV: 12 — Omskaya 20, 13 — Lutescense 70, 14 — Il'inskaya, 15 — AVIADa, 16 — Chernyava 13, 17 — Ikar, 18 — SKENT 3, 19 — Riks; group V: 20 — Tulunskaya 12, 21 — Novosibirskaya 15, 22 —

Iren', 23 — Novosibirskaya 29. Trial fields of the Research Institute of Agriculture of Northern Trans-Urals (Tyumen, 57°09' N, 65°32' E, 2005-2012), The average regression line is marked in red.

In variety Milturum 321, at b_i = 0.83, the regression line is steeper, with a slope of 39°, therefore, it responds more strongly to changes in environmental conditions. The foregoing is well interpreted through the indexes of environmental conditions Ii (see Table 4, Fig.). The index of homeostasis (Hom), reflecting the adaptability of the variety to varying external conditions, was higher in the variety Milturum 321 (Hom = 0.94), lower in the variety Lutescens 956 (Hom = 0.42) and very low in the variety Cesium 111 (Hom = 0.26). This indicates their insufficient adaptability to the agro-climatic conditions of the Northern Trans-Urals and serves as one of the explanations for the fact that not a single variety was created with the participation of the Cesium 111 variety using classical breeding methods. We used the yield and variability of these varieties as basic indicators for further evaluation and interpretation of test results.

In semi-intensive varieties cultivated in 1950-1970 (group II), the average yield was 24.9 c/ha with higher limit values than in group I. The R values in most varieties from group II remained within the limits for the genotypes described above. The exception was the late-ripening variety Milturum 553 with a high maximum yield value (43.3 c/ha) with R = 29.8 c/ha. The variety Milturum 553 had a low yield stability (S^2d_i = 35.4) and a lower homeostatic index (Hom = 0.42), which indicates the inefficiency of its cultivation in the region. The remaining varieties of this group, Lutescens 758, Saratovskaya 29 and Skala showed a certain stability in yield formation (S^2d_i of 8.9, 9.9, and 19.9) and plasticity (b_i of 0.76, 0.81, and 0.92). The regression lines reflecting plasticity (see Fig.) run higher than for the Milturum 553 variety, and at a less steep slope (37-39° vs. 45° for the Milturum 553 variety) and cross the y-axis higher (at 24.9 c/ha). Therefore, these varieties respond to improved cultivation conditions, but at yields above 25 c/ha, they are prone to lodging. Variety Skala turned out to be quite resistant to lodging and well adapted to local conditions (Hom = 0.93), due to which it was cultivated in the West Siberian region for many years.

Intensive mid-season varieties of group III (1971-1990) gave good average yields over the years (29.6-35.2 c/ha). The exception was the variety Novosibirskaya 67 with the average yield over the years of 22.3 c/ha. Its limiting values, as well as the homeostatic index (Hom = 0.51) were close to those of a number of varieties from group II, which refers the variety Novosibirskaya 67 to genotypes less adapted to the conditions of the zone. The grain of variety Novosibirskaya 67 (var. *albidum*) germinates in the ear in autumn in humid conditions. All this influenced the removal of the variety from zoning in the Northern Trans-Urals. Strela and Tyumenskaya 80 varieties (S^2d_i = 4.8 and S^2d_i = 6.1) were the most stable varieties created in the Trans-Urals in terms of yield. They turned out to be well adapted to the conditions of the zone (Hom = 1.50 and Hom = 1.90). Variety Rang in terms of ecological variability of yield was identical to varieties Strela and Tyumenskaya 80, but, unlike them, it was less homeostatic (Hom = 0.98). The narrow-local variety Rang, due to its high resistance to lodging, was cultivated only in the conditions of the northern forest-steppe of the Tyumen region under intensive farming with the use of mineral fertilizers at high doses (100-120 kg a.i./ha). With their decrease, the yield of the Rang variety drops sharply. According to the plasticity in varieties Rang, Strela and Tyumenskaya 80 (b_i = 0.83, b_i = 0.97 and b_i = 1.11), it can be seen that these varieties quite noticeably respond to changes in environmental conditions. Their regression lines had a steeper slope (39°, 42° and 47°). When the environmental conditions improved, the productivity of these varieties increased adequately, and when the environmental conditions worsened, they

similarly reduced it. This is well demonstrated by the averaged regression line, which crosses the y-axis at 29.6 c/ha, being significantly higher than for the previous two groups (see Fig.). Along with the practical significance, the genotypes of this group are widely used in hybridization. Thus, the Strela, Novosibirskaya 67 and Rang varieties, among 15 genotypes, were included in the regional Interdepartmental DIAS program (study of the genetics of spring wheat productivity traits in Western Siberia, 1973-1984) [34] with a wide ecological scope (in eight zones of Siberia). A large-scale hybridological analysis (Heyman diallel analysis) followed by hybrids' and parental forms' testing at eight geographical sites, made it possible to study the genetics of quantitative traits of varieties in the ecological gradient. These genotypes show good variety-forming ability. With their participation, a number of released and registered varieties were created under this program: DIAS-2, Lutescens 70, Altaiskaya 88, Altaiskaya 92, Altai prostor, Kazakhstanskaya early ripening, Kazakhstanskaya 17, Baganskaya 93, Kantegirskaya 89 [34]. In Krasnoufimsk, the spring wheat variety Gornouralskaya was created (allowed for the regional cultivation in 2009), in Tyumen — the varieties Riks, Tyumenskaya 29, Grenada (allowed for the regional cultivation in 2011, 2014, 2018) [34] and the variety Atlanta 1, which has been under State variety testing since 2018. Mid-season intensive varieties cultivated since the late 1990s, the Omskaya 20, Lutescens 70, Chernyava 13, AVIAD, Ikar, SKENT 3 and Riks have increased their yields (x = 34.3 c/ha, i.e., +14.1 c/ha, or +70% to the baseline which was significantly higher vs. the previous group with +4.7 c/ha). The minimum yield in dry conditions remained at the same level as that of varieties from group III well adapted to local conditions. Their maximum yield was significantly higher than that of the compared varieties (48.9-58.0 c/ha), due to which the dispersion index also increased significantly (R = 30.2-39.6 c/ha), which associated with high GEI levels [46]. From the indicated set, the varieties Lutescens 70 and Ikar widely distributed in the Trans-Urals (S^2d_i = 8.7, S^2d_i = 8.6) were distinguished by the stability of crop formation. Variety Chernyava 13 with a good average yield (34.2 c/ha) showed a rather high minimum yield (26.0 c/ha), which indicates its good drought resistance. The maximum yields of the Chernyava 13 variety are significantly less than those of other varieties (44.0 c/ha), which is due to the tendency to lodging. Because of this, the yield value dispersion in this variety is almost 2 times less than that of others (R = 18.0 c/ha). Cultivar Chernyava 13 was distinguished by plasticity (b_i = 0.56), its regression line had a small slope angle (29°) and crossed the y-axis at a high mark (34.2 c/ha) (see Fig.). Consequently, variety Chernyava 13 responds less than other varieties to the deterioration of environmental conditions. All other varieties from group IV had high rates of ecological plasticity (b_i = 1.21-1.40). In graphical form, this was reflected by regression lines with slopes of 50°-54°. This characterizes a strong response to improved conditions for growing varieties and indicates their intensity.

Modern early-ripening varieties Tulunskaya 12, Novosibirskaya 15, Iren', Novosibirskaya 31 (group V) turned out to be less productive than cultivated mid-ripening ones (x = 31.2 c/ha, or –3.1 c/ha compared to mid-ripening varieties). In early ripe varieties, a lower maximum yield (39.2-42.3 c/ha) and a smaller R = 20.8-25.2 c/ha were noted. Varieties Tulunskaya 12 and Novosibirskaya 15 were unstable in terms of yield formation (S^2d_i = 26.6 and S^2d_i = 29.0). The regression lines for varieties Novosibirskaya 15 and Iren' were flatter with slope angles of 37°-38° (b_i = 0.79-0.78) (see Fig.), which indicates a more pronounced homeostatic yield in these varieties compared to variety Tulunskaya 12 (b_i = 0.98), in which the regression line is rather steep (slope angle 44°) and close to the average for the experiment. Cultivar Novosibirskaya 29 in terms of yield (x = 33.3 c/ha), its R = 29.8 c/ha, values of plasticity, stability and homeostaticity parameters (b_i = 1.26,

S^2d_i = 9.1 and Hom = 1.40) was similar to the intensive mid-ripening varieties Lutescens 70 and Ikar, widespread in the Trans-Urals and adapted to local conditions. The low homeostasis index, which we found in the varieties Tulunskaya 12 and Novosibirskaya 15 (Hom = 0.65), indicates their low adaptation to the conditions of the zone due to poor drought resistance in the early summer period, rapid growth, accelerated passage of the tillering phase, and the tendency to pre-harvest germination of grain in the ear.

Our findings confirm the conclusions of many breeders that varieties with high potential productivity under favorable conditions are more likely to respond to its decline in unfavorable conditions than less productive varieties [13].

So, based on an 8-year study of five groups of soft spring wheat varieties that have been used in the Northern Trans-Urals over an 80-year period, we have proposed an approach to quantitatively describe the changes that occur during long-term selection. Monitoring is based on several important characteristics of varieties. i.e., yield variation over the years of testing; effects of response to environmental conditions, changing the ranks of yields over the years; parameters of variety plasticity (yield homeostaticity); relationship between maturity and productivity. The variability of yield over years for one variety and the change in yield ranks over the years observed between varieties (effects of response to environmental conditions) largely depend on the adaptive genetic systems of each variety which ensure yield, when the limiting environmental factors vary from year to year or between geographical locations. The information obtained by the proposed method makes it possible to predict the behavior of varieties in changing environmental conditions and indicates the optimal directions for selection. Varieties adapted to local conditions with high contributions of adaptability systems and response effects to the formation of yield should be used as the starting material in breeding work.

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CORRELATION DEPENDENCES BETWEEN CROP REFLECTION INDICES, GRAIN YIELD AND OPTICAL CHARACTERISTICS OF WHEAT LEAVES AT DIFFERENT NITROGEN LEVEL AND SEEDING DENSITY

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Abstract

Improvement of crop remote sensing application in precision agriculture systems and development of algorithms for satellite and aerial imagery interpretation necessitate comparing remote sensing and ground-based survey data. This paper is the first to report data on spectral characteristics of the leaf diffuse reflection in spring wheat, their relationship with plant productivity, colorimetric characteristics and reflection indices of the crop vegetation cover, depending on the crop management technologies. The first research objective was to assess the dependence of crop canopy optical characteristics and productivity on seeding density and the rates of pre-treatment with nitrogen fertilizer. The second objective was to reveal correlations between the canopy remote sensing data and the leaf diffuse reflection parameters registered by a contact sensor (on the example of spring wheat *Triticum aestivum* L. cv. Daria). The plants were grown on the test plots (Menkovo experimental station of Agrophysical Research Institute, Leningrad Province, Gatchina District) in 2020-2021 years. In total, six test plots with an area of 100 m^2 were assigned. Nitrogen rates ranged from 0 (no fertilizers applied) to 200 kg/ha with increments of 40 kg/ha, and seeding rates of 500 and 600 seeds per m^2. The diffuse light reflection of leaves was registered in situ on stages BBCH 30-31 "booting" and BBCH 53-55 "earing" by a fiber-optical spectroradiometric system (Ocean Insight, USA) in the range from 350 to 1000 nm with a step size of 0.3 nm. After the reflectance spectra recording, the plants were dried to constant weight and each plant was weighed to assess correlations with the optical parameters of leaves. The light diffusion index R_{800} was determined from the spectra of reflected radiation. The reflectance indices calculated were the following: ChlRI (chlorophyll index), PRI (photochemical index), FRI (flavonoids index), WRI (water content index), ARI (anthocyanins content index) and FRI (flavonoids content index). These indices estimate the intensity of the photosynthetic apparatus function and the efficiency of light use in photosynthesis. The crop canopy remote sensing was performed at BBCH 25-27 ("tillering"), BBCH 30-31 ("booting"), BBCH 53-55 ("earing"), and BBCH 61-65 ("blossoming") stages using two synchronized digital cameras Canon G7X (Canon Inc., Japan) mounted on a Geoscan 401 quadcopter (Geoscan, Russia). From a height of 75-120 m, the digital images were obtain in the visible and near infrared spectral ranges. The vegetation indices NDVI (Normalized Difference Vegetation Index) and ARVI (Atmospherically Resistant Vegetation Index) were calculated based on the optical characteristics. For quantitative interpretation of the colorimetric characteristics of leaves and the crop canopy, we used a three-dimensional model of the CIELAB color space. Plants were weighted during the growing season, and, after harvesting, the grain productivity was estimated for the plants sampled from 0.25 m^2 reference plots. The obtained results indicate that at the early stages of plant development when the vegetation cover remains open, NDVI characterizes

the degree of nitrogen supply rather exactly and identifies areas with underdeveloped plants. However, with the development of plants and the formation of a closed vegetation cover this index fails to provide reliable results. ADVI also fails to provide reliable information about the state of spring wheat plants and identifies areas that require additional fertilize application. A close linear correlation between the rate of applied nitrogen fertilizer, the net productivity of plants and spectral characteristics of leaves measured in situ occures until the late stages of development (BBCH 53-55 “earing”, BBCH 61-65 “blossoming”). Crop monitoring based on colorimetric characteristics made it possible to detect changes in the crop canopy associated not only with the plant development and crop density, but also with the spectral characteristics of the diffuse reflection of plant leaves. A comparison of the remote and contact sensing data allows us to conclude that the ChlRI, PRI, FRI and WRI indices can successfully identify areas in which nitrogen deficiency has developed during the closed canopy formation, when the commonly used indices, for example, NDVI, fail to be reliable.

Keywords: spring wheat, reflection indices, spectral characteristics, remote monitoring, nitrogen deficiency, precision agriculture

The spatial variability of the state of crops, which is determined remotely, is due to many factors (soil, climatic, biological, and technological) and depends on the optical properties of leaves, plant architectonics, crop structure. The main function of the vegetation cover is to intercept radiation for photosynthesis and other metabolic processes. This interception occurs with varying degrees of efficiency, which depends mainly on the area of the formed leaves and their orientation relative to the sun. Since the growth and yield of plants are determined mainly by photosynthesis, they are directly dependent on the amount of absorbed light and the efficiency of its conversion in chloroplasts. Agrophytocenosis, the structure of which provides greater absorption of light and its most efficient use, is usually characterized by a higher intensity of plant photosynthesis and yield. For the development of precision farming systems, a fundamentally new methodological, physical, technical and experimental base is currently needed, which will become the main structural component in assessing the variability of crop characteristics and monitoring their condition [1].

The radiation reflected by leaves and other phytoelements carries complete information about the biochemical composition, tissue architecture, physiological state of plants, and specific changes in their spectral characteristics and the amount of reflected radiation make it possible to evaluate the response to the action of various stressors [2-4] and make a yield forecast [5]. Deciphering the information embedded in the optical characteristics of plants underlies the remote and ground-based sensing of vegetation, but the complex nature of the optical systems of plants makes this process ineffective [3]. Over the past decades, approaches have been developed to gain insight into some aspects of plant structure and function from multi- and hyperspectral reflectance data. The content of photosynthetic pigments, the presence of anthocyanins, flavonoids and water in tissues determine the activity of photosynthesis processes, the spectral characteristics of diffuse scattering of leaves and plant cover as a whole. Previously, the most promising reflection indices were described and their use for diagnosing the physiological state of plants even in the absence of visible symptoms of growth inhibition and oppression was considered [6, 7].

The pattern of reflection of different wavelengths of light by leaves in the range of photosynthetically active radiation and near infrared radiation of the electromagnetic spectrum is very different from that for the soil [8]. Differences in the reflectance spectra of the soil and the vegetation cover formed by agrophytocenosis have a clear potential for remote diagnostics of the state of plants both in breeding programs when screening genotypes for efficiency [9] and in assessing the feasibility and volume of using various agricultural practices. The usually observed spatial heterogeneity of the optical characteristics of agrophytocenoses is associated with differences in the content of water, nutrients and other soil properties, as well

as with the features of agricultural technologies used in the cultivation of crops (seeding rates of seeds, their quality, the degree of contamination of crops, etc.)

Insufficient use of remote sensing tools significantly limits the possibilities of precision farming technologies. Largely, this is due to the lack of problem-oriented databases and a significant backlog of methods for decrypting information received from sensors and devices that monitor crops. A simple metric for measuring reflectance differences is the normalized difference vegetation index (NDVI), which was proposed by J.W. Rose et al. [10] and was used to assess the state of vegetation using multispectral remote sensing [11, 12]. N. Oppelt et al. [13] used NDVI in hyperspectral studies to monitor the physiological parameters of wheat. They found that NDVI becomes insensitive to chlorophyll content below 0.3 g/m^2 and also above 1.5 g/m^2. It is probably the most commonly used index for analyzing crop health and provides indirect estimates for leaf area index, light absorption and potential photosynthetic activity [8, 14, 15]. Since NDVI responds to changes in vegetation cover during crop growth and development, it is often used to predict yields [16, 17].

Remote monitoring makes it possible to significantly improve the methods of crop forecasting and operational control over the state of crops both globally and locally [18, 19]. At the initial stages of development and improvement of remote diagnostic methods, it is necessary to compare the obtained data with the results of a ground survey of crops [20]. The data obtained during remote and ground survey of crops at the same time, in the same areas of the fields, are necessary to increase the accuracy of diagnostics and identify criteria and identification indicators of the state of crops [6, 7, 20]. In remote and ground monitoring methods are widely used to evaluate the optical characteristics of leaves, leaf area index and/or projective soil cover, characteristic of a particular crop.

In this work, for the first time, we submit data on the spectral characteristics of the diffuse reflectance of spring wheat leaves, the relationship of the spectral characteristics with plant productivity, colorimetric characteristics, and vegetation reflection indices of the vegetation cover formed by this crop, depending on the applied crop management technologies.

The purpose of the work is to evaluate the dependence of the optical characteristics and crop productivity on the seeding rate and the dose of nitrogen fertilizers applied before sowing on the example of spring wheat (*Triticum aestivum* L.). We also aimed to reveal correlations between the remotely measured optical characteristics of the vegetation cover and the spectral characteristics of diffuse leaves reflection using a contact sensor.

Materials and methods. The studies were carried out on crops of spring wheat (*Triticum aestivum* L.) cv. Daria. The plants were grown in the field of the Menkovsky branch of the Agrophysical Research Institute (AFI) (Leningrad Region, Gatchina District) in 2019-2020. The soil was soddy-weakly podzolic medium-cultivated light loamy with a humus content of 2.07%, exchangeable calcium of 8.38 mmol/100 g, magnesium of 2.88 mmol/100 g, mobile compounds of phosphorus and potassium of 565 and 140 mg/kg, ammonium and nitrate nitrogen of 12.37 and 8.21 mg/kg; pH_{KCl} 5.7. The thickness of the arable layer is 22 cm.

Six test plots with an area of 100 m^2 were laid, on which the dose of evenly applied nitrogen (nitrogen rate, NR) varied from 0 (without fertilization) to 200 kg/ha with a step of 40 kg/ha. The azophoska fertilizer were applied before sowing ($^2/_3$ of nitrogen dosage) and ammonium nitrate was applied as top dressing at the stage BBCH 25-27 ("tillering") (the remaining $^1/_3$ of nitrogen dosage). To create crops of different density, each of the test plots was divided into two 50 m^2 plots. The seeding density (SR) on one of them was equal to 6.0 million/ha, on the

second to 5.0 million/ha, that is, 500 and 600 seeds/m^2 (SR500 and SR600, respectively).

The spectral characteristics of the diffuse reflectance of leaves were determined in situ at stages BBCH 30-31 ("booting") and BBCH 53-55 ("earing") using a fiber-optic spectroradiometric system (Ocean Insight, USA) in the range from 350 to 1000 nm with a step 0.3 nm. To select plants for the purpose of analyzing the spectral characteristics of the leaves, the sowing on the test plots was conventionally divided into 4 parts, and 5-6 plants (20-30 plants in total) were selected from the center of each part, which were transported with a clod of moist earth to the laboratory. To record diffuse reflectance spectra, leaves that had completely finished growing were used, placing the sensor in the middle part of the leaf blade, to the left and right of the central vein. The recorded spectra (at least 20 spectra for each variant) were processed in the Microsoft Excel 2013 program, where the average reflectance values were calculated for all wavelengths of the measured range of 350-1000 nm. After recording the reflectance spectra, the plants were dried to constant weight at a temperature of 85 °C and weighed individually. The resulting parameter (biomass of one plant, B_{1p}) was used to study correlations with the optical characteristics of leaves.

From the spectra of reflected radiation, we determined the measure of leaf light scattering by the R_{800} [21] and calculated the reflection indices characterizing the activity of the photosynthetic apparatus: the content of chlorophyll (ChlRI), the photochemical activity of the photosynthetic apparatus (PRI), the content of water (WRI), anthocyanins (ARI) and flavonoids (FRI).

We used the following formulas:

$$ChlRI = (R_{750} - R_{705})/(R_{750} + R_{705} - 2R_{445}) \text{ [21]},$$
$$PRI = (R_{570} - R_{531})/(R_{570} + R_{531}) \text{ [22]},$$
$$ARI = R_{750}\,(1/R_{550} - 1/R_{700}) \text{ [23]},$$
$$FRI = [(1/R_{410}) - (1/R_{460})] \times R_{800} \text{ [24]},$$
$$WRI = (R_{970} - R_{920})/(R_{970} + R_{920}) \text{ [25]},$$

where R is the reflection value, the subscripts are the wavelengths reflected from the sheet surface.

For the convenience of presenting data and obtaining positive index values, the constant value C was used in the calculation formulas for PRI, ARI and FRI, from which the values of the listed indices were subtracted. The modified reflection indices were obtained: $PRI_{mod} = C1 - PRI$, $ARI_{mod} = C2 - ARI$, $FRI_{mod} = C3 - FRI$. The experimentally selected values of the constants C1, C2 and C3 were equal to 0.5, 0.7 and 0.7, respectively [26]. Correlation dependences between all listed reflection indices and net productivity under the action of various abiotic stressors on wheat [5] and barley [26] plants were considered earlier.

During the main development stages of spring wheat (tillering, BBCH 25-25; booting, BBCH 30-31; earing, BBCH 53-55; blossoming, BBCH 61-65), the crops were photographed remotely. Digital images in the visible and near infrared spectral ranges were obtained from a height of 75=120 m using two synchronized Canon G7X digital cameras (Canon, Inc., Japan) mounted on a Geoscan 401 quadrocopter (Geoscan, Russia). Aat least three images of crops were obtained at each of the test sites.

Analysis of digital images of crops and registration of their spectral characteristics were carried out using the Erdas Imagine program (Erdas, Inc., USA).

When processing the optical characteristics, the vegetation indices NDVI (normalized difference vegetation index) and ARVI (atmospheric resistant vegetation index) were calculated:

$$NDVI = (NIR - RED)/(NIR + RED),$$
$$ARVI = (NIR - R_b)/(NIR + R_b),$$

where NIR and RED are reflections in the near infrared and red regions of the spectrum, respectively, Rb = RED – a × (RED – BLUE), as a rule, a = 1, with little vegetation cover and an unknown type of atmosphere a = 0.5 [27].

To quantify the colorimetric characteristics of leaves and plant cover formed by crops, the CIELAB 3D color space model adopted by the International Commission on Illumination was used [28].

During the growing season at the stages BBCH 25-27, BBCH 30-31, BBCH 53-55 and BBCH 61-65, the plant biomass (Bp) was determined by the weight method. For this purpose, record plots with an area of 0.25 m^2 were allocated on test plots. All plants from the record plot were cut at the level of the root collar, dried to constant weight at 85 °C, and weighed. At the end of the growing season, the grain productivity of plants was determined (Yg, grain weight, g/m^2). In this case, plants were collected from selected 0.25 m^2 plots. The repetition of the experiment in determining Bp and Yg was 3-fold.

Statistical processing was performed using Microsoft Excel 2013 and Statistica 8 software (StatSoft, Inc., USA). The average values of the studied parameters were determined. The significance of differences between the variants was assessed by parametric (Student's *t*-test) and non-parametric (Spearman's rank correlation coefficient and Wilcoxon's pairwise comparison test) statistics. Differences between the variants were considered statistically significant at $p \leq 0.05$. The strength of the factor effect (η^2, partial eta-squared) for the level of nitrogen nutrition and seeding rate was determined as a percentage as the ratio of the corresponding sum of squared deviations of the studied optical parameters from their average values to the total sum of squares.

Results. The data of the measurements are presented at http://www.agrobiology.ru.

Remote assessment of crops by vegetation indices (NDVI and ARVI) and analysis of their relationship with net productivity. It is shown that a yield forecast can be made based on the results of assessing the state of crops in the early stages of development. One of the main criteria for such an assessment is the amount of accumulated biomass, which can be determined both by the direct weight method and by non-invasive optical methods, including remote sensing.

The relationship between grain productivity (Yg, g/m^2) and the biomass of wheat plants formed at booting (B_{p1}, g/m^2), earing (B_{p2}, g/m^2), and blossoming (B_{p3}, g/m^2) was linear and credible:

$Yg = 48.76 + (0.30 \times Bp_1)$ ($r = 0.64$; $p = 0.024$; $R^2 = 0.42$),

$Yg = 36.06 + (0.15 \times Bp_2)$ ($r = 0.82$; $p = 0.001$; $R^2 = 0.67$),

$Yg = 97.41 + (0.13 \times Bp_3)$ ($r = 0.88$; $p = 0.0001$; $R^2 = 0.78$).

Such a close correlation between grain yield and biomass values, even at the early stages of plant growth, allows us to conclude that biomass can serve as one of the main parameters in predicting grain yield. Its evaluation using remote methods creates good prospects for monitoring crops and developing new technologies to manage the production process of plants.

1. Relationships of plant biomass with normalized difference vegetation index (NDVI) **and atmospherically resistant vegetation index** (ARVI) **in spring wheat (*Triticum aestivum* L.) cv. Daria over plant growth stages** (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

Relationship equations	PC, %	*r*	p	R^2
Tillering (BBCH 25-27)				
Bp = –0.913 + (2.674 × NDVI)	43.7	0.927	0.008	0.86
Bp = –0.104 + (0.257 × ARVI)		0.801	0.056	0.54

Continued Table 1

	Booting (BBCH 30-31)				
Bp = –2.015 + (5.729 × NDVI)		62.4	0.810	0.009	0.66
Bp = –0.526 + (0.703 × ARVI)			0.798	0.057	0.64
	Earing (BBCH 53-55)				
Bp = 0.711 + (3.75 × NDVI)		65.0	0.138	0.795	0.019
Bp = 0.235 + (1.032 × ARVI)			0.545	0.263	0.297
	Blossoming (BBCH 61-65)				
Bp = –15.66 + (33.84 × NDVI)		48.1	0.730	0.097	0.533
Bp = –0.707 + (2.044 × ARVI)			0.843	0.035	0.716

Note. Bp — plant biomass, kg/m^2, PC — projective cover of plants (maximum value for each of six 100-m^2 test plots at seeding density of 600 seeds per 1 m^2). Relationship equations between Bp and vegetation indices NDVI and ARVI are based on the average values of the parameters measured on three record plots allocated on each of the six test plots.

Analysis of remote monitoring data indicates the dependence of vegetation indices on sowing density and nitrogen nutrition. The values of the parameters were maximum at booting stage (0.56-0.63 on average for the options) and minimum at tillering stage (0.49-0.56). For SR500, the NDVI values increased up to the earing stage, while for SR600, the increase in the index occurred only up to the booting stage. This is probably due to the faster closing of the vegetation cover at SR600. A significant relationship between plant biomass and the vegetation index NDVI occurred only at the early stages of plant development, and there was no statistically significant relationship between the traits after the main stem extention (Table 1). There was no significant relationship between the biomass of plants formed per 1 m^2 of the test plot and ARVI for all survey periods, with the exception of blossoming stage. It can be concluded that this index is not suitable for monitoring spring wheat crops in order to detect field areas that require additional application of nitrogen fertilizers and adjustment of the nitrogen nutrition regime of plants.

At the late phases of growh (blossoming stage), the correlation coefficients between plant biomass and vegetation indices NDVI and ARVI were quite high (0.730 and 0.843, respectively). However, due to the strong variability of NDVI values for the vegetation cover formed on test plots, the relationship with NDVI was unreliable, whereas the relationship between Bp and ARVI was statistically significant.

The content of chlorophyll (chlorophyll index, ChlRI) in wheat leaves changed significantly depending on the dose of applied nitrogen and the seeding density (SR500 or SR600) (Table 2). The strength of the factorial influence of nitrogen nutrition on ChlRI was high and was equal to 35.1% of the total variability of the indicator in the experiment. The seeding rate had a small (3.7%) but significant effect on ChlRI (see Table 2). No statistically significant interaction between the dose of applied nitrogen fertilizers and the seeding density was found.

The introduction of nitrogen fertilizers, in addition to increasing the content of chlorophyll, also changed the photosynthetic efficiency. One of the parameter used in the work, the photochemical reflectance index (PRI_{mod}), made it possible to evaluate the efficiency of the photosynthetic apparatus of plants and the regulation of light flux in pigment-protein complexes [22]. The photochemical reflectance index, based on the results of testing on various crops [29, 30], provides a quick and non-destructive diagnostic of the effectiveness of photosynthetic processes in leaves or vegetation cover. This index does not depend on the leaf structure and is determined by the concentration of carotenoids and the activity of their conversion in the xanthophyll cycle, which ultimately determines the efficiency of the use of light energy and its conversion in photosystem II [22].

2. Reliability and the degree of influence of technological factors on leaf reflectance indices measured with a contact sensor in spring wheat (*Triticum aestivum* L.) cv. Daria plants at earing stage BBCH 53-55 (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

Reflectance indices	Factors					
	SR		NR		SR × NR	
	p	η^2	p	η^2	p	η^2
ChlRI	< 0.001	3.7	< 0.001	35.1	0.185	0.8
SIPI	0.051	0.9	< 0.001	11.8	0.108	1.1
R_{800}	0.001	2.5	0.111	1.1	0.001	3.1
PRI_{mod}	0.630	1.0	< 0.001	30.4	0.661	0.1
ARI_{mod}	0.872	0.006	0.97	0.01	< 0.001	3.3
FRI_{mod}	0.001	2.4	< 0.001	27.0	0.043	1.5
WRI	< 0.001	28.7	< 0.001	9.5	0.96	0.1

N o t e. SR — seeding rate (2 options), NR — nitrogen rate (N dosage, 6 options), SR × NR — interaction of the factors; η^2 — the effect size for the factor or factors' interaction, p — the significance level for reliable effects. Reflectance indeces for chlorophyll (ChlRI), for carotenoids to total chloorophylls (SIPI), for light scattering in leaf bades (R_{800}), photochemical reflectance index (PRI_{mod}), anthocyanin reflectance index (ARI_{mod}), flavonoid reflectance index (FRI_{mod}), and water reflectance index (WRI, water content) are submitted. Reliability and the degree of the influence of SR и NR parameters on the leaf reflectance indices were assessed by their averages for 2 options for SR and 6 options for NR, 12 options in total. The average value of reflection indices was determined in a sample of at least 20 plants.

Based on the relationship of PRI with leaf area and chlorophyll content in corn and soybeans, it was concluded that the dynamics of changes in PRI during plant growth results from a combination of the activity of xanthophyll cycle and the total pool of chlorophylls and carotenoids which are formed during adaptation to environmental conditions [31].

Usually, an increase in PRI values marks an increase in thermal dissipation due to a decrease in the amount of absorbed light energy, which is used by the plant in the photochemical processes of photosynthesis.

Nitrogen nutrition had the strongest effect on PRI_{mod} (see Table 2). This factor contribution exceeded 30% influence. With a deficiency of nitrogen nutrition (N_0), the photochemical reflectance index had the highest value, indicating the dissipation of light energy not used for photosynthesis, that is, the lowest efficiency of its use. The presowing application of nitrogen contributed to a more efficient light conversion in the photochemical photosynthetic reactions, as can be seen from the decrease in PRI_{mod} as the dose of nitrogen fertilizers applied before sowing was increased.

There was no statistically significant effect of the seeding rate (SR500 or SR600) on the photochemical activity index PRI_{mod}. However, the trend towards an increase in this indicator occurred under nitrogen deficiency (N_0) and higher seeding density (SR600).

The leaf structure-independent pigment index (SIPI) makes it possible to estimate the ratio of the carotenoid content to the amount of chlorophyll, Car/Chl [21]. G.A. Blackburn [32] confirmed that this index has a non-linear dependence on the Car/Chl ratio, which is best described using a logarithmic model ($R^2 = 0.86$). SIPI lacks sensitivity at low Car/Chl ratios, but becomes more sensitive at high Car/Chl ratios. In the present work, the SIPI values were 11.8% influenced by the rate of nitrogen fertilizers. There were no significant changes in SIPI depending on the seed density.

In addition to chlorophylls and carotenoids, the spectral characteristics of the diffuse reflection of leaves and other phytoelements are determined by the content of phenolic compounds (for example, anthocyanins and flavonoids), the presence of which changes the quality and quantity of light penetrating to chloroplasts. Usually, under oxidative stress, the content of anthocyanins and flavonoids increases, which contributes to the shielding of chloroplasts, preventing

their destruction from absorbing excess light energy and reducing or eliminating the effects of oxidative stress.

We did not find significant changes in the anthocyanin index (ARI_{mod}), both depending on nitrogen nutrition and the seeding rate. The flavonoid index (FRI_{mod}) significantly increased with nitrogen deficiency ($\eta^2 = 27\%$, $p < 0.001$) and in denser sowing ($\eta^2 = 2.4\%$, $p = 0.001$). A small but significant interaction was observed between the dose of applied nitrogen and plant density (see Table 2).

In our experiment, there was no statistically significant effect of nitrogen nutrition on the R_{800} index, the value of which depends on the leaf structure, mainly on the cell size and intercellular space. However, the plant density in the crop had a small, statistically significant effect on this index (see Table 2).

WRI has been tested in a number of studies to assess the water status of plants [25, 33]. M. Gutierrez et al. [34] concluded that WRI reveals genetic differences in drought tolerance at the crop cover level and that WRI can be used to quickly and inexpensively assess plant water status. We found that WRI depends both on the dose of applied nitrogen and on the plant density in the crop. Moreover, the sowing density had a stronger effect ($\eta^2 = 28.7\%$) than nitrogen deficiency ($\eta^2 = 9.5\%$) (see Table 2). It has previously been shown that WRI is related to water content, leaf water potential, stomatal conductance, and canopy temperature under water stress [35]. A strong negative linear relationship between grain productivity and WRI was found for various wheat genotypes, and the ability to predict plant yield using this water band index was shown [38].

Estimation of biomass and leaf reflectance indices (ChlRI, SIPI, PRI_{mod}, and FRI_{mod}) at different stages of plant growth made revelaed their close correlation with the nitrogen content in the soil (Table 3). Characteristically, the significant relationship between the listed traits observed at the early stages of plant development (booting) persisted at later stages (earing). As it was shown earlier, the use of NDVI did not provide detection of differences in the state of wheat plants at stages later than booting for a seeding rate of 500 and 600 pcs/m2 (see Table 1).

3. Spearman's rank correlation coefficients for soil nitrogen, plant biomass and leaf reflectance indexes measured with a contact sensor in spring wheat (*Triticum aestivum* L.) cv. Daria plants at booting and earing stages (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

Parameter	Bootin		Earing	
	NR	B_{1p}, g	NR	B_{1p}, g
B_{1p}	0.95*		0.83*	
ChlRI	0.98*	0.91*	0.93*	0.76*
SIPI	–0.93*	–0.86*	–0.83*	–0.81*
R_{800}	0.07	0.02	–0.59	–0.50
PRI_{mod}	–0.83*	–0.88*	–0.90*	–0.81*
ARI_{mod}	0.24	0.38	–0.28	–0.48
FRI_{mod}	–0.91*	–0.79*	–0.83*	–0.67*
WRI	0.63*	0.64*	0.72*	0.74*

N o t e. NR — the rate of nitrogen fertilizers from 0 to 200 kg/ha with a 40 kg/ha step, B_{1p} — plant biomass. Reflectance indeces for chlorophyll (ChlRI), for carotenoids to total chloorophylls (SIPI), for light scattering in leaf bades (R_{800}), photochemical reflectance index (PRI_{mod}), anthocyanin reflectance index (ARI_{mod}), flavonoid reflectance index (FRI_{mod}), and water reflectance index (WRI, water content) are submitted. The Spearman correlation coefficient was calculated for the average values of the reflectance and biomass (B_{1p}) indices of plants, measured in 6 NR options in 2-fold repetition (12 options in total). The average values of the reflection indices and B_{1p} for each option were determined in a sample of at least 20 plants.

* Correlation between traits is significant at $p < 0.05$.

The optical characteristics of wheat leaves depend on the content of chlorophyll (ChlRI), carotenoids (SIPI), some phenolic compounds (ARI and FRI), structure of leaves (R_{800}) and their water content (WRI). A change in each of these features is accompanied by a modification of the spectral characteristics of

the diffuse reflectance of leaves and a change in their colorimetric characteristics. For example, with a decrease in the concentration of chlorophyll, which absorbs blue and red radiation, the proportion of yellow and blue-green radiation in the diffuse reflectance spectrum of a leaf increases. This inevitably leads to a change in the colorimetric characteristics (color) of leaves.

The results obtained indicate that at the early stages of plant development, while the formed cover remains open, NDVI provides accurate assessment of the degree of nitrogen supply of plants and identifies areas of the field where plants are less developed. According to the results of field tests [12, 17], NDVI is closely related to plant biomass and leaf area index. This allows us to consider this vegetation index as a reliable indicator of plant health which can be assessed remotely. However, as the plants develop and close canopy is formed, the results are no longer reliable. The close relationship between the colorimetric characteristics of the closed vegetation cover and the leaves of the upper layer of wheat plants (see Table 3) suggests that the ChlRI, PRI, FRI, and WRI indices can be successfully used for remote assessment of crop areas in which nitrogen nutrition is deficient.

Assessment of the crop state by colorimetric parmeters of their digital images. For a timely assessment of the nitrogen needs of plants, some researchers used images of crops obtained with digital cameras, followed by an assessment of the colorimetric characteristics of the vegetation cover [6, 7, 33, 36]. Digital imaging techniques have also been used to determine the degree of projective soil cover, which is closely related to leaf area index, aboveground biomass, and nitrogen content in the early stages of plant development [19, 37, 38].

According to the CIELAB 3D color space model [21], L denotes the lightness, A denotes the red/green component, and B denotes the yellow/blue component. The maximum value of 100 corresponds to an ideal reflective diffuser (white color), the minimum value of L is zero which corresponds to black color. The A and B axes are numerically unlimited. Positive values of A are inherent in the red object, negative values are green. A yellow object has positive B values, while a blue object has negative B values.

The results of our study showed that the colorimetric characteristics of the vegetation cover formed by wheat plants during the transition to earing changed significantly depending on nitrogen nutrition. A remote assessment of colorimetric characteristics showed that an increase in the dose of nitrogen applied before sowing from 40 to 200 kg/ha (or 5-fold), the L decreased on average from 48.3 to 33.2 (1.45 fold), A increased from –9.7 to –7.7 (1.26-fold), and B decreased from 17.2 to 10.7 (1.64-fold). Measurements done during the earing period with a contact sensor that was placed on the upper leaves also revealed the variability of their colorimetric characteristics depending on the pre-sowing nitrogen dosage. With an increase in the nitrogen dosage from 40 to 200 kg/ha, the parameter L changed from 49.2 to 41.7 (1.18-fold), A from –9.7 to –7.18 (1.35-fold), B from 21.4 to 10.9 (1.96-fold). The results obtained indicate that B is the most sensitive indicator characterizing plant provision with nitrogen.

A statistically significant correlations of the L, A, B values for the upper leaves with the plant nitrogen supply were found both when the colorimetric characteristics were measured contactly and by the remote assessment (Table 4).

The value of the parameter L of the CIELAB 3D color model increased as the dose of applied nitrogen increased, That is, with a nitrogen deficiency, the formed vegetation cover became lighter. Chromatic components A and B were also sensitive to changes in plant nitrogen nutrition. An increase in the B parameter is a marker of nitrogen deficiency and yellowing of the leaves. A shift towards

large negative values along the A-axis as the dose of nitrogen nutrition increases indicates that wheat leaves have a more saturated green color. During the remote examination of crops at the beginning of booting when the vegetation cover had not yet closed, there was no close relationship between the NR applied before sowing and the chromatic components A and B ($p \le 0.05$). There was a statistically significant relationship between the L value and the nitrogen level in the soil ($r = -0.942$, $p = 0.0049$, $R^2 = 0.887$), apparently, due to an increased contribution of the colorimetric characteristics of the background soil in crops with less developed plants to the colorimetric characteristics of the formed vegetation cover.

4. Correlations of soil nitrogen concentration with colorimetric parameters of crop and leaves in spring wheat (*Triticum aestivum* L.) cv. Daria plants at earing stage (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

CIELAB 3D colorimetric parameters	r	p	R2
Ld	–0,933	0,0065	0,811
Ad	0,977	0,0007	0,956
Bd	–0,977	0,0002	0,955
Lc	–0,943	0,0015	0,889
Ac	0,964	0,0019	0,930
Bc	–0,984	0,0001	0,969

N o t e. Parameters of CIELAB 3D color model measured from digital images of crops obtained distantly (Ld, Ad, Bd) and of upper leaves obtained with a contact sensor (Lc, Ac, Bc). The correlation coefficients between the dose of nitrogen fertilizers NR and the average values of the remotely and contact measured parameters of the LAB model were calculated for 6 NR options in 2 repetitions (12 options in total). Mean Lc, Ac, and Bc values were determined for a sample of 20 plants in each option; mean Ld, Ad, and Bd values were determined for three crop images for each NR option.

The relationship between the parameters of the CIELAB 3D model, measured remotely from a height of 75-100 m (Ld, Ad and Bd) and in the upper leaves (Lc, Ac and Bc) was linear and especially significant between the chromatic components Ld and Lc, and also Bd and Bc (Table 5).

5. Relationship between CIELAB 3D parameters of crops measured distantly (Ld, Ad, Bd) **and of upper leaves measured with a contact sensor** (Lc, Ac, Bc) **in spring wheat (*Triticum aestivum* L.) cv. Daria** (Menkovsky branch of the Agrophysical Research Institute AFI, Leningrad Region, Gatchina District, 2019-2020)

Relationship equations	*r*	p	R^2
Ld = –31.463 + (1.585 × Lc)	0.845	0.034	0.714
Ad = –2.541 + (0.715 × Ac)	0.969	0.0014	0.939
Bd = 5.598 + (0.527 × Bc)	0.959	0.0006	0.919

N o t e. For options and sample sizes, see Table 4.

Chromatic components A and B were closely associated with ChlRI, FRI_{mod}, and PRI_{mod} indeces ($p < 0.05$), while the L value correlated with WRI and, to a lesser extent, with ChlRI.

Our results indicate that at the early stages of plant growth, while the canopy cover remains open, NDVI makes it possible to accurately diagnose the degree of nitrogen supply to crops and identify field areas where plants are less developed. In numerous field trials [12, 17], NDVI is closely related to plant biomass and leaf area index. However, for a closed vegetation cover, the use of NDVI does not provide reliable assessment of the plant nitrogen status. The close relationship between the colorimetric characteristics of the closed vegetation cover and the leaves of the upper layer of wheat plant (see Table 5) suggests that the ChlRI, PRI, FRI, and WRI indices can be successfully used for remote assessment of crop areas where nitrogen nutrition is deficient.

Thus, on the example of spring wheat cv. Daria, the dependence has been established of the vegetation cover optical characteristics on the plant standing density and, to the greatest extent, on the dose of nitrogen fertilizers applied. It was

found that the normalized difference vegetation index (NDVI), with a high degree of reliability ($R^2 = 0.85$, $p = 0.009$), reflects the accumulated plant biomass value in the first half of the growing season up to the BBCH 31 stage (booting). When the projective soil cover is 60% or more, it is impossible to reliably estimate the biomass value by NDVI. The use of the atmospheric resistant vegetation index (ADVI) also does not allow obtaining reliable information about the state of spring wheat plants and identifying planting areas that require additional fertilizers. It has been shown that with nitrogen deficiency and a high seeding rate, the intensity of photosynthesis decreases, as evidenced by the lower chlorophyll accumulation (ChlRI) in the leaves. In addition to reducing the intensity of photosynthesis, unfavorable vegetation conditions lead to a decrease in the efficiency of using light in photosynthesis. An increase in the photochemical reflectance index (PRI_{mod}) and flavonoid index (FRI_{mod}) indicates a decrease in the efficiency of light use and inhibition of plant growth. The pre-sowing application of nitrogen contributed to more efficient light conversion in the photosynthetic photochemical processes. The higher seeding rate (600 vs. 500 seeds per 1 m_2) had a negative effect on the efficiency of light use. The water index WRI was found to increase with denser seeding and nitrogen deficiency. Such changes indicate a lower water content and likely inducate leaf aging and a decrease in their photosynthetic activity. The increased seeding rate has the most significant negative impact on WRI. The negative impact of the increased seeding rate remained in the variants with pre-sowing application of nitrogen. Diagnostics of the state of crops by colorimetric characteristics (parameters L, A, and B of the three-dimensional CIELAB model) makes it possible to detect changes in the vegetation cover associated not only with plant growth stage and density, but also with the spectral characteristics of the diffuse reflection of leaves.

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INTENSITY OF PHOTOSYNTHESIS AND TRANSPORT OF ASSIMILATES IN *Solanum tuberosum* UNDER THE ACTION OF 24-EPIBRASSINOLIDE

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Abstract

Brassinosteroids are a unique class of steroid hormones. They have a wide functional activity, combining the properties of growth stimulants and inducers of protective reactions that reduce the damaging effect of stressors on the plant organism. They regulate the processes of ethiolation, the synthesis of other groups of phytohormones. There is information about the participation of brassinosteroids in the expression of light-regulated photosynthetic genes and regulation of the functioning of the photosynthetic apparatus. Nevertheless, information regarding the content of pigments is conflicting. Activation of the enzymes of the Calvin cycle is shown. Some researchers noted stimulation of CO_2 uptake and a positive effect on the yield of various crops. However, there are practically no data on the effect of brassinosteroids on the transport of assimilates to the attracting centers of plants. In this work, we established the effect of brassinosteroids on the rate of photosynthesis of potato plants and for the first time revealed their participation in the regulation of the transport of assimilates to tubers through changes in the content of ABA in the basal zone of the stem and cytokinins in tubers. Our work aimed to study the effect of 24-epibrassinolide on the intensity of the photosynthesis process, the content of assimilates in different zones of the stem, and to reveal the participation of ABA and cytokinins in the outflow of assimilates into tubers in potato plants. The *Solanum tuberosum* L. cv. Skoroplodny plants were grown in a growing house in soil culture. At flowering phase, the plants were sprayed with a 1.47×10^{-8} M solution of 24-epibrassinolil (Institute of Bioorganic Chemistry of the National Academy of Sciences of Belarus); the control plants were sprayed with water. The intensity of photosynthesis was assessed using the ^{14}C radioactive isotope generated in a gasholder from a mixture of radioactive and non-radioactive sodium bicarbonate. At the end of flowering, a clothespin chamber was attached to an intact leaf of the eighth layer. In the chamber, the leaf was exposed to $^{14}CO_2$ atmosphere (10 ml, 0.6% $^{14}CO_2$, 0.0334 mBq/nM) for 10 minutes. To determine the content of ^{14}C-assimilates, sections of the stem zones were fixed 48 hours after exposure to the $^{14}CO_2$ atmosphere. The radioactivity was measured (a T-25-BFL end counter, Isotope, Russia). The sucrose content was measured refractometrically (an RPL-3 refractometer, OAO Kyiv plant "AnalytPribor", Ukraine). The concentration of abscisic acid (AA) in the zones of the stem and cytokinins in the tubers were determined by enzyme-linked immunosorbent assay (ELISA) method. AA and zeatin (Serva, Germany) served as standard solutions of phytohormones. The content of chlorophylls a, b and carotenoids was determined in 80% acetone extracts (a KFK-3-01 photometer, AO ZOMZ, Russia). The thickness of the phellema was measured on intravital cross sections in the middle part of the tuber using an eyepiece micrometer (a Biolam microscope, LOMO, Russia). After the end of flowering, epibrassinolide increased the intensity of $^{14}CO_2$ assimilation by 23 % ($p \le 0.05$). The treated leaves exposed to $^{14}CO_2$ contained more sucrose as compared to the control leaves. An increase in the content of chlorophylls a, b and carotenoids occurred. The concentration of sucrose and ^{14}C-assimilates differed between various stem zones. In the basal zone, the concentrations were lower than in the middle part. Epibrassinolide increased the gradient of ^{14}C- assimilates and sucrose between the zones of the stem, which may indicate an increase in their outflow into tubers. Simultaneously, the level of endogenous abscisic acid in the basal zone increased, which facilitates unloading of phloem endings. Under the influence of epibrassinolide, the AA gradient between the zones was 41 % vs. 26 % ($p \le 0.05$) in the

control. In tubers, due to the exogenous epibrassinolide, the level of cytokinins which exhibit an attracting effect was higher compared to the control. The brassinosteroid increased the productivity of potato plants by 25 % ($p \leq 0.05$) and stimulated phellema formation in the tubers. The research data obtained suggest that epibrassinolide regulates the intensity of the photosynthesis process, the outflow of assimilates into the forming tubers through the participation of AA in the creation of a gradient of assimilates in the stem zones and an increase in cytokinins in tubers, which attract assimilates. This ultimately affects the productivity of potato plants.

Keywords: 24-epibrassinolide, photosynthesis, pigments, sucrose, ^{14}C-assimilates, abscisic acid, cytokinins, stem zones, *Solanum tuberosum*

Brassinosteroids are a unique class of steroid hormones. They have a wide functional activity, combining the properties of growth stimulants [1, 2] and inducers of protective response that reduce the damaging effect of stressors on the plant [3-5], and also regulate etiolation [6-8] and the synthesis of other groups of phytohormones. [9]. There is information about the participation of brassinosteroids in the expression of light-regulated photosynthetic genes [10] and the regulation of the functioning of the photosynthetic apparatus [11]. In particular, a significant increase in the content of chlorophylls a, b and carotenoids was shown due to the activation of enzymes involved in the biosynthesis of chlorophylls [10]. In studies on corn seedlings [12] and rhododendron plants [5], there is a slight effect of epibrassinolide on the content of pigments.

A positive effect of brassinosteroids on increasing the photochemical efficiency of photosystem II was established [5, 10, 13], which is confirmed mainly by the parameters of chlorophyll fluorescence. In some works, there are data on the activation of the enzymes of the Calvin cycle, carbonic anhydrase and RuBisCO [10, 13-15]. According to E.O. Fedina [16], the effect of the hormone on the activity of RuBisCO is due to the reduction of tyrosine phosphorylation of its subunits.

Few studies have shown stimulation of CO_2 uptake when plants are treated with epibrassinolide [5, 15]. The authors attribute this effect to an improvement in stomatal conductance. A positive effect of brassinosteroids on the productivity of various types of crops has been established]17]. However, there is practically no information in the special literature on the effect of phytohormones of a steroid nature on the transport of assimilates in plants. C. Wu et al. [18] in their work on transgenic rice plants with increased synthesis of brassinosteroids revealed an increase in the outflow of assimilates into underdeveloped grains of the upper part of the inflorescence. The participation of brassins in the control of sucrose transport into grapes is also noted [19].

This work shows for the first time the participation of 24-epibrassinolide in the regulation of assimilate transport into potato tubers through changes in the content of abscisic acid (AA) in the basal zone of the stem and cytokinins in tubers.

Our goal was to study the effect of 24-epibrassinolide on the intensity of the photosynthesis process, the content of assimilates in the stem zones, and to reveal the involvement of AA and cytokinins in the outflow of assimilates into potato tubers.

Materials and methods. Potato plants (*Solanum tuberosum* L.) of the Skoroplodny variety (selection of the Lorkh FRC of potato, Russia) were grown in a growing house (agro-bio station of the Turgenev Oryol State University, 2018-2019) in soil culture. Soil was gray forest medium loamy. Nitrogen, phosphorus and potassium were added in the amounts optimal for potatoes, 230, 70, and 310 mg/kg of soil, respectively. Each plant was grown in individual pot with 10 kg of soil, the humidity was maintained at 60% of full moisture content.

Plants at flowering stage were sprayed with a 1.47×10^{-8} M solution of 24-

epibrassinolide (Institute of Bioorganic Chemistry, National Academy of Sciences of Belarus); control plants were sprayed with water.

At the end of flowering, average samples of leaves of the 8th tier and stem zones 7 cm long (medium part between leaves of the 7th-8th tiers and basal part between leaves of the 1st-2nd tiers) were collected for analysis:; at the end of the growing season, tubers were collcted.

The intensity of photosynthesis was studied using the radioactive isotope ^{14}C [20] which was obtained in a laboratory gas holder from a mixture of 4 mg of radioactive and 2525 mg of non-radioactive sodium bicarbonate. At the end of plant flowering, an intact leaf of the 8th tier was exposed for 10 min to $^{14}CO_2$ under natural conditions (at 11.00 am and a temperature of 17-19 °C, a clothespin chamber into which 10 ml of $^{14}CO_2$ was injected from a gas holder using a medical syringe). Air humidity was 49%, illumination was 340 μmol photons $\cdot m^{-2} \cdot s^{-1}$. The specific radioactivity in the $^{14}CO_2$ atmosphere was 0.0334 mBq/nM, the $^{14}CO_2$ concentration $^{14}CO_2$ was 0.6%. To determine the rate of photosynthesis in a leaf that received $^{14}CO_2$, the extreme part of the leaf was split off, fixed for 30 min at 105 °C and dried at 60 °C. To assess the content of 1^4C-assimilates, segments of stem zones were fixed for 48 h after exposure to $^{14}CO_2$. Radioactivity was measured on a T-25-BFL end counter (Isotope, Russia).

The content of sucrose was estimated refractometrically (an RPL-3 refractometer, JSC Kiev Plant AnalytPribor, Ukraine), taking into account the refractive index of cell sap. The amount of abscisic acid (AA) in stem zones and the content of zeatin in tubers were measured by enzyme-linked immunosorbent assay [21]. After binding protein conjugate of the hormone, serum with antibodies and the experimental samples were added into the wells of the polystyrene plate. The amount of antibodies specifically bound to the protein conjugate of the hormone was determined using ram antibodies against rabbit immunoglobulins, labeled with peroxidase. Ortho-phenylenediamine was used to assess the activity of bound peroxidase. The intensity of the chromophore response was determined (a Dombi plate microphotometer, Latvia; $\lambda = 492$ nm). Reagents from Uralinvest (Russia) were used for the analyses. AA and zeatin (SERVA Electrophoresis GmbH, Germany) were standard solutions of phytohormones.

The pigments were extracted from the leaves with 80% acetone solution. The content of chlorophylls a, b and carotenoids was determined (a KFK-3-01 photometer, AO ZOMZ, Russia) [22].

The thickness of the phellema (cork) was measured on lifetimt transverse sections in the middle part of the tuber (an ocular micrometer MOV-1-15x, a Biolam microscope, LOMO, Russia).

Statistical processing of the experimental data was carried out using the Microsoft Excel 2010 program. The figures and the table show the arithmetic mean values (*M*) and their standard errors (±SEM) from five biological replicates. Analytical repetition is 5-fold. The significance of the results was assessed using the Student's *t*-test at a confidence level above 0.95.

Results. Spraying potato plants at the end of flowering with a solution of 24-epibrassinolide increased the intensity of $^{14}CO_2$ assimilation (Fig. 1) by 23% ($p \leq 0.05$). As a result, the leaves of the 8th tier, which received $^{14}CO_2$, contained 34% more sucrose compared to the control ($p \leq 0.05$).

An increase in the intensity of photosynthesis occurred with an increase in the pigment content in leaves (Fig. 2). The amoint of chlorophylls a and b increased by 25%, while carotenoids by 14% ($p \leq 0.05$). I.F. Golovatskaya et al. [11] reported that the enhancement of photosynthesis in regenerated potato plants upon root treatment with 10 nM epibrassinolide is associated with a significant

increase in the assimilation index. The effect of brassinosteroids on the intensity of photosynthesis could be due to their effect on the hormonal balance in the plant. An increase in the content of cytokinins in wheat plants was shown under the influence of treatment with a 0.4 μM solution of epibrassinolide [9]. Cytokinins are known to increase the intensity of photosynthesis [23].

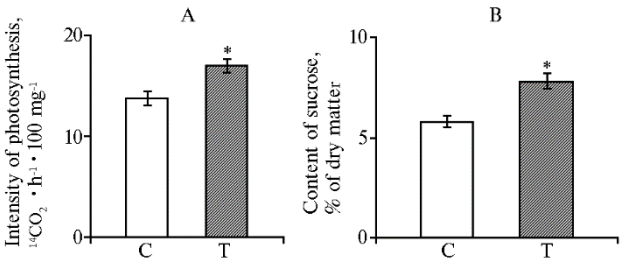

Fig. 1. Intensity of photosynthesis (A) **and sucrose content** (B) **in leaves of potato (*Solanum tuberosum* L.) cv. Skoroplodny plants in control** (C) **and upon spraying plants with 1.47×10^{-8} M 24-epibrassinolide** (T) **at blossoming** ($M\pm$SEM, $n = 5$, $N = 5$).
* Differences with control are statistically significant at $p \le 0.05$.

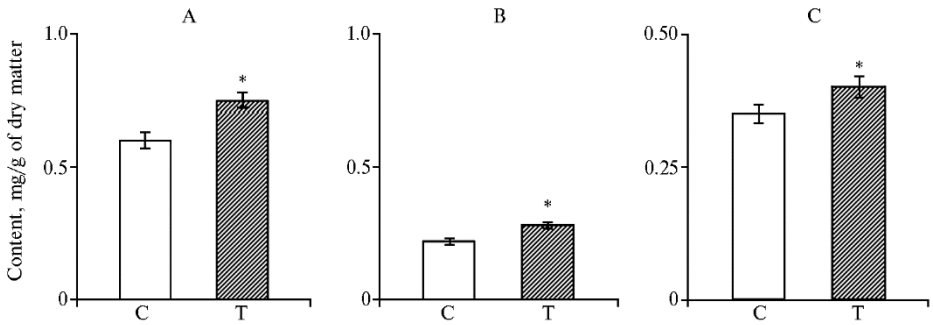

Fig. 2. Content of chlorophyll a (A)**, chlorophyll b** (B) **and carotenoids** (C) **in leaves of potato (*Solanum tuberosum* L.) cv. Skoroplodny plants in control** (C) **and upon spraying plants with 1.47×10^{-8} M 24-epibrassinolide** (T) **at blossoming** ($M\pm$SEM, $n = 5$, $N = 5$).
* Differences with control are statistically significant at $p \le 0.05$.

For the formation of economically valuable plant organs, it is important not only the prodution of assimilates in photosynthesis, but also their directed transport [24]. It is known that the distribution of photosynthesis products into attracting centers is determined by donor-acceptor interaction. We studied the distribution of ^{14}C-assimilates and sucrose which is the main transport form of assimilates in the middle and basal zones of the potato stem. Differences in the content of sucrose in the stem zones were found 48 h after the leaf exposure to $^{14}CO_2$ (Fig. 3). In the basal zone, the sucrose concentration was 24% less than in the middle zone ($p \le 0.05$). The value of the sucrose gradient between the middle and basal zones of the stem increased under the influence of 24-epibrassinolide to 44% ($p \le 0.05$).

A decrease in the content of sucrose in the basal zone could indicate an increase in its outflow. The distribution of ^{14}C-assimilates over stem zones turned out to be similar to the sucrose gradients in the control and in upon the variant with 24-epibrassinolide. That is, a basipetal gradient of sucrose and ^{14}C-assimilates was revealed in the stem.

The mechanisms of assimilate transport are widely discussed with special attention also paid to phytohormones. First of all, their role in attracting centers is considered {25}. Less attention is paid to the stem, although it is there that the redistribution of the outflow of assimilates to different organs occurs. There is no

information about the effect of brassinosteroids on the content of phytohormones in different zones of the stem. In our experiment, after the flowering, when there was an intensive growth of young potato tubers, abscisic acid accumulated in the basal zone of the stem, and to a greater extent under the influence of 24-epibrassinolide (Fig. 4).

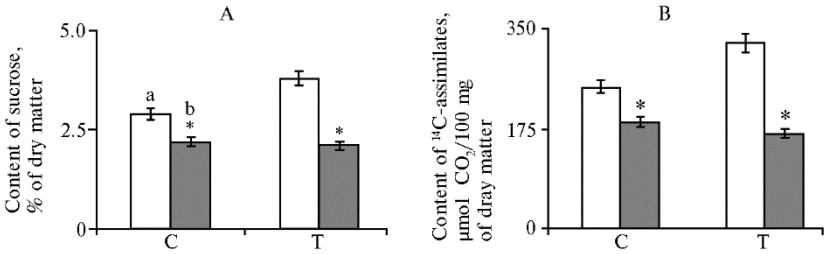

Fig. 3. Content of sucrose (A) **and ^{14}C-assimilates** (B) **in middle** (a) **and basal** (b) **stem zones in leaves of potato (*Solanum tuberosum* L.) cv. Skoroplodny plants in control** (C) **and upon spraying plants with 1.47×10^{-8} M 24-epibrassinolide** (T) **at blossoming** ($M\pm$SEM, $n = 5$, $N = 5$).
* Differences with control are statistically significant at $p \le 0.05$.

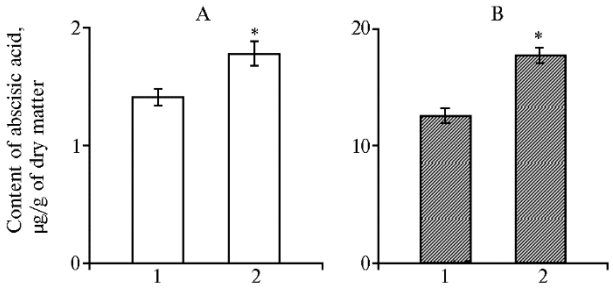

Fig. 4. Content of abscisic acid (AbA) in middle (1) **and basal** (2) **stem zones of potato (*Solanum tuberosum* L.) cv. Skoroplodny plants in control** (A) **and upon spraying plants with 1.47×10^{-8} M 24-epibrassinolide** (B) **at blossoming** ($M\pm$SEM, $n = 5$, $N = 5$).
* Differences with control are statistically significant at $p \le 0.05$.

Thus, in the control, the accumulation of endogenous AA in the basal zone was 26% higher than in the middle zone, and with 24-epibrassinolide, it was 41% higher ($p \le 0.05$). As a result, the AA gradient between the middle and basal zones was almost 2 times higher than that in the control.

According to T.H. Thomas [26], abscisic acid contributes to the unloading of phloem endings and, as a result, stimulates the accumulation of assimilates in the storage organ [27]. It should be noted that the formation of a positive sucrose gradient in the stem in the second half of the plant vegetation is facilitated by the accumulation of not only AA, but also auxins in the basal zone. Such data were obtained by us earlier [28]. According to the hypothesis put forward by E. Munch [29] and currently recognized, the osmotic pressure gradient is the driving force for the directed transport of assimilates.

Strengthening the outflow of assimilates into growing tubers under the influence of 24-epibrassinolide increased the productivity of potato plants grown in soil culture by 25% ($p \le 0.05$) (Fig. 5). An increase in the mass of tubers upon 24-epibrassinolide treatment may have been associated not only with an increase in the transport of assimilates, but also with the content of cytokinins in tubers, which are known to have an attracting effect. When plants were enriched with 24-brassinosteroid, a significant (more than 2-fold) increase in the content of zeatin cytokinin in tubers occurred (see Fig. 5).

For potato tubers, the formation of the secondary integumentary tissue of the periderm, primarily the phellema (cork) which regulates gas exchange and

protection against pathogens, is of great importance. Treatment of plants with 24-epibrassinolide increased the thickness of the phellema by 40% compared to the control ($p \leq 0.05$). Apparently, in this case, the influence was exerted by an increase in the content of cytokinons in tubers, which regulate cell division of phellogen, a secondary tissue that lays phellema layers outside the tuber.

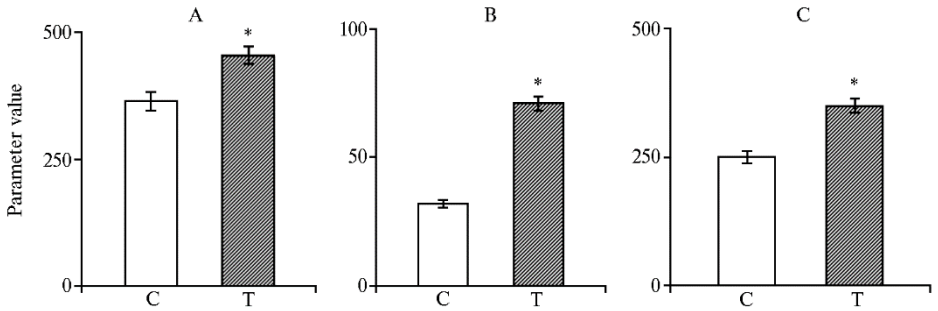

FIg. 5. Weight (g/plant, A)**, content of zeatin** (ng/g of dry weight, B) **and .phellema thickness** (μm, C) **in tubers of potato (*Solanum tuberosum* L.) cv. Skoroplodny in control** (C) **and upon spraying plants with 1.47×10^{-8} M 24-epibrassinolide** (T) **at blossoming** ($M\pm$SEM, $n = 5$, $N = 5$)**.**
* Differences with control are statistically significant at $p \leq 0.05$.

Thus, spraying potato plants of cv. Skoroplodny with a 1.47×10^{-8} M solution of 24-epibrassinolide at flowering stage of growth leads to an intensification of $^{14}CO_2$ absorption and an increase in the content of pigments in the leaves. At the end of flowering, with an increase in the content of abscisic acid (AA), the amount of ^{14}C-assimilates and sucrose decreased in the basal zone of the stem compared to the middle zone, which indicates the participation of AA in the unloading of phloem endings. The influx of assimilates into tubers under the influence of epibrassinolide was due to an increase in the content of cytokinins in the tubers. The data obtained allow us to conclude that 24-epibrassinolide regulates the intensity of the photosynthesis and outflow of assimilates into the developing tubers through the participation of AA in the formation of an assimilate gradient in stem zones and an increase in the content of cytokinins in tubers, which ultimately affects the productivity of potato plants.

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ANTIOXIDANT ACTIVITY AND BIOCHEMICAL COMPOSITION OF *Morus alba* AND *Morus nigra* SPECIES

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Abstract

Mulberry (*Morus* L.), a woody plant popular in Russia is of great economic benefits. On the territory of the Dagestan Republic, there are two *Morus* species, the *Morus alba* L. and *Morus nigra* L. The mulberry fruits, from leaf, bark and root extracts have powerful antioxidant, anti-inflammatory, antimicrobial properties and analgesic effects. In this paper, we revealed for the first time a high antioxidant status for two species, *M. alba* and *M. nigra* (cv. Hartut) growing in the Republic of Dagestan. Biochemical analysis revealed variability of the antioxidant level in the mulberry fruit pomace, leaves, bark, and roots. It was revealed that fruit pomace contains the required amount of mono-, disaccharides and bioactive compounds (e.g., water-soluble vitamins, organic acids) and has a rich mineral composition. The aim of the research was to assess the biological activity and biochemical composition of the fruit pomace, leaves, bark, and roots of mulberry plants grown in Dagestan. Plant material was sampled in the second decade of June 2020 (OOO Nizam, the suburbs of the city of Makhachkala, settlement Leninkent, Republic of Dagestan). The total content of antioxidants was measured amperometrically (a Tsvet Yauza 01-AA device, OAO NPO Khimavtomatika, Russia). The concentrations of water-soluble vitamins, organic acids and sugars were measured by capillary electrophoresis (a Kapel-105M system, OOO Lumex-marketing, Russia). Micro- and macro elements were quantified by atomic absorption spectrometry using ordinary acetylene-air flame atomization (a SavantAAΣ atomic absorption spectrometer, GBC Scientific Equipment Pty Ltd., Australia). The research results showed a high antioxidant status of fruit pomace, especially for white-fruited *M. alba* (400.73 mg/g) and *M. nigra* cv. Hartu (363.77 mg/g). A high level of antioxidants also occurred in leaves, bark, and roots. The antioxidant concentration in the leaves and roots of black-fruited form of *M. alba* was the highest, 44.56 mg/g and 71.79 mg/g, respectively. M. *nigra* plants have the highest amount of antioxidants in the bark, 36.33 mg/g. The quantitative determination revealed a higher content of vitamins C (ascorbic acid) and B_9 (folic acid) in mulberry fruit pomace, and the white-fruited form of *M. alba* (31.4 and 5.2 mg%) and *M. nigra* cv. Hartut (29.0 and 6.0 mg%) were most prominent. Qualitative and quantitative analysis revealed 10 chemical elements in plants, of which five (Na, K, Ca, Mg, Fe) were of leading importance. Potassium in a larger amount (342.6 mg%) occurred in *M. alba* (black-fruited form), a high concentration of Ca (50.6 mg%) was characteristic of *M. alba* (pink-fruited form). *M. nigra* stood out by the accumulation of Mg (54.6 mg%). *M. alba* (black-fruited) contained the largest amount of Na (16.5 mg%). *M. alba* (pink-fruited) was distinguished by the content of Fe (3.1 mg%). As to organic asides of *M. alba* and *M. nigra*, in the samples, we revealed only malic and citric acids. In all samples, the content of citric acid was 1.5-2 times higher than that of malic acid, except for the pink-fruited *M. alba*. Of mono- and disaccharides (fructose, glucose, sucrose), glucose dominated quantitatively in all samples, especially in black-fruited *M. alba* (10.40 %). Our findings indicate the high biological value of the *M. alba* and *M. nigra* species and their high potential for the development of therapeutic and prophylactic food products, bioactive additives, etc. Therefore, more phytochemical studies are necessary to search and reproduce the most valuable forms for practical use and improvement.

Keywords: antioxidants, *Morus alba*, *Morus nigra*, cv. Hartut, macro elemns, microelevents, vitamins, organic acids, sugars

Oxidative stress disrupts the body natural antioxidant system, increases risks for various dangerous diseases, and reduces life expectancy [1-4)]. It is known

that antioxidants from their natural sources (medicinal plants, fruits, vegetables, seeds, berries) and derived functional products can neutralize the excess of free radicals. Currently, plant materials containing antioxidants which have a mild effect on the body are increasingly used for preventive purposes [5-7].

Antioxidants slow down free radical reactions, protect cell membranes and DNA from destruction. In medicine, the use of plant materials is largely due to the content of bioflavonoids which have anti-inflammatory, antiviral, antimutagenic, antitumor and hepatoprotective effects [8-11]. Traditionally, for the design of a healthy diet, fruit and berry raw materials are used, which are characterized by high biological and pharmacological activity [12, 13].

In recent years, interest in the phytochemical characteristics and pharmacological value of the mulberry culture (*Morus* L.) has increased [14, 15]. It is known that the genus *Morus* consists of 10-16 species distributed in the temperate and subtropical zones of Asia, Africa, and North America. Mulberry is a woody plant of great economic importance. Fruits of the genus *Morus* have powerful antioxidant properties, extracts from the bark and roots have an analgesic effect [16, 17]. Extracts from the leaves and flowers of the white mulberry have antituberculosis activity and immunomodulating properties [18, 19]. The roots, bark and fruits of *Morus* plants contain a powerful antioxidant resveratrol which normalizes cellular metabolism and enhances oxygen transport, regulates fat metabolism in the liver, strengthens the walls of blood vessels, improves blood rheology, and also has anti-allergic, radioprotective, anti-inflammatory, antimicrobial properties [20-23]. Due to powerful biological potential, *Morus* plants are attractive as a source of raw material for functional nutrition products.

In the present work, we have for the first time established the high antioxidant status of fruit pomace in two *Morus* species, *M. alba* and *M. nigra* (cv. Hartut), growing in the Republic of Dagestan. The data obtained assess the degree of variability in the amount of antioxidants not only in fruit pomace, but also in other parts of the plant (leaves, bark, and roots). We have found that fruit pomace contains the required amount of mono-, disaccharides and bioactive substances (vitamins, organic acids), and also has a rich mineral composition.

Our goal was to study the antioxidant activity and biochemical composition of fruit pomace, leaves, bark, and roots in two species, *Morus alba* and *M. nigra* (cv. Hartut) under the conditions of the Republic of Dagestan.

Materials and methods. Mulberry pomace and parts of plants (leaves, bark, and roots) of *M. alba* (white-fruited form), *M. alba* (dark-fruited form), *M. alba* (pink-fruited form), and *M. nigra* (cv. Hartut) were used in the study.

Samples were collected in 2020 during mass fruit ripening in the second decade of June in a private nursery OOO Nizam located in the suburbs of Makhachkala (Leninkent village, Republic of Dagestan). The trees of the same age (27 years old) were standing separately. The site is irrigated, the soils are chestnut, loamy, 2-3% humus.

The total content of antioxidants in fruit pomace and plant parts was measured amperometrically (a Tsvet Yauza 01-AA device, OAO NPO Khimavtomatika, Russia), based on the electric current in the electrochemical cell, which occurred when a certain potential was applied to the electrode. For a calibration graph, in order to exclude random results, solutions of gallic acid trihydrate (purity > 98.5%) (Sigma-Aldrich, China) were prepared with a mass concentration of 0.2; 0.4; 2.0; 4.0 mg/l to be consecutively measured in five repetitions of which three estimates were used in statistical processing. Orthophosphoric acid (Komponent-reaktiv, Russia) with a molar fraction of 0.0022 mol/dm^3 was an eluent. Using calibration, the signals of the studied extract were compared to the signals of the reference sample, the gallic acid. The total concentration of antioxidants

was expressed in mg/g [24].

The concentratio of water-soluble vitamins (Methodology M 04-72-2011, https://www.lumex.ru/methodics/20ARU03.13.03-1.pdf), organic acids and sugars in fruit pomace was estimated based on the separation of the ionic forms of the analyzed components by the capillary electrophoresis (a Kapel-105M system, OOO Lyumex-marketing, Russia). The amount of water-soluble vitamins was determined at an electric field voltage of 25 kV and λ = 200 nm, organic acids at –20 kV and λ = 254 nm (Method M 04-47-2012, https://www.lumex.ru/methodics/20ARU03 .01.09-1.pdf), sugar content at 25 kV and λ = 254 nm (Methodology M 04-69-2011, https://www.lumex.ru/methodics/20ARU03.15.03-1.pdf).

The mineral composition was assayed by the atomic absorption method with atomization in an acetylene-air flame (a SavantAA Σ atomic absorption spectrometer, GBC Scientific Equipment Pty Ltd., Australia). The concentration of macro-, micro- and ultramicroelements was expressed in mg% (MU 01-19/47-11).

The obtained data were statistically processed using the Microsoft Excel package and the Statistika 5.5 program (StatSoft, Inc., USA). The mean values (*M*) and relative standard deviation (RSD) values were determined, which in our case did not exceed 0.1% (with an allowable 5%). Based on the RSD, the error of the arithmetic mean SEM was calculated (the sample error estimate of the mean is RSD/$\sqrt{n}$). The processing was performed by the one-way analysis of variance. Significance between sample means was assessed by Fisher's *F*-test. The probability of confirming the null hypothesis (p-level) was also calculated.

Results. In our experiment, we evaluated plants of the *M. alba* species of folk selection with different fruit colors. This is especially important because *M. nigra* plants are rare in Dagestan both in cultivation and as a wild form and are commonly confused with dark-fruited forms of *M. alba*. As a model for the *M. nigra*, we chose the ancient Iranian black mulberry cv. Hartut, the original area of which is the Derbent District of the Republic of Dagestan, where immigrants from Northern Iran live.

1. Total content of antioxidants (mg/g) **in fruit pomace and in parts of plans of genus *Morus*** (N = 3, M±SEM; settlement Leninkent, Republic of Dagestan, 2020)

Plant	Fruit pomace	Plant part		
		leaves	bark	roots
Morus alba (white-fruited form)	400,73±0,051	21,29±0,053	15,00±0,002	59,15±0,060
Morus alba (dark-fruited form)	266,00±0,135	44,56±0,012	16,00±0,052	71,79±0,014
Morus alba (pink-fruited form)	257,07±0,076	5,04±0,003	19,18±0,024	17,51±0,005
Morus nigra (cv. Hartut)	363,77±0,014	6,71±0,041	36,33±0,033	30,18±0,062

The antioxidant concentration in the fruit pomace of the studied samples was high and ranged from 257.07-400.73 mg/g, with the white-fruited form of *M. alba* being especially prominent (Table 1). It should be noted that in the work of researchers from Pakistan, the total antioxidant activity for *M. nigra* was 1.19-1.25 mmol trolox/g, for *M. alba* 0.75-0.78 mmol trolox/g [25]. According to the content of antioxidants in the leaves and roots, the dark-fruited form of *M. alba* was distinguished, 44.56 mg/g and 71.79 mg/g, respectively, which was statistically ($p \leq 0.01$) higher than in other samples. For antioxidants in the bark, *M. nigra* prevailed with the value of 36.33 mg/g. According to the literature, all parts of *Morus* plants are used in medicine, and extracts obtained from them have antioxidant, antiinflammatory, antibacterial, and antiviral properties [26, 27].

The one-way analysis of variance shows high reliability of differences between the samples in the total content of antioxidants (Table 2).

It should be noted that the accumulation of antioxidants was higher in the samples of *Morus* compared to spicy-aromatic and essential oil plants [28]. Thus, a route survey of wild-growing populations of cumin (*Carum carvi*) revealed that

its seeds contain from 0.76 to 1.47 mg/g of antioxidants. In another ecogeographic examinations, the total amount of antioxidants in coriander seeds was 1.2-3.7 mg/g, depending on the variety, and in dill seeds 6.5-12 mg [28, 29]. In addition, the total content of antioxidants in the samples we studied exceeded those in foods, juices, teas and coffees [30].

2. One-way analysis of variance of total content of antioxidants in fruit pomace and in parts of plans of genus *Morus* ($N = 3$, $M \pm$SEM; settlement Leninkent, Republic of Dagestan, 2020)

Plant part	SS	df_A	mS_A	SS_E	df_E	MS_E	F	p
Pomace	46104.26	3	15368.09	1.346067	8	0.168258	91336	0.000000
Leaves	557.05	3	185.68	0.020362	8	0.002545	72952	0.000000
Bark	896.07	3	298.69	0.000874	8	0.000109	2733050	0.000000
Tooys	5680.34	3	1893.45	0.491826	8	0.061478	30799	0.000000

N o t e. SS — sum of squares, df_A — degree of freedom, mS_A — mean square, SS_E — sum of squares of error, df_E — degree of freedom of error, MS_E — mean square error, F — Fisher's test, p — significance level for the null hypothesis of no difference between averages.

3. Accumulation of water-soluble vitamins in fruit pomace in plans of genus *Morus* ($N = 3$, $M \pm$SEM; settlement Leninkent, Republic of Dagestan, 2020)

Plant	Content of vitamin, mg%						
	B_1	B_2	B_6	C	B_3	PP	B_9
Morus alba (white-fruited form)	0.04±0.000	0.02±0.000	0.03±0.000	31.4±0.00	0.5±0.00	0.8±0.00	5.2±0.01
Morus alba (dark-fruited form)	0.03±0.001	0.04±0.000	0.07±0.002	27.8±0.00	0.4±0.00	0.7±0.00	4.7±0.01
Morus alba (pink-fruited form)	0.02±0.000	0.04±0.000	0.06±0.001	25.6±0.00	0.3±0.00	0.8±0.00	4.4±0.00
Morus nigra (cv. Hartut)	0.04±0.002	0.03±0.000	0.05±0.001	29.0±0.00	0.1±0.00	0.7±0.00	6.0±0.00

All *Morus* samples had a high content of vitamins C (ascorbic acid) and B_9 (folic acid) (Table 3). The highest accumulation of vitamins C and B_9 occurred in the white-fruited form of *M. alba* (31.4 and 5.2 mg%, respectively) and *M. nigra* (29.0 and 6.0 mg%). It is known that two phenolic groups in the structure of the ascorbic acid molecule allows it to participate in redox processes as a hydrogen donor and acceptor. Vitamin C reduces the amount of hydroxyl and peroxide radicals, restoring the active form of vitamin E and glutathione [31].

The total content of antioxidants in fruit pomace we obtained for plants of the genus *Morus* cannot be explained only by a high amount of ascorbic acid. Additional studies are needed on the accumulation of strong antioxidants in this crop capable of generating strong amperometric signal. In our opinion, these may include the hydrocarbon stilbene and its derivatives. Chemical compounds based on folic acid (folates, vitamin B_9) are involved in methylation of proteins, hormones, lipids, neurotransmitters, enzymes and other essential components of metabolism, nucleotide synthesis and DNA replication, cell division and normal growth of the body [32-3].

Qualitative and quantitative analysis of *Morus* raw materials revealed 10 chemical elements (Table 4) of which five were of leading importance (Na, K, Ca, Mg, and Fe). A comparative analysis showed that the content of chemical elements in *Morus* samples varied within different limits. Potassium, which regulates the state of the cytoplasm of plant cells and accelerates photosynthetic phosphorylation, was found in a greater amount (342.6 mg%) in the dark-fruited form of *M. alba*. A high concentration of Ca, which is part of the plant cell wall, was found in pink-fruited form of *M. alba*, 50.6 mg%. According to the accumulation of Mg, a cofactor of many enzymes, *M. nigra* stood out with 54.6 mg%. The amount of Na, which regulates the transport of carbohydrates in the plant, was higher in dark-fruited form of *M. alba*, 16.5 mg%. The content of Fe involved in the creation of chlorophyll and the process of plant respiration, was the highest in pink-fruited form of *M. alba*, 3.1 mg%. The sufficient amount of essential minerals

in *Morus* culture can be considered as a bioavailable complex that plays a physiological role in the functioning of many body systems [36, 37].

4. Mineral composition of fruit pomace in plans of genus *Morus* (N = 3, M±SEM; settlement Leninkent, Republic of Dagestan, 2020)

Element	Concentration, mg%			
	Morus alba (white-fruited form)	*Morus alba* (dark-fruited form)	*Morus alba* (pink-fruited form)	*Morus nigra* (cv. Hartut)
Macroelements				
Na	15.2±0.03	16.5±0.07	12.8±0.06	14.0±0.06
K	286.3±0.14	342.6±0.49	328.6±0.34	309.8±0.20
Ca	42.2±0.20	45.1±0.17	50.6±0.06	28.7±0.08
Mg	48.3±0.05	45.9±0.29	42.4±0.17	54.6±0.25
Microelements				
Fw	2.5±0.02	2.6±0.00	3.1±0.00	2.4±0.03
Cu	0.02±0.000	0.05±0.001	0.01±0.000	0.03±0.001
Zn	0.31±0.001	0.28±0.002	0.35±0.041	0.38±0.004
Cr	0.003±0.0000	0.003±0.0000	0.005±0.0000	0.004±0.0000
Mn	0.012±0.0001	0.024±0.0000	0.010±0.0000	0.03±0.001
Al	0.32±0.002	0.41±0.003	0.35±0.002	0.44±0.005
Ni	0.01±0.000	0.02±0.000	0.01±0.000	0.02±0.001
Iodides	0.001±0.0000	0.002±0.0000	0.001±0.0000	0.002±0.0000
Ultramicrorlrmrnts				
Pl	0.002±0.0000	0.002±0.0000	0.004±0.0000	0.003±0.0000

Among organic acids (Table 5), we detected only malic and citric acids. In all samples, the concentration of citric acid was 1.5-2 times higher than that of malic acid, except for the pink-fruited form of *M. alba*. The amount of free organic acids in *Morus* samples was several times higher than the requirements established by the State Pharmacopoeia of the Russian Federation (at least 2.6%). This is of importance for food biotechnology, since these acids provide optimal conditions for a full-fledged digestion and have the ability to suppress the development microorganisms due to the concentration of hydrogen ions.

5. Accumulation of organic acids and sugars in fruit pomace in genus *Morus* (N = 3, M±SEM; settlement Leninkent, Republic of Dagestan, 2020)

Plant	Organic acids, mg%		Sugars, %		
	malic acids	citric acids	fructose	glucose	sucrose
Morus alba (white-fruited form)	10,30±0,110	54,40±0,060	4,36±0,004	7,68±0,000	0,68±0,004
Morus alba (dark-fruited form)	20,40±0,052	57,90±0,050	7,66±0,010	10,40±0,005	0,71±0,006
Morus alba (pink-fruited form)	24,76±0,083	14,40±0,030	4,80±0,001	8,21±0,003	0,70±0,001
Morus nigra (cv. Hartut)	12,16±0,031	21,90±0,030	4,91±0,000	8,07±0,002	0,75±0,003

According to accumulation of mono- and disaccharides (fructose, glucose and sucrose) in fruit pomace, glucose dominated quantitatively in all samples, especially in the dark-fruited form of *M. alba*, 10.40% (see Table 5). It is known that the fruits of *M. nigra* are 82.9-86.2% water and 10.9-12.7% sugars. In dried fruits, carbohydrates accounted for approx. 73.3-83.7% [38]. In our study, the required levels of mono- and disaccharides additionally makes the culture attractive.

The results of one-way analysis of variance showed (Table 6) that the differences between the studied samples in terms of water-soluble vitamins, organic acids, and sugars were highly significant.

6. One-way analysis of variance of concentration of water-soluble vitamins, organic acids and sugars in fruit pomace in genus *Morus* (N = 3, M±SEM; settlement Leninkent, Republic of Dagestan, 2020)

Substances		F	p
Vitamins	B_1	315	0.000000
	B_2	297	0.000000
	B_6	948	0.000000
	C	150492	0.000000
	B_3	2576	0.000000
	PP	500	0.000000
	B_9	4831	0.000000

Continued Table 6

Organic acids	Apple acid	7309.50	0.000000
	Lemon acid	197474.10	0.000000
Sugars	Fructose	18954.63	0.000000
	Glucose	41603.08	0.000000
	Sucrose	40.17	0.000036
	Sum of sugars	52633.17	0.000000

N o t e. F — Fisher's test, p — significance level for the null hypothesis of no difference between averages.

It should be noted that the mulberry is a not used fruit resource. It requires additional research to develop a technology for its field harvesting and processing. It is necessary to search for the genetic resources of both species and create a collection of local assortment of cultivars to develop programs for breeding large-fruited varieties. It also requires an inventory of local methods of processing mulberry fruit products (drying, production of anhydrous syrups, juices and low-alcohol drinks, etc.) for the development of technical conditions and GOST standards.

Thus, we have established a high antioxidant status of fruit and berry raw materials of the *Morus* plants, in particular, the white-fruited form of *M. alba* (400.73 mg/g) and *M. nigra* cv. Hartut variety (363.77 mg/g), growing in the territory of the Republic of Dagestan. The high antioxidant activity of the leaves and roots of the dark-fruited form of *M. alba* and the bark of *M. nigra* have been also shown. The revealed value of fruit pomace in *M. alba* and *M. nigra* cv. Hartut is due to the accumulation of biologically active compounds (vitamins, organic acids), as well as a rich mineral composition. Our findings indicate that the *Morus* crops are valuable fruit and medicinal sources of interest for phytochemistry and the food industry.

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FIRST DETECTION OF FUNGUS *Fusarium coffeatum* IN THE TERRITORY OF THE RUSSIAN FEDERATION

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Abstract

According to the data of Rosstat, cereals and pulses took 17.2-20.2 % of agricultural production in Russia in 2014-2018, and their gross harvest in the same period was from 105.2 to 135.5 million tons. At the same time, the problem of contamination of grain and grain products by plant pathogens of different nature, including toxigenic Fusarium fungi, is still actual. Estimation of composition of species, affecting agricultural crops in different regions, is one on the key measures against *Fusarium*-induced infections spread. A complex investigation, which includes both traditional microbiological procedures and analysis of nucleotide sequences of marker genes followed by their comparison with reference ones from GenBank database, has been widely used for species-specific identification. This work is devoted to the description of the fungus of the genus *Fusarium* strain ION-3/4, isolated from wheat grain in Tula region of the Russian Federation (2014). DNA extraction and purification were performed by DNAeasy® Plant Pro Kit (Qiagen, Germany). Sanger sequencing of marker fragments of translation elongation factor 1 alpha (*TEF1α*, fragment size 587 b.p.) and RNA polymerase II subunit gene (*RPB2*, fragment size 689 b.p.) was carried out on an automated sequencer ABI PRISM 3730 (Applied Biosystems, USA). To analyze *TEF1α* and *RPB2* sequences, BLAST algorithm was used (https://blast.ncbi.nlm.nih.gov/Blast.cgi). Phylogenetic analysis and phylogenetic tree constructions were performed by MEGA-X software (https://www.megasoftware.net/) using maximum likelihood method and Kimura two-parameter model. Macromorphological characteristics of the isolate were studied on several culture media, micromorphology was studied using an Olympus CX33 microscope (Olympus Corporation, Japan). Complex of the results made it possible to identify the isolate — strain ION-3/4 as recently described *Fusarium coffeatum* species, belonging to the *F. incarnatum-equiseti* species complex. Phylogenetically the isolate (strain ION-3/4) formed a separate group with ex-type *F. coffeatum* strain 635.76. Key morphological characters (mycelium type, conidia shape and size, structure of mono- and polyphialides) also corresponded to the typical features of this species. As *F. coffeatum* is a member of *F. incarnatum-equiseti* species complex, which includes plant pathogens and mycotoxin producers, this species also can be considered as a potential cause of plant diseases and needs serious attention and further investigations.

Keywords: *Fusarium coffeatum*, *Fusarium incarnatum-equiseti* species complex, phylogenetic analysis, DNA-markers, morphology

Species identification of fungi of the genus *Fusarium*, especially in the case of closely related species, is often difficult due to the high similarity of key morphological structures. This can be confirmed by the fact that during the history of the study of the genus *Fusarium*, up to 10 taxonomic systems were proposed based on the analysis of morphological traits, in which the number of species ranged from 9 to 75 [1-4]. Molecular genetic methods based primarily on the sequencing of short marker DNA segments ("barcodes") [5, 6], phylogenetic analysis, and PCR identification are currently of great importance in resolving these problems.

Phylogenetic species recognition based on genealogical concordance (Genealogical Concordance Phylogenetic Species Recognition, GCPSR) are considered the most reliable method of molecular taxonomy [7-10]. This method is used to establish species boundaries and is based on multilocus phylogenetic analysis. The genes *TEF1*α (translation elongation factor 1 alpha gene) [11] and *RPB2* (RNA polymerase II subunit gene) [12] are mostly used as phylogenetically informative DNA markers for fungi of the genus *Fusarium*. Importantly, the molecular genetic methods cannot replace classical microbiological procedures, but should be a reasonable tool which significantly expands the arsenal of analytical capabilities of a researcher. Examples of a successful combination of microbiological and molecular approaches are works devoted to the detection of previously unidentified species *F. torulosum* [13] and *F. globosum* [14] in Russia.

Since the late 1990s, the use of multilocus analysis has allowed for deeper species differentiation, as a result of which many morphologically similar isolates (strains) of *Fusarium*, previously considered as representatives of the same species, were re-classified and assigned to a group or complex of species (species complex) [15]. One of the largest and most intensively studied is the *Fusarium incarnatum-equiseti* (FIESC) species complex [16] which currently includes more than 30 phylogenetic species of two clades, *F. equiseti* and *F. incarnatum* [17-20]. Researchers are interested in FIESC representatives due to their high genetic variability, ecological plasticity, and ability to synthesize a wide range of secondary metabolites, including mycotoxins [21].

In 2014, as part of the annual monitoring of the contamination of grain raw materials with mycotoxins and the study of their producers, carried out at the Federal Research Center for Nutrition and Biotechnology, fungus isolate was obtained from a wheat plant grown in the Tula region. The isolate was given the working name ION-3/4. A preliminary analysis of morphological features did not allow unambiguous determination of its species, which initiated subsequent studies using molecular genetic tools.

The purpose of this work is to determine the species status of the ION-3/4 isolate based on an integrated approach which includes the analysis of nucleotide sequences of marker genes and an extended study of cultural, macro- and micro-morphological properties.

Materials and methods. The *Fusarium* strain ION-3/4 was isolated during the grain mycological survey from the mycelium grown directly from the caryopsis. After successive passages, a monospore isolate was obtained. At present, the strain ION-3/4 is stored in the collection of microorganisms in the Laboratory of Biosafety and Nutrimicrobiome Analysis (Federal Research Center for Nutrition and Biosafety).

For molecular genetic studies and phylogenetic analysis, in addition to strain ION-3/4, we used strains of other morphologically similar species of the genus *Fusarium*: *F. equiseti* 64803 and *F. equiseti* 97001 (collection of the All-Russian Institute of Plant Protection, St. Petersburg—Pushkin), *F. incarnatum* F-2681 (collection of the Pushchino Scientific Center for Biological Research RAS) and *F. graminearum* F-892 (collection of the National Research Center Kurchatov Institute—GosNIIgenetika, Moscow), the species identity of which was confirmed in a previous study [22].

DNA from monospore cultures of fungi was isolated using a DNAeasy® Plant Pro Kit (Qiagen, Germany) according to the manufacturer's protocol. The DNA concentrations and purification from protein and low molecular weight impurities were determined (a NanoVue spectrophotometer, GE HealthCare, USA).

The design of universal primers for sequencing marker regions was carried out by aligning the nucleotide sequences of the *TEF1*α and *RPB2* genes of the

genus *Fusarium* fungi deposited in the database of the National Center for Biotechnology Information (GenBank, http://www.ncbi.nlm.nih.gov/GenBank). Algorithm ClustalW [23] was used for alignment. Calculation of the annealing temperature and evaluation of the physicochemical properties of oligonucleotides were performed using the Oligo.6.71 program (https://www.oligo.net/). As a result, of the following primers were designed: TEF30F — 5′-CGTCGTCATCGGCCACGT-3′, TEF650R — 5′-ACCAATGACRGTGACATAGTAGC-3′; RPB2F — 5'-ATGRTCMRCMGAGGYATGGAAGT-3', RPB2R — 5'- TTGTGATCGGGGAADGGA-3′.

PCR was performed in a Tertsik amplifier (DNA-technology, Russia). For a pair of TEF30F-650R, the following amplification program was used: 93 °C, 90 s (1 cycle); 93 °C, 20 s, 64°C, 5 s, 67 °C, 5 s (5 cycles); 93 °C, 1 s, 64 °C, 5 s, 67 °C, 5 s (40 cycles); for a pair of RPB2F-2R, the program was as follows: 93 °C, 90 s (1 cycle); 93 °C, 5 s, 60 °C, 10 s, 72 °C, 5 s (45 cycles). A set of reagents for PCR was used, including Taq polymerase UP (5 units/μl, OOO DNA Technology, Russia). The composition of the PCR buffer and the concentrations of the components of the reaction mixture have been described previously [24].

PCR products were cloned into the pAL2-T plasmid vector using the Quick-TA kit (ZAO Eurogen, Russia) according to the manufacturer's protocol. Sequencing was performed by a modified Sanger method using a fluorescent label (an ABI PRISM 3730 automatic sequencer, Applied Biosystems, USA; sequencing was performed at ZAO Eurogen). The BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to analyze the marker sequences of the genes *TEF1*α and RPB2 and to compare these sequences with the NCBI database (https://www.ncbi.nlm.nih.gov/genome/).

Phylogenetic analysis was performed using the MEGA-X program [25] using the maximum likelihood (ML) method and Kimura's two-parameter model [26]. The reliability of the topologies of phylogenetic trees was confirmed by bootstrap analysis with 1000 repetitions. The analysis included the sequences of the *TEF1*α and *RPB2* genes of fungi of the genus *Fusarium* deposited in the NCBI database: *F. equiseti* NRRL 264 919 (GQ505599, GQ505777), *F. scirpi* NRRL 36478 (GQ505654, GQ505832), *F. compactum* NRRL 36323 (GQ505648, GQ505826), *F. lacertarum* NRRL 20434 (GQ505593, GQ505771), *F. incarnatum* CBS 132.73 (MN170476, MN170409). The sequences of the type strain *F. coffeatum* CBS 635.76 (MN120736, MN120755) were also used.

The macro- and micromorphological characteristics of the ION-3/4 monospore isolate were studied on several types of nutrient media: potato sucrose agar (PSA) [1], potato dextrose agar (PDA) (Himedia Laboratories Pvt Ltd., India; Neogen Corporation, USA), Czapek-Dox agar (CDA) (Himedia Laboratories Pvt Ltd., India), oatmeal agar (OA) (Difco Laboratories, Fisher Scientific, USA), carnation-leaf agar (CLA) [1] and Nirenberg medium (SNA) [3]. Macromorphological features (mycelium structure and pigmentation) were assessed on PSA, PDA, OA and CDA media on day 7 of growth, in darkness and variable lighting (16 h day/8 h night) at 5 °C, pigmentation changes were observed up to 3 weeks The horizontal growth rate was studied in culture on PDA, CDA, OA, and SNA media in Petri dishes at 20, 25, and 30 °C. The diameter of colonies on days 3, 5, and 7 was measured in two transverse directions for three inoculations ($n = 3$). Mean values (M) were calculated, the standard deviation for all measurements was 5.3%, calculations were performed using Microsoft Office Excel software. Micromorphological characteristics (shape and size of conidia, types of phialides) were studied on CLA medium at 25 °C on days 7-14 using an Olympus CX33 microscope (Olympus Corporation, Japan). Conidia sizes are presented as mean and smallest

to largest size ranges (min-max) in longitudinal and transverse directions obtained from at least 10 measurements made using ToupView software http://toup-tek.com/product/ showproduct.php?lang=en&id=103.

The morphological features of strain ION-3/4 were compared with descriptions of fungi of the genus *Fusarium* in guides [2, 3] and in publications [20, 27].

Results. Nucleotide sequences of two marker genes showed 100% (*TEF1α*) and 99.45% (*RPB2*) similarity of the analyzed fragments with the corresponding sequences of the type strain *F. coffeatum* CBS 635.76. A study of the topology of the phylogenetic trees presented in Figure 1 showed that strains ION-3/4 and CBS 635.76 form a separate cluster with bootstrap support for the *TEF1α* and *RPB2* genes of 100% and 99%, respectively. Importantly, the *F. coffeatum* cluster belonged to the phylogenetic clade *F. incarnatum*, which is consistent with the data published earlier [20, 27].

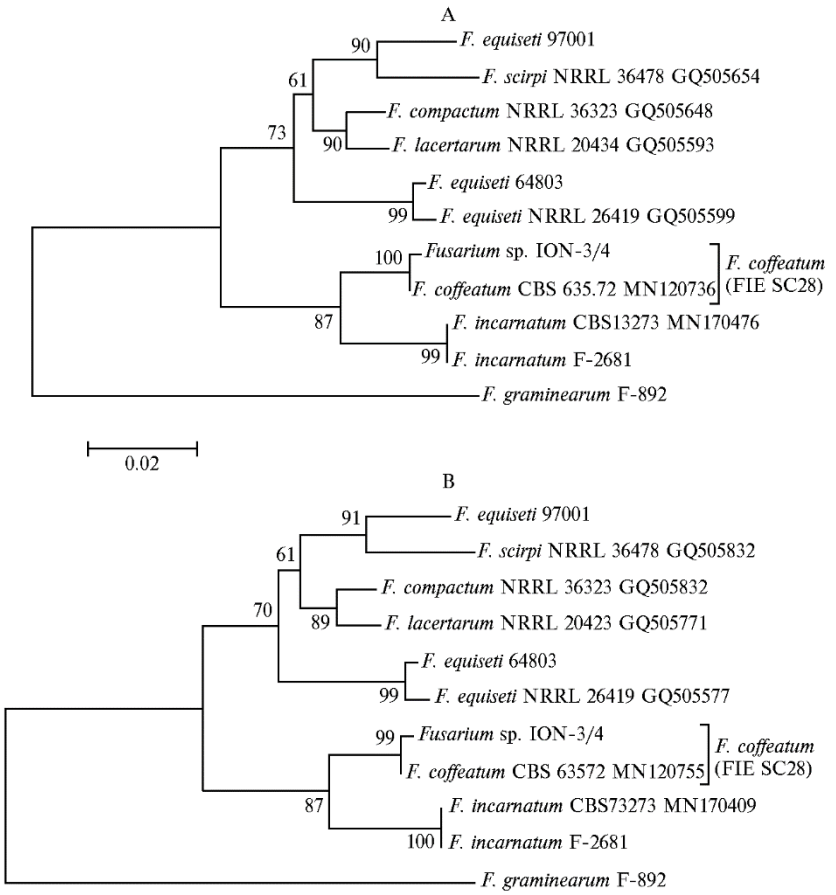

Fig. 1. Phylogenetic trees constructed based on the marker sequences of the *TEF1α* gene (A) and *RPB2* gene (B) of the ION-3/4 isolate and reference strains of seven species of fungi of the genus *Fusarium* using the maximum likelihood method (ML) and Kimura's two-parameter model [26] in program MEGA-X [25]. Only bootstrap values greater than 50% for 1000 replicates are shown. *F. graminearum* F-892 strain sequences are used as an "outgroup".

The macromorphological characteristics of strain ION-3/4 were studied in cultures on PSA, PDA, OA, and CDA nutrient media. On PSA and PDA, there was an abundant dense low aerial mycelium of milky white color with a slight creamy tint was noted, the reverse had a more saturated creamy peach color. As the culture aged (at more than 2 weeks of age), a more pronounced yellow color appeared, which then turned into light brown ("coffee with milk") (Fig. 2, A).

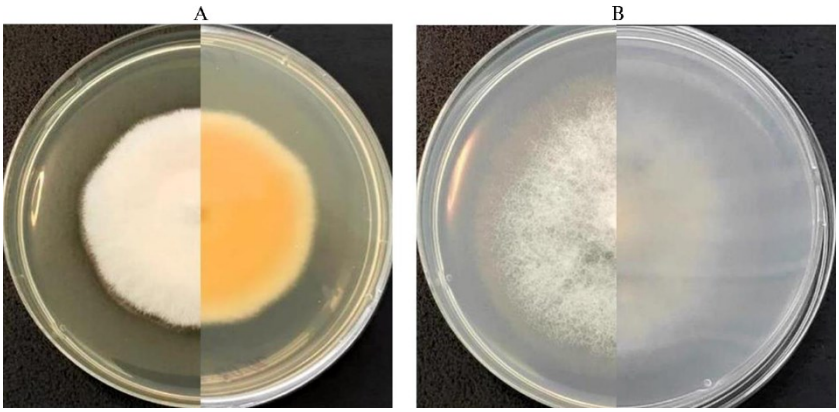

Fig. 2. ***Fusarium coffeatum*** **(strain ION-3/4), day 4 of culture, 25 °C. potato-sucrose agar** (PSA) (A) **and Czapek-Dox agar (**CDA) ***(B)*****.**

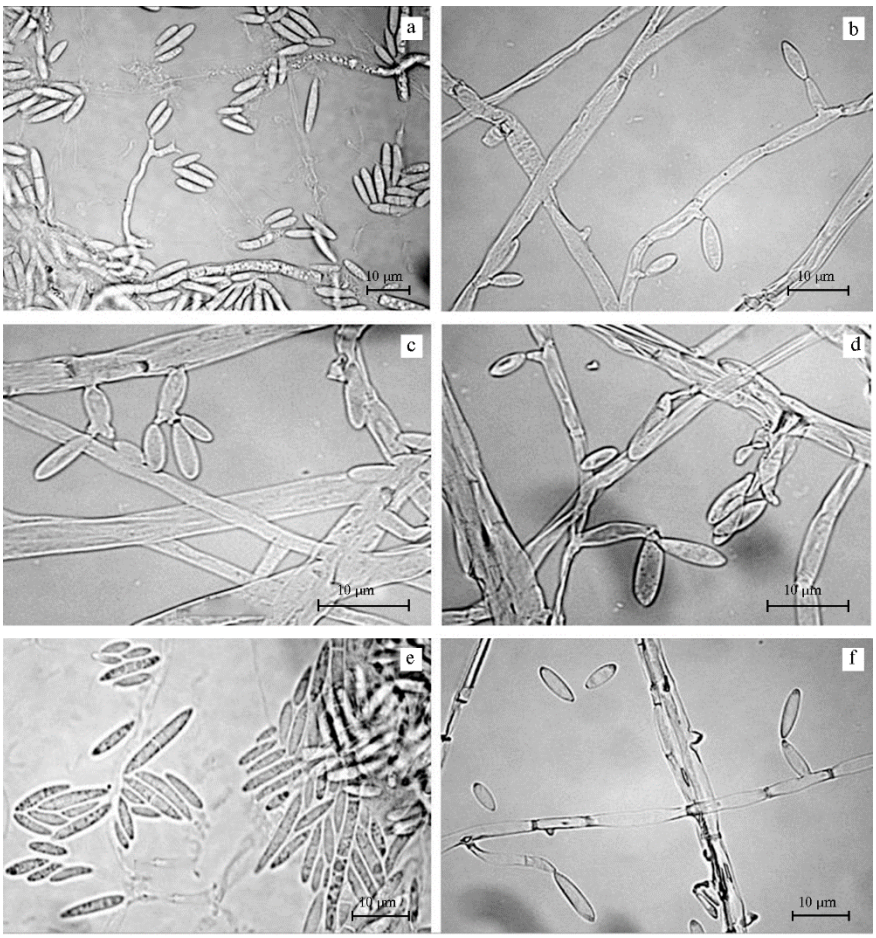

Fig. 3. ***Fusarium coffeatum*** **(strain ION-3/4) micro- and mesoconidia formed in aerial mycelium on mono- and polyphialides on carnation-leaf agar** (CLA)**: a, c — microconidia on polyphialides; b, f — microconidia on monophialides; d — microconidia on mono- and polyphialides; e — micro- and mesoconidia** (Olympus CX33 microscope, Olympus Corporation, Japan).

On potato extract-based nutrient media from different manufacturers, the color saturation of the reverse varied. A lighter reverse was observed during growth on PSA prepared under laboratory conditions from potato broth according to recipe [2], the most intense bright yellow-orange color of the colony was on PDA

medium manufactured by Himedia Laboratories Pvt Ltd. Growing under conditions of variable illumination (16 h day/8 h night) on PSA and PDA media led to the formation of a more saturated colors of the reverse, in contrast to conditions without light. The aerial mycelium always remained milky white regardless of the light conditions. When grown on OA medium, aerial mycelium was less dense, and on CDA it had a loose, flaky structure, less developed than on PDA. The color of aerial mycelium and reverse on OA and CDA remained milky white (see Fig. 2, B).

Micromorphological structures were evaluated on CLA at 25 °C on days 7-14. In aerial mycelium, the culture formed abundant micro- and mesoconidia (Fig. 3), macroconidia were rare. The sizes of conidia are shown in Table 1. The formation of sporodochia was not observed.

1. Size (μm) **of *Fusarium coffeatum* (strain ION-3/4) conidia on carnation-leaf agar** (CLA) (n = 10)

Type	Length (L), average (min-max)	Width (W), average (min-max)	L/W
Microconidia	6.7 (6.0-7.4)	1.6 (1.0-2.1)	4.2
Mesoconidia :			
without a septum	15.7 (8.6-16.0)	2.7 (2.3-3.1)	5.8
with one septum	15.2 (9.4-21.0)	2.4 (2.0-2.7)	6.3
Macroconidia (with two septa)	24.5 (24.0-25.0)	3.8 (3.5-4.0)	7.3

Macroconidia formed on CLA, mostly with two septa, almost straight, with a slight curve on the dorsal side, the terminal cells had a weakly expressed shape (papilla-shaped in the apical part, foot-shaped in the basal part).

In the aerial mycelium, there was an abundance of spindle-shaped mesoconidia without a septum or with one septum (see Fig. 3, e), as well as oval or obovate microconidia (see Fig. 3, a-d, f) formed in young cultures on monophialides (see Fig. 3, b, f), and as it grows, also on polyphialides (see Fig. 3, a, c, d). Polyphialids had two or more loci for the formation of conidia. The size of the phialides varied from 6 to 60 μm (see Fig. 3, a-d, f). Chlamydospores were not found.

When compared to the morphologically similar species *F. chlamydosporum* Wollenweber & Reinking [3], the *F. coffeatum* isolate (strain ION-3/4) shows similarities in the reduction or absence of formation of sporodochia and macroconidia in the form of conidiogenic cells (mono- and polyphialids), in the abundance and shape of microconidia, the formation of white aerial mycelium, however, ION-3/4 cultures lack the pink and burgundy color of the reverse.

In terms of the abundance of straight spindle-shaped mesoconidia in the aerial mycelium on mono- and polyphialides, the isolate of *F. coffeatum* (strain ION-3/4) is similar to *F. semitectum* Berkeley & Ravenel (syn. *F. incarnatum*) [3], the *F. coffeatum* (strain ION-3/4) and *F. semitectum* also show similarity in a reduced formation or the absence of chlamydospores, which, like the formation of sporodochia, is a strain-specific trait. However, unlike *F. semitectum*, the isolate *F. coffeatum* (strain ION-3/4) produces abundant microconidia in young cultures, while in *F. semitectum* in old cultures [3], and beige or brown color of the reverse appears only in the old culture.

The obtained characteristics for the studied isolate (strain ION-3/4) are consistent with the description of a typical strain of *F. coffeatum* (CBS 635.76) given earlier [27]. This concerns, first of all, micromorphological characteristics (shape and size of conidia formed in aerial mycelium, structure of mono- and polyphialids, and absence of sporodochia on CLA). The strain *F. coffeatum* (CBS 635.76) lost the ability to produce pigments (from beige to coffee-brown) on PDA and OA media, and there were no sporodochia on CLA, which, according to the

authors, is a consequence of its degeneration. In the isolate of *F. coffeatum* obtained by us (strain ION-3/4), as described above, the pigment is produced on PDA media, but the intensity of pigmentation varies on media from different manufacturers. There is currently no more detailed description of *F. coffeatum* in scientific publications.

The growth rate of the ION-3/4 strain culture was studied for 7 days when cultured in Petri dishes on four types of nutrient media at 20, 25, and 30 °C (Table 2).

2. Radial growth of *Fusarium coffeatum* (strain ION-3/4) colonies (mm) depending on various nutrient media at different temperatures (n = 3, M±5.3 %)

Days	PDA			CDA			OA			SNA		
	20 °C	25 °C	30°C	20 °C	20 °C	30 °C	20 °C	25 °C	30 °C	20 °C	25 °C	30 °C
3	22	35	23	30	40	35	18	32	23	23	30	17
5	50	65	52	60	77	72	45	72	60	46	53	40
7	75	86	75	80	86	86	67	86	70	64	80	60

N o t e. PDA — potato dextrose agar (Himedia Laboratories Pvt Ltd., India), CDA — Czapek-Dox agar (Himedia Laboratories Pvt Ltd., India), OA — oatmeal agar (Difco Laboratories, Fisher Scientific, USA), and Nirenberg medium (SNA) [3].

The highest growth rate occurred at 25 °C. On day 7, the colonies reached their maximum size (86 mm on PDA, CDA, OA, 80 mm on SNA). At 20 °C and 30 °C, the culture grew much more slowly on PDA, OA, and SNA, while on CDA, the colony size at 30 °C was the same as at 25 °C, and at 20 °C it was somewhat smaller. Thus, it was found that the temperature of 25 °C is the most favorable for the growth of the *F. coffeatum* isolate (strain ION-3/4) on all types of nutrient media used.

Thus, the main result of this study is the first detection of a *Fusarium coffeatum* fungus strain in Russia and a detailed description of its phenotypic characteristics. According to GenBank (NCBI), so far monospore strains of *F. coffeatum* have been isolarted in Australia (FIESC28_10703), South Africa (type strain CBS 635.76), and Romania (CBS 430.81). Based on the data obtained, it can be noted that this species is cosmopolitan and has a wide ecological plasticity, which allows it to exist in various climatic conditions. The discovery of *F. coffeatum* expands the understanding of the species diversity of fungi of the genus *Fusarium* in the Russian Federation and adds new knowledge about the area of this species. However, it is not yet clear whether *F. coffeatum* is capable of infecting crops and synthesizing mycotoxins. The study of these aspects may be the goal of future research.

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AN EFFECTIVE AFLATOXIN B_1 REDUCTION IN WHEAT GRAIN CONTAMINATED BY *Aspergillus flavus* VIA COMBINING THE BIOLOGICAL DEGRADATION OF THE TOXIN WITH INHIBITION OF ITS BIOSYNTHESIS

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Abstract

Decontamination of forage grain polluted with mycotoxins is one of the relevant problems of the forage safety provision. In recent years, the frequency of a severe contamination of forage grain and other fodder with aflatoxin B_1 (AFB1) in Russia significantly increased. The probability of the AFB1 contamination of the grass stand and forage grain produced in the central and northern regions of Russia may increase in the future due to the further expansion of *Aspergillus flavus* fungus, the main AFB1 producer, into these regions as a result of climate change. One of the promising approaches to decontaminate grain contaminated with AFB1 is the toxin catabolization by various microorganisms producing enzymes able to degrade AFB1. Another approach includes the treatment of grain contaminated with the AFB1 producers with compounds able to inhibit the aflatoxigenesis. In the present work, it was shown for the first time that the treatment of the cultural broth of *Rhodococcus erythropolus* AC-884 with the supernatant after the treatment with compactin almost completely prevents the accumulation of mycotoxin in the infected grain. The aim of the work is to evaluate the effectiveness of reducing the content of mycotoxin in wheat grain artificially contaminated with aflatoxin B_1 after treatment with actinobacteria of the genus *Rhodococcus* or an inhibitor of aflatoxygenesis, the compactin, as well as a combination of these methods. The present study reports the results of investigation of the AFB1 destruction capability in four *Rhodococcus* strains (*Rhodococcus* sp., AC-1260, *R. erythropolus* AC-1269 and AC-884, and *R. ruber* AC-1801). Quantitative analysis of AFB1 by high performance liquid chromatography revealed that the most active mycotoxin degradation occurred in the cell-free cultural broth supernatant of AC-884 (CBS-884). Only trace amounts of AFB1 added in CBS-17 to a final concentration of 0.2 μg/ml were detected in this supernatant after 48-h incubation at 30 °C, whereas cultural broth supernatants of other studied strains contained from 15 to 50 % of the added AFB1 after its incubation under the same conditions. A 72-h treatment of wheat (*Triticum aestivum* L., cv. Daria) grain artificially contaminated with AFB1 (1.0, 2.5, or 5.0 μg/g) with CBS-884 removed 60 % of the toxin, while the use of cultural broth supernatants or cell suspensions of AC-1260, AC-1269 or AC-1801 strains did not result in any changes in the AFB1 content comparing to the control. Using a consecutive treatment of grain infected with a toxigenic *Aspergillus flavus* strain by compactin, inhibiting the AFB1 production in this fungus, and then by CBS-884, we first demonstrated that the approach based on application of AFB1 biosynthesis inhibitors followed by the toxin biodegradation allowed an efficient decontamination of grain if the use of inhibitors alone did not result in a complete suppression of the aflatoxigenesis. Grain treatment of with the supernatant of AC-884 was more effective than the treatment with a similarly obtained supernatant of another AFB1-destroiyng agent, *Phoma glomerata* PG-41. In our experiments, the AFB1 content in wheat grain contaminated with *A. flavus* A11 reduced twice compared to the control in 7 days after compactin (0.05 mg/g) application. In 24 h after the treatment of the contaminated grain with CBS-884 alone (0.25 ml/g), the AFB1 amount produced by *A. flavus* for six post-inoculation days was reduced almost thrice. Combination of both treatments (compactin, 0.05 mg/g, and then CBS-884, 0.25 ml/g) resulted in a more than 200-fold reduction of the AFB1 content and the achievement of almost complete grain

decontamination. Therefore, an approach based on a combination of biodegradation with inhibition of aflatoxigenesis can provide effective decontamination of grain contaminated with AFB1 producers in cases where the use of inhibitors does not lead to complete suppression of mycotoxin biosynthesis.

Keywords: aflatoxin B_1, forage, grain, decontamination, *Aspergillus flavus*, compactin, *Rhodococcus erythropolus*

In recent years, samples of feed grain, silage and other feed contaminated with mycotoxins [1-3] collected in Russia have increasingly been found to contain aflatoxin B_1 (APB_1) [4, 5], including in amounts exceeding its maximum allowable concentrations [2, 6, 7]. Since global warming promotes the migration of heat-loving micromycetes to zones with a temperate and cold climate, we should expect an expansion of the range of *Aspergillus flavus* and other APB_1-producing aspergillus species in these regions and, accordingly, an increase in the likelihood of aflatoxin contamination of grass stands and fodder grains of cereals cultivated there.

Like other mycotoxins, AFB_1 enters the food chain and can end up in food, posing a serious threat to human health due to high hepatotoxicity and hepatocarcinogenic activity [8]. Entering the animal body with food, AFB_1 is metabolized with the formation of DNA-damaging exo-8,9-epoxide and aflatoxin M1 [9, 10], which accumulates in poultry eggs, cow milk and, like AFB_1 itself, has carcinogenic, teratogenic, mutagenic and immunosuppressive effects [11].

Due to the high risk of AFB_1, the development of effective methods for reducing the contamination of feed grains used in various livestock industries is one of the most important tasks in ensuring safety of agricultural feed. To solve it, researchers involve both physical, chemical, and biological methods of decontamination [12, 13]. Among the latter, a special place is occupied by biodegradation, an approach based on the destruction or detoxifying transformation of AFB_1 by degrading microorganisms [14, 15] which secrete metabolites with the corresponding enzymatic activity [16-18].

The ability to biodegrade aflatoxins has been found in many microorganisms, including actinobacteria of the genus *Rhodococcus* [19-22]. Nevertheless, the study of the destructive potential of this taxonomic group toward FB1 in order to identify the most effective strains is still relevant, since the ability to produce aflatoxin-degrading metabolites is not species-specific. Both *Rhodococcus* species and strains within the same species differ significantly in productivity [20, 22]. In addition, almost all studies reported only the ability of these actinobacteria to degrade AFB_1 when added to the nutrient medium, and only in very rare cases, the possibility of using bacteria to degrade AFB_1 extracted from contaminated grain or grain processing waste [23].

The content of AFB_1 in grain contaminated with its producers can also be reduced with synthetic or biological inhibitors of aflatoxygenesis [12] treatment with which will prevent the accumulation of the toxin in the infected substrate. The promise of this approach was demonstrated by us in previous studies which showed that compactin, one of the secondary metabolites of *Penicillium citrinum*, is able to suppress the formation of AFB_1 in *Aspergillus flavus* [24]. However, with an increase in temperature and humidity in granaries, conditions are often created that are favorable for the rapid and intensive development of aspergillus, which can significantly reduce the effectiveness of inhibitors due to insufficiently complete suppression of toxigenesis. To ensure the necessary decontamination, toxin residues in inhibitor-treated grain can be eliminated through biodegradation.

In the present work, it was shown for the first time that the treatment of the culture liquid of *Rhodococcus erythropolus* AC-884 with the supernatant following the treatment with compactin almost completely prevents the accumulation of mycotoxin in the infected grain.

The purpose of this work is to evaluate the effectiveness of reducing the content of mycotoxin in wheat grain artificially contaminated with aflatoxin B_1 after treatment with actinobacteria of the genus *Rhodococcus*, an inhibitor of aflatoxygenesis (compactin), or their combination.

Materials and methods. Strains of *Rhodococcus* sp. AC-1260, *R. ruber* AC1801, *R. erythropolus* AC-1269, and AC-884 (All-Russian Collection of Industrial Microorganisms, State Research Institute of Genetics and Breeding of Industrial Microorganisms, Kurchatov Institute, Moscow) were cultured on R1 medium containing (g/l) peptone 15.0, yeast extract 6.0, NaCl 1.0, glucose 1.0; pH 7.0, which is used in testing bioactivity of actinobacteria of the genus *Rhodococcus* [25]. Equal amounts of bacterial inoculum (10^9 cells/ml) were introduced into 50 ml flasks with 10 ml of nutrient medium and grown for 48 h (an Excella® E-25R orbital shaker, New Brunswick, USA) at temperature of 30 °C and 290 rpm. Flasks with nutrient media without actinobacteria were kept under the same conditions (control). The bacterial biomass (number of cells/ml) was determined at the end of growth.

Toxin-degrading activity of metabolites of *Phoma glomerata* strain PG-41 (the State Collection of Phytopathogenic Microorganisms of the All-Russian Research Institute of Phytopathology), the AFB_1 biodestructor previously discovered by us [26] was compared with the activity of *R. erythropolus* AC-884.

To assess the ability of the above collection strains of actinobacteria to produce metabolites degrading AFB_1, bacterial cultures were centrifuged for 30 min at 10,000 g (5702 R, Eppendorf AG, Germany). Prior to toxin addition, the resulting supernatants were pH adjusted to 7.0 to prevent non-enzymatic reversible lactone ring opening in AFB_1, which occurs in an alkaline environment at high pH values [27], This can lead to false positive results in high performance liquid chromatography (HPLC) analysis. The supernatants were filtered through membranes (0.22 μm, Millipore, USA). AFB_1 (Sigma-Aldrich, USA) was added to the supernatants free from bacterial cells, as well as to the supernatants of control media to a concentration of 0.2 μg/ml, the mixture was incubated at 30 °C with stirring on a shaker for 24 h at the conditions indicated above, and the content of the toxin in the mixture was measured.

In experiments on the degradation of AFB_1 upon artificial contamination, grain of soft spring wheat (*Triticum aestivum* L.) cv. Darra, placed in 250 ml shaking flasks (20 g per flask), were soaked in water (10 ml per 20 g of grain) and autoclaved for 1 h at 1 atm. After sterilization, 1 ml of AFB_1 stock solutions in 20% ethanol were added to the flasks to final concentrations of 1.0; 2.5 and 5.0 μg/g of grain and vigorously shaken for 10-15 min to evenly distribute the toxin. Then 1 ml of 20% ethanol was added to the control flasks. *Rhodococcus* cultures were grown and centrifuged as described above, the supernatants were separated, and the pellets were suspended in 20 ml of sterile water. Concentration-equalized cell suspensions of the tested bacterial strains were added to the flasks with grain at the rate of 0.05 ml of suspension per 1 g of grain or 0.25 ml of supernatants. The same volumes of sterile water were added to the control. When comparing the toxin-degrading activity of the cell suspension and the *R. erythropolus* AC-884 culture liquid supernatant, the grain contaminated with the maximum concentration of AFB_1 (5.0 μg/g) was treated with supernatant.

In experiments with decontamination of grains infected with toxigenic *A. flavus*, flasks with sterile grains were infected with strain A11 (107 conidia/ml, 1 ml suspension/20 g grain) and divided into four batches. Compactin was added to the first batch to a final concentration of 0.05 mg/g. In the second batch, the grain, 6 days after inoculation with a conidial suspension of the same concentration, was treated with the supernatant of the culture liquid (CL) of strain AC-884

(0.25 ml/g). In the third batch, the grain was first treated with compactin (0.05 mg/g), and in 6 days, the above amount of supernatant was added. Control flasks were supplemented with 20% ethanol, sterile water, or both ethanol and water.

The flasks with grain artificially contaminated with the toxin were kept in a TSO-1/80 thermostat (OAO Smolenskoye SKTB SPU, Russia) at 30 °C for 3 days after the addition of biodestructors, and the flasks with contaminated grain were kept at 27 °C for 7 days after inoculation. Control flasks were incubated for 3 or 7 days under the same conditions.

Spores of toxigenic *A. flavus* A11 for wheat grain inoculation were obtained as previously described [24]. Compactin was obtained from *P. citrinum* (strain 18-12) CL using the method described by S.N. Ukraintseva et al. [28].

Cultures of the fungus *P. glomerata* PG-41 secreting AFB_1-degrading metabolites were grown in a liquid nutrient medium as described previously [26], and supernatants were obtained from the CL filtrate by centrifuging and sterilizing it in the same way as in experiments with bacterial cultures. *P. glomerata* supernatants were used in experiments on the degradation of AFB_1 and decontamination of grain infected with *A. flavus* A11, similarly to the protocol described above for bacterial strains.

Residual amounts of APB_1 extracted from supernatants of CL and wheat grain by extraction with chloroform were mwasured by high performance liquid chromatography (Waters 1525 Breeze HPLC SYSTEM, Waters Corp., USA) [26, 28]. The limit of detection of AFV_1 was 0.005 μg/g of grain (maximum concentration limit in grain was 0.01-0.5 μg/g), the completeness of extraction from CL was at least 80%.

Experiments (at least 3 repetitions per option in each) were repeated three times. Statistical processing was performed using the STATISTICA 6.1 program (StatSoft, Inc., USA). Mean (M) and standard deviation (±SD) values were calculated. Significance of differences between the control and experimental variants at $p \leq 0.05$ was determined using a t-test for independent variables.

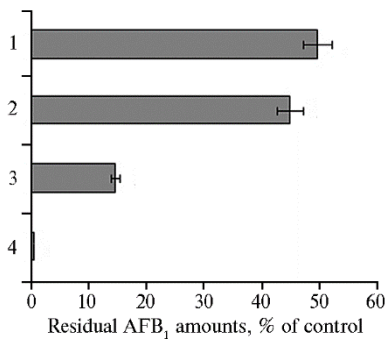

Fig. 1. The ability of various strains of *Rho*dococcus spp. to reduce the content of aflatoxin B_1 (AFB_1) added to the supernatant of their culture liquid. Average values for three experiments: 1 — *R. ruber* AC1801, 2 — *Rhodococcus* sp. AC-1260, 3 — *R. erythropolus* AC-1269, 4 — *R. erythropolus* AC-884 (n = 9, M±SD).

Results. All *Rhodococcus* strains actively grew on the R1 medium. Their biomass on day 2 reached 10^{10} bacterial cells/ml. By the end of the test period, pH values of CL did not differ among the strains and ranged from 8.8 to 9.0. These results indicate that the potential AFB_1 biodegraders in our experiments were cultured under conditions that provided a correct comparative assessment of their ability to produce metabolites with the target activity and could not prevent the realization of this ability in a particular strain.

Analysis of the residual amounts of AFB_1 after its incubation in CL supernatants showed that among the tested *Rhodococcus* cultures, the *R. erythropolus* AC-884 was the most active producer of toxin-degrading metabolites. After incubation of AFB_1 in the CL supernatant of this strain at neutral pH for 24 h, only trace amounts of the toxin were detected (Fig. 1). Under the same conditions, the CL supernatants of three other strains retained from 15 to 50% of the added AFB_1 (see Fig. 1).

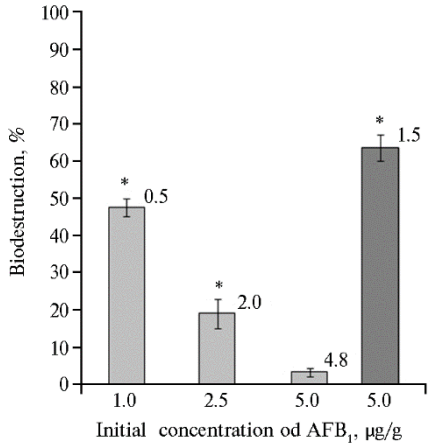

Fig. 2. Efficiency of degradation of aflatoxin B_1 (APB_1) added to grain of wheat (*Triticum aestivum* L.) cv. Daria, using a cell suspension (light gray columns) **or supernatant of the culture liquid** (dark gray column) **of *Rhodococcus erythropolus* AC-884, depending on the initial concentration of the toxin** (n = 9, M±SD). The numbers next to the columns show the residual amounts of AFB_1 in the grain (μg/g) 3 days after treatment. *Differences from control are statistically significant at $p \leq 0.05$ (marked with an asterisk).

In the analysis of grain artificially contaminated with commercial AFB_1, a statistically significant decrease in its content compared to the control was found (at $p \leq 0.05$)only if the treatment was carried out using strain AC-884. Three days after the introduction of the bacterial suspension, the amount of AFB_1 in the grain decreased by almost 2 times compared to the control (degradation efficiency 47.6%) if the final concentration of the toxin was 1.0 rg/g grain. Statistically significant differences with the control after treatment with the cell suspension ($p = 0.003$) remained at the AFB_1 concentration of 2.5 μg/g.

The treatment of grain contaminated with the maximum dose of the toxin (5.0 μg/g) with CL AC-884 supernatant led to the removal of 60 to 70% of AFV_1 within 3 days (Fig. 2). In addition, the degradation of AFB_1 in the CL supernatant of *R. erythropolus* AC-884 occurred faster than in the similarly obtained extracellular supernatant of the culture of the fungus *P. glomerata* PG-41, another AFB_1 biodegrader that actively removed the toxin from model incubation media [26]. However, unlike the supernatant, CL AC-884 proved to be ineffective when used on contaminated grain (Fig. 3).

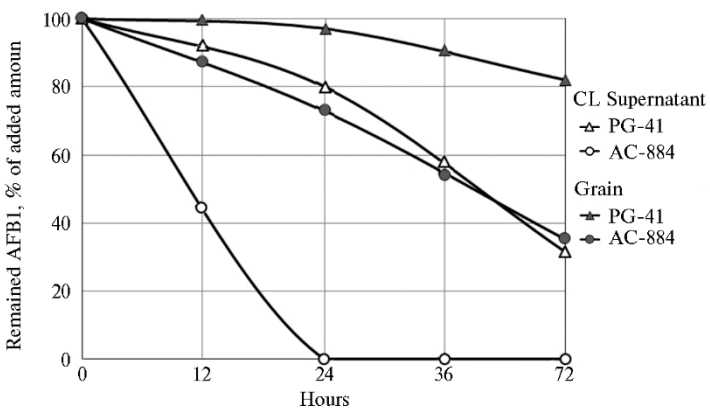

FIg. 3. Dynamics of degradation of aflatoxin B_1 (AFB_1) for 3 days in the supernatant of the culture liquid of *Rhodococcus erythropolus* AC-884 or *Phoma glomerata* PG41 and in treated wheat (*Triticum aestivum* L.) cv. Daria grain artificially contaminated with the toxin.

The high toxin-degrading activity of metabolites secreted by strain AC-884 in CL was confirmed by experiments with infection of wheat grains with AFB_1-producing *A. flavus* strain (Table). One day after treatment with the supernatant of grain samples on which the fungus developed for 6 days, the toxin content in the extracts obtained from these samples decreased by almost 3 times compared to the control, and the use of an inhibitor led to a 5-fold decrease in contamination.

Accumulation of aflatoxin B_1 (AFB_1) in *Aspergillus flavus* A11-infected grain of wheat (*Triticum aestivum* L.) cv. Daria after separate or combined treatment of grain with compactin and metabolites of *Rhodococcus erythropolus* AC-884 ($n = 9$, $M \pm SD$)

Treatment	AFB_1, μg/ml	Decontamination, %
Compactin, 0.05 mg/g grain	7.45±1.34	78.5
Culture liquid supernatant (CL) AC-884, 0.25 ml/g grain	12.10±1.27	65.1
Compactin, 0.05 mg + CL supernatant AC-884, 0.25 ml/g grain	0.13±0.09	99.6
Control (no processing)	34.66±3.07	

Note. Differences from control and between treatments are statistically significant at $p < 0.05$.

As a result of the successive application of compactin and supernatant CL AC-884, the concentration of AFB_1 in the extracts decreased by more than 200 times (see Table), that is, the combination of two treatments provided almost 100% grain decontamination. Since the supernatant freed from bacterial cells was used in these experiments, the decrease in the content of AFB_1 in the grain is due to the action of metabolites secreted by bacteria in the CL, and not to the often occurring adsorption of the toxin by biodestructor cells [29] that can be reversible [30] and led to the fact that this decontamination method does not always retain its effectiveness [31].

The results of our previous studies [26], the sensitivity of the detected AFB1-degrading activity to proteolysis, and the analysis of literature data suggest that the activity of AC-884 supernathans is of an enzymatic nature. Thus, enzymes that catalyze the degradation of fungal toxins, for example, ergot alkaloids (ErgA hydrolase, ErgB amidase) [32], or are involved in the catabolism of toxic polyaromatic compounds (for example, biphenyl dioxygenase and dehydrogenase), have been identified in a number of *R. erythropolus* strains. It has been established that the genes encoding these enzymes are grouped into clusters, and enzymatic degradation occurs through a cascade of reactions, including the cleavage of aromatic rings. Since APB_1 is also a polyaromatic compound, it is hypothesized that it may be targeted by similar enzymes and be degraded in a similar way [33].

The high decontaminating potential of AC-884 CL supernatant in our experiments is consistent with previously obtained data on the ability of extracellular supernatants of other *R. erythropolus* shams to degrade AFB_1, while significantly reducing its genotoxicity [33]. Apparently, the synthesis of AFB_1-degrading enzymes is a constitutive property of many *R. erythropolus* strains, although, unlike AC-884, not all of them are able to secrete such CL enzymes [22].

In our studies, the use of the AFB_1 biosynthesis inhibitor together with the subsequent removal of residual amounts of toxin through their biodegradation led to almost complete decontamination of contaminated grain. The possibility of practical implementation of this approach will be tested in further experiments by processing samples of stored grain collected in areas at risk of contamination by aflatoxin producers and contaminated silage. It is also very promising that some strains of *R. erythropolus* are able to degrade mycotoxins T-2 [20] and zearalenone [18], as well as to use ochratoxin A as a source of phenylalanine [34]. Since we have recently discovered that a compactin analogue can inhibit the formation of zearalenone [35], it seems reasonable to test the ability of AC-884 to degrade this fusariotoxin and other mycotoxins. If such an ability is discovered, the scope of the developed approach can be significantly expanded. The availability of sources of target metabolites, namely, the aflatoxin-degrading activity in the CL supernatant of *R. erythropolus* AC-884 and the ability of *P. citrinum* 18-12 to secrete compactin in vitro [26], is a factor that in the future may contribute to the development of a relatively simple technology for a two-component biological product

for decontamination of feed grains and other feeds.

Thus, as a result of the study of the toxin-degrading ability of four strains of *Rhodococcus* (*Rhodococcus* sp. AC-1260, *R. ruber* AC1801, *R. erythropolus* AC-1269 and *R. erythropolus* AC-884) and its comparison in experiments with grain treatment with culture liquid supernatant (CL) of *Phoma glomerata* PG-41, the most active biodegrader of aflatoxin B_1 (AFB_1), *R. erythropolus* AC-884, was selected. The efficiency of decontamination of grain artificially contaminated with a toxin preparation after treatment with AC-884 culture liquid supernatant could reach 70%, and after application on grain infected with toxigenic *Aspergillus flavus*, it averaged 65%. The treatment of such grain with compactin reduced the content of AFB_1 with an efficiency of almost 79%. Combined treatment, i.e., addition of supernatant CL AC-884 after compactin, actually completely prevented the accumulation of mycotoxin in wheat grain infected with *A. flavusinfected*.

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SPECIES COMPOSITION OF FUNGI OF THE GENUS *Fusarium* Link ON GARLIC PLANTS IN MOSCOW REGION

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Abstract

Garlic (*Allium sativum* L.) cultivation is restrained by the lack of seed propagation and high sensitivity to pathogenic fungi, bacteria, viruses and nematodes. Currently, *Fusarium* dry rot caused by soil fungi of the genus *Fusarium* is the most harmful disease of the crop. For the first time, in a multidisciplinary study, we have quantified the ratio of the main *Fusarium* dry rot pathogens affecting garlic in the Moscow region. Data shows the percentage of occurrence of key pathogens, high harmfulness and a dominant position of dry rot of the species *F. proliferatum* in the complex of pathogens. The work aimed to investigate the species composition of plant pathogenic fungi of the genus *Fusarium* on a garlic (*Allium sativum* L.) crop in the Moscow region. Diseased bulbs (n = 1108) of winter garlic cv. Gladiator were collected during 2019 to 2021 (All-Russian Research Institute of Vegetable Growing) and divided into sample sets based on the same symptoms of bulbs and cloves damage. Two hundred garlic cloves from each sample were surface sterilized by common methods and used to isolate pathogens. Scrapings from damaged clove tissues were placed on Czapek's medium in sterile Petri dishes and incubated for 12 days at 25 °C. The colonies were subcultured until monospore cultures were obtained. The isolates were identified using taxonomic keys. The frequency of occurrence of pathogens was calculated. Analysis of the marker sequences of the *TEF1α* gene (translation elongation factor 1 alpha) (~ 550 bp) and *MCM7* (gene encoding minichromosome maintenance protein 7) (~ 650 bp) was performed to confirm the taxonomic identification of the isolates. Molecular identification of the isolates was performed by quantitative PCR using species-specific primers for *F. proliferatum*. NCBI's BLAST algorithm (https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to analyse the nucleotide sequences of the *TEF1α* and *MCM7* genes. Seven genera of fungi, the *Fusarium*, *Penicillium*, *Botrytis*, *Embellisia*, *Aspergillus*, *Alternaria*, and *Sclerotium* were isolated from the garlic bulbs with varying degrees of occurrence. Among these pathogens, the genus *Fusarium* prevailed with a frequency of more than 44 %. Of six identified species, the *F. proliferatum*, *F. oxysporum*, *F. poae*, *F. verticilloides*, *F. culmorum* and *F. acuminatum*, the *F. proliferatum* predominated at a frequency of 61.4-75.6 %. Sequencing of DNA markers confirmed identification of the *F. proliferatum* isolates. The similarity to the sequences deposited in the NCBI database was 99-100 % for *TEF1α* gene (reference sequence numbers MN158137, KP267240, MN012923, KT224299) and 98-99 % for *MCM7* gene (reference sequence numbers XM031230017, XM0311M36726). qPCR analysis using specific primers also confirmed these results. Our findings add on the available data on the prevalence and dynamics of changes in the species composition of fungi of the genus *Fusarium* in the Moscow region. The data are also practically important for the development of more effective methods to prevent and control

Fusarium diseases of garlic.

Keywords: garlic, *Fusarium genus*, *Fusarium* dry rot, frequency of occurrence, DNA markers, polymerase chain reaction

Garlic (*Allium sativum* L.) is a vegetatively propagated plant which leads to the gradual accumulation of pathogens and their transmission to offspring. Despite the fact that garlic has bactericidal properties, it itself is susceptible to fungal, bacterial, nematode and viral diseases. The greatest harm is caused by fungi that accumulate in the tissues of planting material, which leads to a decrease in yield and degeneration of varieties [1-3].

Garlic diseases caused by representatives of the genus *Fusarium* Link which are called dry bottom rot are among the most harmful and common. In some years, the frequency of occurrence of *Fusarium* wilt on garlic reaches 10-80%, which causes yield losses from 17 to 60% [3-6]. Currently, the genus Fusarium includes more than 300 species [7-9]. Among the species characterized by the ability to infect garlic, *F. oxysporum* and *F. solani* are the most common [10, 11].

To date, the State Register of Breeding Achievements of the Russian Federation [12] does not contain garlic varieties that are resistant to fungi of the genus *Fusarium*. This is largely due to the high plasticity of the latter and the ability to remain viable for several years, form chlamydospores or sclerotia, and acquire resistance to chemical or biological fungicides [13-15].

The composition of pathogens varies depending on the host plant, agrotechnical and meteorological growing conditions. The weather conditions of the Moscow region are favorable both for the growth of garlic plants and for the development of fungi of the genus *Fusarium*, and therefore in some years there is a high infection of plants with *Fusarium* pathogens [16].

To identify fungi of the genus *Fusarium*, morphological and biological approaches together with DNA analysis and gene systematics are used. It is the combination of these methods that gives the most reliable results [7].

In this work, for the first time, a quantitative assessment of the ratio of the main pathogens of *Fusarium* dry rot affecting garlic in the Moscow region was carried out using a multidisciplinary approach. The percentage ratios of the occurrence of key pathogens are shown, high harmfulness and a dominant position in the complex of pathogens of dry rot of the species *F. proliferatum* are revealed.

The aim of the work was to study the species composition of phytopathogenic fungi of the genus Fusarium on a garlic culture in the Moscow region.

Materials and methods. The studies were carried out at the All-Russian Research Institute of Vegetable Growing, a branch of the Federal Scientific Center for Vegetable Growing (Ramensky District, Moscow Province), on winter garlic cv. Gladiator variety, included in the State Register of Breeding Achievements of the Russian Federation, approved for use since 2011 [12]. Plants were grown according to conventional methods [17]. In the period from 2019 to 2021, 1108 diseased bulbs were selected and divided into samples with the same symptoms of damage to the bulbs and cloves. To isolate pathogens, 200 cloves were taken from each sample and surface-sterilized [18].

After sterilization, scrapings were made from infected tissues, the material was plated on Czapek's medium in Petri dishes with and incubated at 25 °C for 12 days.

Pathogen colonies were serially subcultured until a monospore culture was obtained. The isolates were then classified using the taxonomic keys of the *Fusarium* laboratory manual [19]. The frequency of occurrence of pathogens was determined by the formula: $A = B/C \times 100\%$, where B is the number of samples in which *F. proliferatum* was found, C is the total number of samples analyzed.

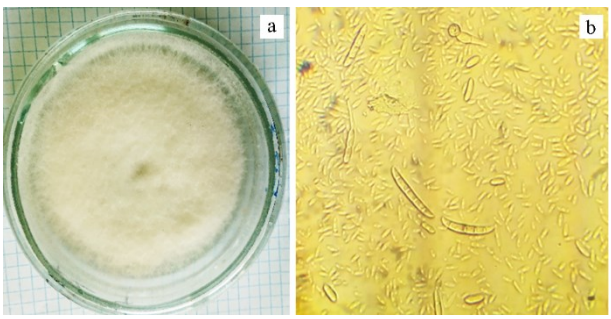
To confirm the taxonomic identification of the isolates, molecular genetic analysis of the marker sequences of the *TEF1α* gene (TEF30F: 5′-CGTCGTCATCGGCCACGT-3′, TEF650R: 5′-ACCAATGACRGTGACATAGTAGC-3′) and the *MCM7* gene (MCM7F: 5′-GCACCTGTCAGCTATGAGAAGC-3′, MCM7R: 5′-CAAGTTCCCTGCGTCCAC-3′) was performed, the primers were designed in this study.

DNA isolation and cloning of PCR products were performed according to the previously described procedure [20, 21]. For PCR amplification, a universal protocol was used: 90 s at 93 °C; 20 s at 93 °C, 5 s at 60 °C, 5 s at 67 °C (5 cycles); 1 s at 93 °C, 5 s at 64 °C, 5 s at 67 °C (40 cycles). DNA molecules were sequenced on an ABI PRISM 3730 automatic sequencer (Applied Biosystems, USA). To analyze the decoded nucleotide sequences of the *TEF1α* and *MCM7* genes, we used the BLAST algorithm on the NCBI website (https://blast.ncbi.nlm.nih.gov/Blast.cgi/).

DNA analysis of the isolates was also performed using quantitative PCR with *F. proliferatum* specific primers: FprolF — 5′-GTCCTCCCTCGAGACTGCC-3′, FprolR — 5′-GTTCTTCTTCGTGGAGTAGCCG-3′; fluorescent labeled probe 5′-(BHQ1)-ACGCAGACGT(FAMdT)CTTACAATCCCCCGAAA-3′. Amplification was carried out in a detecting cycler DT-96 (DNK-technology, Russia) in accordance with the following program: 90 s at 93 °C; 1 s at 93 °C, 5 s at 64 °C, 5 s at 67 °C (45 cycles).

Results. Our studies have shown that even if the necessary agrotechnical requirements are met, in some years up to 14.5% of garlic plants can be affected by fungal infections. The analysis of affected samples detected representatives of 7 genera of fungi (*Fusarium*, *Penicillium*, *Botrytis*, *Embellisia*, *Aspergillus*, *Alternaria*, *Sclerotium*) which occur with different frequencies. Among these pathogens, fungi of the genus *Fusarium* were the most common. During the growing season and storage, the frequency of their occurrence was more than 44%.

From garlic samples affected by *Fusarium*, 280 isolates belonging to 6 species were isolated: *F. proliferatum*, *F. oxysporum*, *F. poae*, *F. veticilloides*, *F. culmorum*, and *F. acuminatum*. The highest frequency of occurrence (from 61.4 to 75.6%) was characteristic of the species *F. proliferatu*m. The frequency of occurrence of *F. oxysporum* was lower and amounted to 15.1-32.3%. Fungi of the species *F. verticilloides* (9.3%), *F. poae* (4.4-7.7%), *F. culmorum* (1.9-5.1%) and F. *acuminatum* (2.6%) were less common.



A colony (a) **and spores** (b, Olympus CX33, Olympus Corporation, Japan) **of *Fusarium proliferatum* isolated from infected plants of winter garlic (*Allium sativum* L.) cv. Gladiator in the Moscow Province** (Chapek's medium, 12 days, 25 °C).

Microbiological analysis showed that the colonies of the fungus *F. proliferatum* on day 12 of growth on Czapek's medium had white mycelium, oval microconidia, 2.2-3.5×8.0-10.0 μm in size, slightly curved macroconidia with 3-5 septa, 3.3-4.1×30-46 μm in size (Fig.).

In molecular genetic analysis, genes of translation elongation factor 1 alpha (*TEF1α*) and minichromosome maintenance protein 7 (*MCM7*) were chosen as informative DNA markers to confirm the species of the studied strains. The

*TEF1*α gene is considered as the "gold standard" of molecular taxonomy of fungi of the genus *Fusarium*. The *MCM7* gene for the taxonomic classification of the genus *Fusarium* has not been practically used, but its taxonomic potential has been demonstrated using other fungi as an example [20].

Molecular genetic analysis of the marker sequences of the *TEF1*α and *MCM7* genes confirmed the identification of the studied strains as *F. proliferatum*. The correspondence with the sequences deposited in the NCBI database (https://www.ncbi.nlm.nih.gov/) was 99-100% for the *TEF1*α gene (reference sequence numbers MN158137, KP267240, MN012923, KT224299) and 98-99% for *MCM7* (XM03123 0017, XM031176796, XM31230017). Strain identification was also confirmed by qPCR with specific primers for *F. proliferatum* (Table).

Quantitative PCR analysis of *Fusarium proliferatum* isolated from infected winter garlic (*Allium sativum* L.) cv. Gladiator plants in the Moscow Province with a *F. proliferatum* specific test system

Изолят	Cq, Fam	Test result
Fo6	23.7	+
Fo5	25.4	+
Fo13	19.6	+
Fo10	16.5	+
Fo11	18.5	+
Fo2	17.8	+
Fo4	24.2	+
F09	17.8	+
Fo14	20.6	+
Fo1-1	19.8	+
Positive control	18.1	+
Negative control		–

N o t e. Cq — threshold cycle, "+" — positive control, "–" — negative control (water).

In previous studies, it was found that complexes of *F. oxysporum* and *F. solani* species are the most harmful and widespread on garlic in the Moscow region [3, 4]. Obviously, the dynamics of the species composition of fungi of the genus *Fusarium* can change depending on the natural agroecosystem and weather conditions during the year. However, the frequency of occurrence of the species *F. poae*, *F. culmorum*, *F. verticilloides*, and *F. acuminatum* on garlic culture in the Moscow region remains relatively stable.

Until 2021, there was no information about the damage of garlic by fungi of the species *F. proliferatum* in the Moscow region. However, it is known that abroad this pathogen is one of the most virulent, belongs to polyphages, capable of strongly infecting plants of different families [8], including garlic plants [22-25]. Garlic disease caused by *F. proliferaum* was first reported in 2002 in Germany [25], then in North America [23], Serbia [26], Italy [27], Spain [10, 28], India [29], Egypt [30], and France [31].

Thus, during the growing season in the conditions of the Moscow region, garlic plants can be affected by pathogenic fungi belonging to seven genera, *Fusarium*, *Penicillium*, *Botrytis*, *Embellisia*, *Aspergillus*, *Alternaria* and *Sclerotium* which occur with different frequencies. Among these pathogens, representatives of the genus *Fusarium* are the most common. The frequency of their occurrence was more than 44%. Within this genus, isolates belonging to the species *F. proliferatum*, *F. oxysporum*, *F. poae*, *F. verticilloides*, *F. culmorum*, and *F. acuminatum* were found. The highest frequency of occurrence (from 61.4 to 75.6%) was characteristic of the species *F. proliferatum*. Molecular genetic analysis of the marker sequences of the *TEF1*α and *MCM7* genes confirmed that the isolates belong to the species *F. proliferatum*. The similarity with the sequences deposited in the NCBI database was 99-100% for the *TEF1*α gene and 98-99% for the *MCM7* gene.

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THE STUDY OF *Agrobacterium radiobacter* 10 AND *Pseudomonas fluorescens* PG7 PHOSPHATE-MOBILIZING ABILITIES in vitro

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Abstract

There is a need to improve the phosphorus nutrition of agricultural plants due to the mobilization of phosphorus from hard-to-reach soil compounds and fertilizers by useful rhizosphere microorganisms (PGPB). For this purpose, phosphate-mobilizing bacteria are being selected to create biologicals with a fertilizing action. Here, our research data for the first time show that the strain *Agrobacterium radiobacter* 10 can metabolize phytate to utilize it as a source of carbon and energy in the absence of other sources, and the strain *Pseudomonas chlororaphis* PG7 can solubilize inorganic phosphates (tricalcium phosphate, hydroxyapatite) and organic phosphates (calcium phytate). The aim of the work is to investigate the potential of phosphate-mobilizing ability of two strains, *A. radiobacter* 10 and *P. chlororaphis* PG7. The stock cultures were propagated on pea agar (according to Khotyanovich). The phosphate mobilizing ability of the strains was assessed in vitro on selective nutrient media at 28 °C. Dephosphorylation of sodium phytate was examined in two liquid media. Medium II had the following composition (g/l distilled water): $(NH_4)_2SO_4$ — 1.0, K_2SO_4 — 0.2, Na phytate (Sigma-Aldrich, USA) — 10, corn extract — 0.2, pH 6.8. PSM (phytase screening medium) composition was as follows (g/l distilled water): D-glucose — 15.0, $(NH_4)_2SO_4$ — 5.0, KCl — 0.5, $MgSO_4 \cdot 7H_2O$ — 0.1, NaCl — 0.1, $CaCl_2 \cdot 2H_2O$ — 0.1; $FeSO_4 \cdot 7H_2O$ — 0.01, $MnSO_4 \cdot 7H_2O$ — 0.01; Na phytate (Sigma-Aldrich, USA) — 5, pH 6.5. The content of total phosphorus added to media with Na phytate was determined by the method of E. Truog and A.H. Meyer modified by J.B. Rodriguez et al. (1994) after ashing as per N.E. Ginsburg and G.M. Shcheglova (1960). The growth of strains in liquid media was estimated by the bacteria abundance (CFU/ml of suspension) during incubation. The ability of the strains to solubilize inorganic phosphates (tricalcium phosphate, hydroxyapatite) and organic phosphate (calcium phytate) was carried out on three solid nutrient media, the NBRIP, glucose-aspartic medium (according to G.S. Muromtsev) and PSM. NBRIP (National Botanical Research Institute's phosphate growth medium) composition was as followed (g/l of distilled water): D-glucose — 10, $Ca_3(PO_4)_2$ — 5.0, $MgCl_2 \cdot 6H_2O$ — 5.0, $MgSO_4 \cdot 7H_2O$ — 0.25, KCl — 2.0, $(NH_4)_2SO_4$ — 0.1, agar-agar — 20, pH 6.8. The glucose-aspartic medium with hydroxyapatite (according to G.S. Muromtsev) (43) contained (g/l of distilled water) D-glucose — 10, asparagine — 1, K_2SO_4 — 0.2, $MgSO_4 \cdot 7H_2O$ — 0.2, corn extract — 0.2, $Ca_5(PO_4)_3O_5$ — 4, agar-agar — 20, pH 6.8. The PSM composition is as hereinabove, added with agar-agar 20 g/l, pH 6.5 adjusted to by adding a 1 0% aqueous solution of $Ca(OH)_2$ to convert soluble sodium phytate into insoluble calcium phytate. The formation of halos around the colonies was recorded. The research revealed that *A. radiobacter* 10 cultured in the liquid medium uses phytate as a source of carbon and phosphorus for growth and enzymatically dephosphorylates phytate. This was evidenced by a significant increase in abundance of the bacteria during 4-day growth, a relatively small decrease in pH of the liquid broth compared to the control without inoculation, and the accumulation of immobilized phosphorus in the bacterial cell sediment and free orthophosphate in the liquid medium. *P. chlororaphis* PG7 could not mobilize phytate in the medium II. In particular, despite an increase in the *P. chlororaphis* PG7 aundance, there was no noticeable accumulation

of bacterial cell sediment and free orthophosphate in the liquid medium. It was shown that when cultured in the liquid PSM, both strains actively grew and multiplied, obviously using glucose as a source of carbon and energy. Under these conditions, a significant amount of immobilized phosphorus accumulated in the bacterial cell sediment, while the content of free orthophosphate in the medium remained at the control level. In addition, bacterial growth led to significant acidification of the medium, which contributed to the non-enzymatic hydrolysis of sodium phytate. Therefore, the research data could not drive to an unambiguous conclusion about the ability of strains to enzymatic hydrolysis of sodium phytate when cultured in the liquid PSM with two carbon sources. The halos around the colonies of *P. chlororaphis* PG7 on solid media indicated its ability to dissolve inorganic phosphates and phytin by solubilization. Unlike the *Pseudomonas* strain, the *A. radiobacter* 10 showed no solubilizing ability. This indicates its individual physiological features, since, as follows from special publication, many representatives of the genus *Rhizobium* are potential solubilizers. Thus, the ability of strains to solubilize mineral phosphates should be tested on solid nutrient media, where the formation of halos around colonies is a criterion for evaluating phosphate dephosphorization. The ability of strains to mobilize phosphorus from phytates should be assessed in liquid media in order to avoid false positive or false negative results. The main indicators of the enzymatic hydrolysis of phytates are the accumulation of immobilized phosphorus in the sediment of bacterial cells and free orthophosphate in the medium.

Keywords: phosphate-mobilizing ability, *Agrobacterium radiobacter*, *Pseudomonas fluorescens*, phytate, tricalcium phosphate, hydroxyapatite, selective nutrient media, immobilized phosphorus, orthophosphate

Phosphorus is one of the key elements in plant life. It is part of a number of organic compounds, in particular nucleic acids, nucleotides, nucleoproteins, vitamins, phospholipids, phytin, etc., which play a central role in metabolism. Phosphorus deficiency affects almost all life processes of plants. Phosphorus enters the root system and functions in the plant in the form of oxidized compounds, mainly orthophosphoric acid residues ($H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}) [1, 2]. Mycorrhizal fungi involved in the transport of phosphorus from the soil solution to the roots of the host plant also absorb phosphorus mainly in the form of the $H_2PO_4^-$ ion [3-5].

In soils, phosphorus occurs almost exclusively in the form of orthophosphates and is part of mineral and organic compounds. The gross content of phosphorus in the arable layer of soils is 0.03-0.2%, or 1-6 t/ha. Despite the significant reserves of phosphorus in soils, its availability for plants is largely hindered due to the low content of orthophosphoric acid ions in the soil solution, which is due to the intense retrogradation of phosphates (transition of easily digestible forms into hardly digestible ones) [3, 6]. In the mineral compounds of soils, phosphorus is mainly represented by inactive forms, such as primary minerals of soil-forming rocks, various compounds of secondary origin in the form of salts with alkaline and alkaline earth bases and sesquioxides.

The solubility of mineral phosphates depends on the reaction of the soil. Thus, calcium and magnesium phosphates become insoluble in an alkaline environment, and aluminum and iron phosphates become insoluble in an acidic one. The greatest amount of phosphorus compounds available for plants is present in soils with a slightly acidic and neutral reaction, the pH of which is in the range of 6.5-7.0 [6, 7].

It is known that soil microorganisms (bacteria and fungi) play a key role in the cyclic cycle of soil phosphorus and its availability for plant nutrition [8]. Rhizosphere bacteria involved in the release (solubilization) of phosphates from insoluble inorganic raw materials use various strategies to convert forms inaccessible to plants into available ones. Many bacteria release carbon dioxide and acidify the environment or, utilizing sugars, release low molecular weight organic acids (acetic, malic, gluconic, etc.) which have chelating properties and form organomineral complexes with cations associated with phosphorus, thereby converting it into soluble forms. Mobilization of phosphorus bound in the rhizosphere may result from activation of proton transport from root cells and acidification of

the medium in response to bacterial inoculation. In addition, bacterial siderophores that chelate iron and other metals with the formation of stable complexes can play an important role in increasing the availability of phosphorus [9]. Representatives of the genera *Arthrobacter*, *Bacillus*, *Burkholderia*, *Beijerinckia*, *Mesorhizobium*, *Flavobacterium* [10], *Rhizobium* [11, 12], *Pseudomonas* [13], *Enterobacter*, *Klebsiella*, *Stenotrophomonas* [14], *Streptomyces*, *Leifsonia* [15] and *Lisinobacillus* (16) associated with mycorrhizal fungi participate in the solubilization of inorganic phosphates in the soil.

Soil organic phosphorus makes up from 30 to 50% of its total content and is represented by two groups of compounds that are different in nature, i.e., products of biological synthesis and humus formation. The first group includes nucleoproteins, phytin, phospholipids, phosphoproteins and other organic compounds that are part of living organisms. The largest part (30-60%) among soil organic phosphates is occupied by phytates, the salts of phytic acid (D-myo-inositol-1,2,3,4,5,6-hexakisdihydrophosphoric acid) which is an ester of the cyclic hexahydric alcohol myo-inositol and six residues of orthophosphoric acid. The distribution of phytates depends on the acidity of the soil. Calcium and magnesium phytates are common in neutral soils while iron and aluminum phytates are found in acid soils [17]. Phytates are found in large quantities in plants, especially in seeds, where they represent the main form of phosphorus storage. Phytates enter the soil with plant residues and manure [18].

The most common isomer of inositol phosphate in soil, myo-inositol hexakisphosphate (InsP6), is a strong polyanion chelating agent. It can form insoluble complexes with vital divalent metal cations, as well as with proteins, carbohydrates, amino acids, turning them into insoluble conglomerates [19]. In acidic solutions, protonation (addition of protons to the molecule) of the phosphate groups of phytate promotes the formation of a free form of the molecule. In neutral and alkaline solutions, deprotonation of phosphate groups increases the affinity for divalent metal cations, which significantly reduces the solubility of phytate [20].

Hydrolysis of InsP6 is carried out by phytase enzymes. Phytases are a special group of phosphatases capable of stepwise phytate dephosphorylation with the formation of less phosphorylated derivatives of myo-inositol phosphate, inorganic phosphate, and free metal ions [21]. Based on the pH optimum, phytases are divided into two classes — acidic and alkaline. At present, the biochemical properties and mechanism of catalysis of individual enzymes, representatives of these classes, as well as their structural features, substrate specificity, and temperature dependence have been studied [19].

The main producers of phytase in the soil are microorganisms that make the phosphorus of organic compounds available for plant nutrition [22-24]. Weak phytase activity was also found in plant roots. However, the enzyme is not secreted into the rhizosphere, so plants cannot independently absorb the phosphorus bound in phytate [25]. Among soil microorganisms, the most active producers of extracellular phytase are micromycetes from the genera *Aspergillus*, *Penicillium*, and *Mucor* [26]. Phytases have been found in yeast [27] and bacteria of various taxa, including *Pseudomonas*, *Bacillus*, *Klebsiella*, *Enterobacter* [22]. Bacterial phytases are mainly intracellular enzymes, but bacteria of the genera *Bacillus* [28] and *Enterobacter* [29] are also able to produce extracellular phytases, and in *Escherichia coli* phytase is a periplasmic protein that most likely has access to phytate substrates in vivo (30). The ability to hydrolyze phytates was also found in some representatives of *Arthrobacter* [31], *Flavobacterium* [32], *Burkholderia* [33], and *Pantoea* [34].

According to the G.S. Muromtsev's concept [32], the microbiological mobilization of phosphorus from phytic acid salts proceeds in two phases. These are

the "non-specific" phase (dissolution of Ca, Mg, Fe and Al phytates) is carried out by various acid-forming microorganisms, the "specific" (enzymatic dephosphorylation of phytic acid) is carried out by specific microorganisms, among which there those using phytin or the product of phytin hydrolysis myo-inositol as a carbon source, e,g,, *Bacillus subtilis*, *Enterobacter aerogenes*, *Rhizobium leguminosarum* bv. *viciae*, *Sinorhizobium meliloti*, *Sinorhizobium fredii*, *Corynebacterium glutamicum* and *Lactobacillus casei* [32]. In general, the catabolic pathway of myo-inositol after cellular uptake has been studied in *Bacillus subtilis*. It includes multiple and stepwise reactions involving dehydrogenase, dehydratase and other enzymes. The end result of the myo-inositol catabolic pathway is an equimolar mixture of dihydroxyacetone phosphate, acetyl-CoA, and CO_2 [35].

The ability of rhizosphere bacteria to mobilize hard-to-reach soil phosphates has long been considered by scientists as an important mechanism for the positive effect on plant phosphorus nutrition [36, 37]. It is also known that most phosphate-mobilizing bacteria have a beneficial effect on the growth and development of the plant as a whole. This is due to an increase in the availability of other mineral elements (N, Fe, Zn, etc.), the release of vitamins and phytohormones, the production of antibiotics that inhibit the development of pathogens, the induction of mechanisms of systemic resistance to abiotic and biotic stresses [9, 38], and, finally, due to the formation of intracellular signaling molecules (second messengers), such as InsP3, a positive regulator of many signaling pathways [39] which is important for plant-microbial interaction and specific interaction between plants and nitrogen-fixing bacteria [40).

However, for the effective use of biological preparations, more complete knowledge of the physiological characteristics of the microorganisms included in their composition is needed. Among the most important characteristics of strains of rhizosphere bacteria selected for the development of biological preparations of fertilizer, stimulating or protective action is their ability to mobilize soil phosphates and fertilizers. In the absence of such information, the results of the action of biological preparations on the phosphorus nutrition of plants remain little predictable [7, 13]. Research in this direction will contribute to a better understanding of the potential interactions between PGPB (plant growth-promoting bacteria) and plants, as well as between PGPB and mycorrhizal fungi.

In the present work, the phosphate mobilizing ability of two bacterial strains, *A. radiobacter* 10 and *P. chlororaphis* PG7, of scientific and practical importance, was studied for the first time. The ability of *A. radiobacter* 10 to metabolize phytate, using it as a source of carbon and energy in the absence of other sources, and the ability of *P. chlororaphis* PH7 to solubilize inorganic (tricalcium phosphate, hydroxyapatite) and organic (calcium phytate) phosphates have been established.

Our goal was to study the potential ability of the Agrobacterium radiobacter 10 and Pseudomonas chlororaphis PG7 strains to mobilize phosphorus from mineral and organic compounds that are difficult for plant nutrition.

Materials and methods. The strains of *Agrobacterium radiobacte*r 10 and *Pseudomonas chlororaphis* PG7 stored in the collection of the All-Russian Institute of Agricultural Microbiology (ARRIAM) were used. It is known that the former is capable of fixing atmospheric nitrogen when grown on a nitrogen-free Vinogradsky medium, has a growth-stimulating effect for many types of agricultural plants, and serves as the basis for the commercial biopreparation Agrofil (EKOS BIOPRODUCTS, Russia) [41, 42]. The second, as a potential agent in the biocontrol of phytopathogenic microorganisms, is being tested in the geographical network [42]. The strains were identified based on the analysis of the 16S rRNA gene sequence at the Department of Genomic Technologies of the ARRIAM Center

for Collective Use.

To obtain an enrichment culture, the strains were propagated on bean agar (according to the prescription of A.V. Khotyanovich) with the following composition (g/l of pea broth): sucrose — 10; KH_2PO_4 — 0.5; $MgSO4 \cdot 7H2O$ — 0.3, chalk — 1, agar-agar — 20 (pH 6.8-7.0)

The study of the phosphate-mobilizing ability of the strains was carried out in vitro on selective nutrient media at 28 °C according to the methodological recommendations of G.S. Muromtsev [43], V.F. Pavlova et al. [43], B. Sasirekha et al. [44] and C.S. Nautiyal [45].

The ability of the strains to dephosphorylate sodium phytate was evaluated in two liquid media. Medium II [43] had the following composition (g/l distilled water): $(NH_4)_2SO_4$ — 1.0, K_2SO_4 — 0.2, Na phytate (Sigma-Aldrich, USA) — 10, corn extract — 0.2 (pH 6.8). PSM (phytase screening medium) [44] contained (g/l distilled water) D-glucose — 15.0, $(NH_4)_2SO_4$ — 5.0, KCl — 0.5, $MgSO_4 \cdot 7H_2O$ — 0.1, NaCl — 0.1, $CaCl_2 \cdot 2H_2O$ — 0.1; $FeSO_4 \cdot 7H_2O$ — 0.01, $MnSO_4 \cdot 7H_2O$ — 0.01, phytate-Na (Sigma-Aldrich, USA) — 5 (pH 6.5). For this purpose, 25 ml of media were poured under sterile conditions into 200 cm^3 flat-bottomed flasks; 100 ml of initial suspensions of bacteria were preliminarily prepared in 0.9% aqueous NaCl solution (washing from the surface of one Petri dish from bean agar). Then, 400 μl of initial suspensions of bacteria were added to flasks with media (bacteria were not added in the control) and cultured on a GFL 3015 orbital shaker (LAUDA-GFL, Germany) at 220 rpm for 4 days. The content of total phosphorus introduced into media with Na phytate was determined by the method of E. Truog and A.H. Meyer [46] with modification by J.B. Rodriguez et al. [47] after the preparation was ashed according to the method of N.E. Ginsburg and G.M. Shcheglova [48]. In terms of 1 ml of medium II, it amounted to 3.98 mg P_2O_5, in terms of 1 ml of PSM medium 1.99 mg P_2O_5.

The ability of strains to grow on liquid media was evaluated by the change in titers (CFU/ml) during the incubation period. The titers were determined by the serial dilutions of bacteria suspensions in saline followed by inoculation of 100 μl portions on Petri dishes with bean agar. The growth of colonies was assessed after 2 days of culture at 28 °C. The pH of suspensions was measured using a H1230B combined electrode on a portable pH meter HI 83141 (Hanna Instruments, Inc., USA) and optical density on a KFK-2 photoelectrocolorimeter (OAO Zagorsk Optical and Mechanical Plant, Russia) at λ = 590 nm. Bacterial cells (4 ml suspension) were sedimented by centrifugation in Eppendorf tubes for 5 min at 12000 g in a PE-6926 centrifuge (OOO EKROSKHIM, Russia). The resulting precipitates were washed twice from the culture medium with saline, after removing the supernatant, 1 ml of concentrated H_2SO_4 was poured into the test tubes, and the precipitates were charred for 2 days at 24 °C. The intensity of acid staining in brown color directly depended on the amount of sediment in the test tube. The accumulation of immobilized phosphorus (total phosphorus in the sediment of bacterial cells) was determined by the methods described above, recalculating it in mg P_2O_5 per 1 ml of suspension.

The accumulation of free orthophosphate in the medium during the incubation period was assessed by centrifuging bacterial suspensions for 5 min at 12000 g, then filtering the supernatant through a Whatman FP 30/0.2 CA-S membrane filter (Cytiva, UK). The phosphorus content was determined as per the method of E. Truog and A.H. Meyer [46] modified by J.B. Rodriguez et al. [47].

The ability of strains to dissolve inorganic and organic phosphates was studied on solid nutrient media of the following composition (g/l of distilled water): NBRIP (National Botanical Research Institute's phosphate growth medium) [45] — D-glucose 10, $Ca_3(PO_4)_2$ 5.0, $MgCl_2 \cdot 6H_2O$ 5.0, $MgSO_4 \cdot 7H_2O$ 0.25, KCl

2.0, $(NH_4)_2SO_4$ 0.1, agar-agar 20 (pH 6.8). Glucose-aspartic acid with hydroxyapatite medium (according to G.S. Muromtsev) [43] was as follows (g/l of distilled water): D-glucose 10, asparagine 1, K_2SO_4 0.2, $MgSO_4 \cdot 7H_2O$ 0.2, corn extract 0.2, $Ca_5(PO_4)_3O_5$ 4, agar-agar 20 (pH 6.8). The PSM medium composition is presented above [44], an additional 20 g/l of agar-agar was added and the pH was adjusted to 6.5 by 10% aqueous solution of $Ca(OH)_2$ to convert soluble sodium phytate to insoluble calcium phytate. The formation of zones of enlightenment of the nutrient medium (halo) around the colonies were recorded.

Statistical processing (calculation of mean values, standard deviations, and errors of sample means) was performed using Microsoft Excel 2010. To test the null hypothesis when compared sample means, we used an interval estimate of the distribution parameters, for which we calculated confidence intervals for the general means. Student's *t*-test were used for 5% significance level according to B.A. Dospekhov (the number of degrees of freedom is 3, Table 1 of the appendix) [49].

Results. Analysis of the phosphate mobilizing ability of when cultivated in liquid medium II showed that the *A. radiobacter* 10 strain can enzymatically mobilize phosphorus from sodium phytate. The content of free orthophosphate in the medium increased vs. control without inoculation, and the sediment of bacterial cells significantly accumulated immobilized phosphorus (Table).

The growth of bacteria utilizing phytate as a carbon source was evidenced by an increase in cell titers (from 3.59×10^6 to 9.71×10^8 CFU/ml), turbidity of the suspension, a decrease in the pH of the medium (see Table), and accumulation of bacterial cell biomass (Fig. 1, A, b). To verify the biological nature of phytate dephosphorization, an analysis was carried out for the content of free orthophosphate in a 1% aqueous solution of sodium phytate with a change in pH in the operating range from 6.83 to 6.11. The results did not reveal a significant effect of increasing the acidity of the medium on the hydrolysis of phytate. The obtained results are consistent with the literature data on the presence of extracellular phytase in some members of the genus *Agrobacterium* which can hydrolyze phytic acid with the formation of substrates containing less than six phosphoric acid residues, i.e., the inositol phosphates, inositol and inorganic phosphate [32]. Probably, the ability of the *A. radiobacter* 10 strain to enzymatically hydrolyze phytate under conditions when phytate serves as the only source of carbon in the environment is directly related to the ability to assimilate myo-inositol as a carbon and energy source using the mechanisms described for *Bacillus subtilis* [35].

Dephosphorylation capability of the *Pseudomonas chlororaphis* PG7 and *Agrobacterium radiobacter* 10 strains when cultured in liquid nutrient media with sodium phytate at 28 °C ($n = 4$, day 4)

Treatment	pH	OD_{590}	Free phosphorus concentration in the medium, mg P_2O_5/ml	Total phosphorus in bacterial sediment, mg P_2O_5/ml suspension
		Medium II		
P. chlororaphis PG7	6.47±0.15	0.10±0.01	1.34±0.16	0.0005±0.0002
A. Radiobacter 10	6.12±0.13	0.53±0.09	3.04±0.47	0.0440±0.0100
Without inoculation (control)	6.47±0.09	0.08±0.01	1.34±0.11	0.0001±0.0000[c]
		Medium PSM		
P. chlororaphis PG7	4.25±0.13	1.50±0.22	0.02±0.01	0.0720±0.0180
A. Radiobacter 10	4.52±0.13	1.50±0.18	0.04±0.02	0.0380±0.0070
Without inoculation (control)	6.13±0.12	0.01±0.00	0.04±0.01	0.0001±0.0000[к]

Note. For the composition of the media, see the "Materials and methods" section. The Table shows the confidence intervals for the general means at the 5% significance level; c indicates the control for reagents.

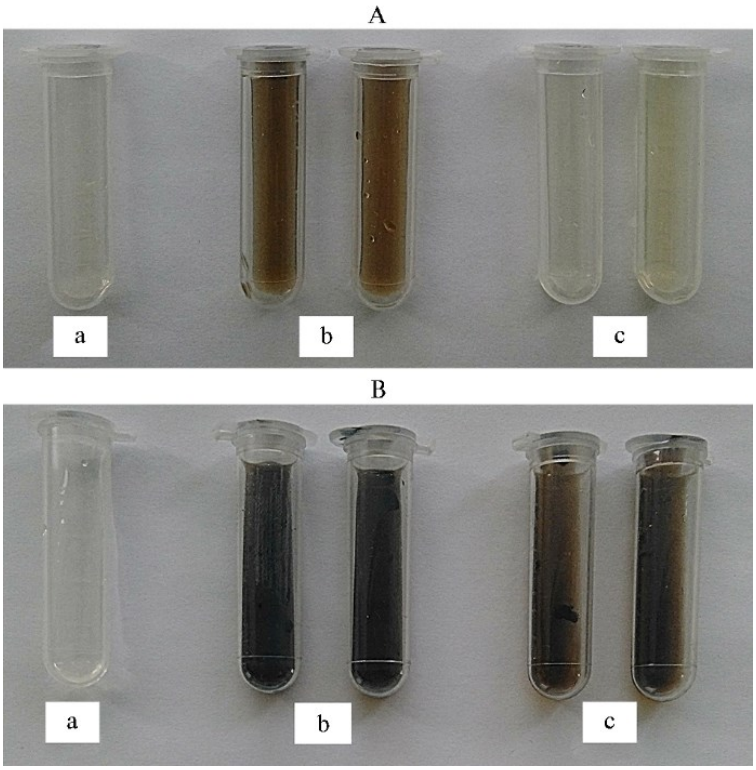

Fig. 1. Bacterial sediments charred with concentrated H_2SO_4 depending on growth medium: A — medium II, B — medium PSM; a — control (without inoculation), b — *Agrobacterium radiobacter* 10, c — *Pseudomonas chlororaphis* PG7. For the composition of the media, see the "Materials and methods" section.

In turn, the strain *P. chlororaphis* PG7 did not show the ability to enzymatic hydrolysis of sodium phytate when cultured in liquid medium II. Despite the increase in titers from 1.03×10^6 to 3.34×10^8 CFU/ml, there was no accumulation of free orthophosphate in the medium, no change in pH and turbidity of the medium, and the formation of a sediment of bacterial cells and the accumulation of immobilized phosphorus in it was insignificant (see Table, Fig. 1, A, c). It can be assumed that bacteria could not use phytate as a source of carbon and energy in the absence of other sources and activated other metabolic pathways.

In liquid PSM medium with two carbon sources, active growth of both strains occurred. Thus, during the incubation period, the titers of *A. radiobacter* 10 and *P. chlororaphis* PG7 increased from 3.59×10^6 to 1.33×10^9 and from 1.03×10^6 to 1.41×10^8 CFU/ml, respectively. The bacterial cultures became significantly turbid and the pH of the medium decreased vs. control; the biomass of cell sediments and the amount of immobilized phosphorus in the sediments increased, while the content of free orthophosphate in the solution remained comparable to the control (see Table, Fig. 1, B, b, c).

In experiments with the *Bacillus subtilis* 60015 strain which is able to metabolize inositol, it was shown that the presence of D-glucose and other easily metabolized carbohydrates in the medium suppresses the production of inositol-2-dehydrogenase (the first enzyme in the myo-inositol catabolic pathway) [30]. Based on these data, we preliminaryly conclude that the strains *A. radiobacter* 10 and *P. chlororaphis* PG7, when cultured in the liquid PSM medium, use glucose rather than phytate as a source of carbon and energy.

The experiments with the PSM liquid medium did not allow us unambiguously draw a conclusion about the ability of the strains to enzymatic hydrolysis

of sodium phytate, since the test of the effect of acidification of the medium on the dephosphorization of phytate in this case gave positive results. With a decrease in the pH of a 0.5% aqueous solution of sodium phytate from 6.50 to 4.19, a significant (by 16.7%) increase in the content of free orthophosphate in the solution occurred, from 0.048±0.001 to 0.056±0.006 mg P_2O_5/ml for 1 h at room temperature. The presence of sodium phytate and glucose in the medium, as well as aeration, can have a significant positive effect on the production of extracellular phytase in microorganisms, as evidenced by the data obtained for *P. aeruginos*a [50], *Mucor racemosus* NRRL (51), and *Bacillus subtilis* DR6 [52]. On this basis, we made a preliminary conclusion that the phosphorus nutrition of the strains in the liquid PSM medium was carried out at the expense of free orthophosphate, the reserves of which were replenished due to both nonenzymatic hydrolysis of phytate upon acidification of the medium and enzymatic hydrolysis of phytate.

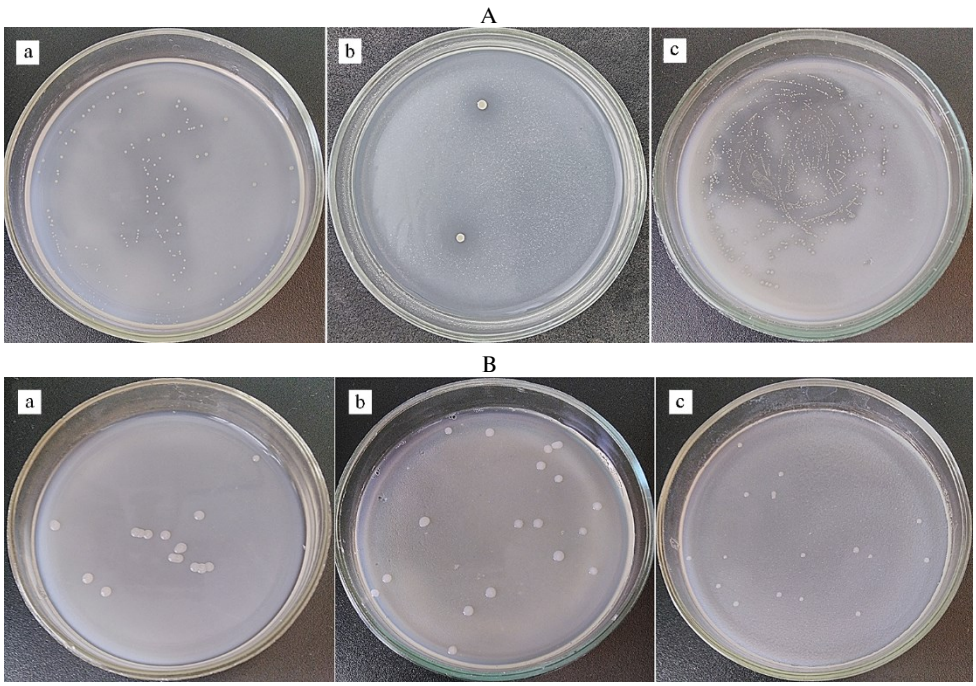

Fig. 2. Growth of the strains *Pseudomonas chlororaphis* PG7 (A) **and *Agrobacterium radiobacter* 10** (B) **on NBRIP medium with tricalcium phosphate** (a)**, glucose-aspartic medium with hydroxyapatite (as per G.S. Muromtsev)** (b) **and PSM medium with calcium phytate** (c)**.** For the composition of the media, see the "Materials and methods" section.

In experiments on solid media, *P. chlororaphis* PG7 is able to dissolve inorganic phosphates (tricalcium phosphate, hydroxyapatite) and organic phosphate (calcium phytate). This was evidenced by the formation of halo zones around the colonies when the strain was cultured on NBRIP, glucose-aspartic (according to G.S. Muromtsev), and PSM media (Fig. 2, A). The results obtained are consistent with data on the ability of some species of Gram-negative bacteria from the genus *Pseudomonas* to dissolve calcium phosphates according to the solubilization scheme [13]. Data on the solubilizing capacity of *P. chlororaphis* in the special literature are very scarce [53]. The data we obtained allow us to consider the strain *P. chlororaphis* PG7 not only as an agent in the biological defense of plants against pathogens, but also as a growth stimulator that improves phosphorus nutrition of plants.

The absence of halo zones around the colonies of the *A. radiobacter* 10 strain on the same solid media indicated that it did not have the ability to solubilize

mineral phosphates and calcium phytate (see Fig. 2, B). As follows from the literature sources, many representatives of the genus *Rhizobium* are such potential solubilizers. They secrete low molecular weight organic acids and dissolve inorganic phosphates [11], which is facilitated by the presence of glucose in the medium [12]. The absence of a halo on the PSM agar medium remains inexplicable, since, as noted earlier, the liquid PSM medium was strongly acidified during culturing the strain (see Table). It can be assumed that this is due to the lower metabolic activity of the *A. radiobacter* 10 strain on a solid medium compared to a liquid one.

Discussing phytase activity in the strains, given the physicochemical properties of phytic acid and its complexes with metals [20, 54], we can conclude that the clear zones around bacterial colonies on a solid medium cannot indicate calcium phytate dephosphorization by phytase, because acid-forming bacteria can dissolve calcium phytate by the solubilization scheme. It is seen in the example of *P. chlororaphis* PG7. False positive results obtained on solid media when testing acid-forming bacteria for the ability to enzymatic hydrolysis of calcium phytate are also known from the literature [55]. Thereof, the ability of bacteria to mobilize phosphorus from phytates should be assessed using liquid rather than solid media or using a technique that allows one to neutralize the halo zones formed as a result of acidification of the PSM medium and preserve those that appeared as a result of the enzymatic hydrolysis of calcium phytate [55].

Thus, the *Agrobacterium radiobacter* 10 strain can use sodium phytate as a source of carbon and phosphorus in the absence of other more available sources, while the *Pseudomonas chlororaphis* PG7 strain does not have this ability. *A. radiobacter* 10 enzymatically hydrolyzes phytate, as a result, free orthophosphate is released into the medium, and immobilized phosphorus accumulates in the sediment of bacterial cells. It was shown that when cultured in a liquid medium with two carbon sources, glucose and sodium phytate, both strains actively multiply using glucose as a carbon source. In this case, a strong acidification of the medium occurs which contributes to the non-enzymatic hydrolysis of phytate. This does not allow us to make an unambiguous conclusion about the ability of strains to enzymatic hydrolysis of phytate. It was shown that the strain *P. chlororaphis* PG7 can dissolve inorganic phosphates (tricalcium phosphate, hydroxyapatite) and organic phosphate (calcium phytate) according to the solubilization scheme when cultured on solid nutrient media, while *A. radiobacter* 10 does not have this property. It is advisable to analyze the ability of bacterial strains to dissolve mineral and organic phosphates according to the solubilization scheme on solid nutrient media with the identification of clear zones around the colonies, while the enzymatic hydrolysis of phytates must be evaluated in liquid media to avoid false positive or false negative results. The main indicator of the ability of strains to perform the enzymatic hydrolysis of phytates is the accumulation of immobilized phosphorus in the sediment of bacterial cells and free orthophosphate in the culture medium.

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CO-CULTURE OF *Pseudomonas chlororaphis* and *Saccharomyces cerevisiae* TO CREATE A COMPLEX BIOLOGICAL PRODUCT

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Abstract

Currently, there has been a trend in agriculture towards an increase in the use of biological preparations, including plant protection means. Developments are actively underway to create and optimize technologies for the production of new biological preparations that contain plant growth stimulating lines of bacteria (Plant Growth Promoting Bacteria, PGPB) or fungi (Plant Growth Promoting Fungi, PGPF). Fungi and bacteria co-inhabit the rhizosphere of higher plants and fungal-bacterial interactions permanently occur. In recent years, biological preparations based on bacteria and fungi (insecticide Biostop, fungicide Sporobacterin) have entered the agrochemical and pesticide market. However, special requirements of bacteria and fungi for nutrient media, aeration, and culture regimes imped production of combined bacterial-fungal biologicals. In this work, we have established for the first time the positive effect of *Saccharomyces cerevisiae* Y-4317 on the growth of *Pseudomonas chlororaphis* subsp. *auerofaciens* B-5326 and revealed the optimal regimes and the composition of the nutrient medium for co-culture of microorganisms of different taxonomic groups (PGPB and PGPF). The high efficiency of using sugar beet molasses for co-culture of *P. chlororaphis* and *S. cerevisiae* has been shown. Our findings revealed the stimulating effect of the liquid culture (LC) on the seed germination energy and germination rate of cereals. The aim of this work was to develop a protocol for co-culture of bacteria *Pseudomonas chlororaphis* subsp. *auerofaciens* B-5326 and yeast *Saccharomyces cerevisiae* Y-4317 to create a biological preparation stimulating seed germination and initial growth of cereal plants. Lyophilized strains *P. chlororaphis* and *S. cerevisiae* were obtained from the All-Russian Collection of Industrial Microorganisms of the National Research Center Kurchatov Institute — GosNIIgenetica (Moscow). Seeds of maize (*Zea mays* L.) hybrid Delitop, wheat (*Triticum aestivum* L.) variety Mironovskaya 808, barley (*Hordeum vulgare* L.) variety Scepter served for testing bioactivity of the biological. After restoring viability, the strains were cultured for 48 hours on a shaker SPH-2102 (BIORUS, Belarus) using three nutrient media differing in the carbon source (glucose, fructose, and sugar beet molasses). Microbial growth (colony-forming units, CFU) and biomass were assessed during co-culture (from 0 to 72 h) on the shaker and in a lab fermenter BIORUS GJ (BIORUS, Belarus) at different temperatures (from 20 to 32 °C) and airflow rates (from 1 to 6 L/h). The seeds were treated by spraying with 1:200-1:25 serial dilutions of the liquid co-culture. After 12 h, the seeds were placed into Petri dishes with water. The germination energy and seed germination rate were determined in 3 and 7 days, respectively. The research data showed that media containing sugar beet molasses completely satisfies the need of co-cultured *P. chlororaphis* and *S. cerevisiae* for basic nutrients, and the titers did not fall below 6×10^8 and 3×10^6 CFU/ml, respectively. The total biomass was 20.4 g/l, or 17-22 % higher than on the media with glucose or fructose. For co-culture, the optimal conditions were 30°C, aeration mode 4 l/h and 24 h of growth. A positive effect of *S. cerevisiae* on the viability of *P. chlororaphis* during 72 h co-culture was demonstrated. The abundance of *P. chlororaphis* in the co-culture with *S. cerevisiae* was 1×10^6 CFU/ml vs. 5×10^4 CFU/ml in pure culture of *P. chlororaphis*. Probably, a higher viability of *P. chlororaphis* and stimulation of its growth is due to phytohormones produced by *S. cerevisiae* during co-culture. Preliminary testing revealed stimulating effect of the biological on the germination energy and seed germination rate for barley, wheat and corn. All serial dilutions of the liquid co-culture exhibited a clear trend towards an increase in seed germination in the cereals tested. The maize seed germination was most stimulated. A 1:200 dilution of the biological led to the maximum increase in seed germination of the crops. Our research data identify the

key parameters of the co-culture of PGPB *P. chlororaphis* and PGPF *S. cerevisiae* and thereby create the prerequisites for the development of a biological based on microorganisms of vadifferent taxonomic groups.

Keywords: *Pseudomonas chlororaphis*, *Saccharomyces cerevisiae*, biological preparation, biomass, culture procedure, maize, wheat, barley, seed germination

In recent years, there has been a trend in global agriculture towards an increase in the use of biological products, including plant protection products (1). Technologies for the production of new biological products are being actively optimized. To ensure the high efficiency of biological preparations, plant growth promoting bacteria (PGPB) and fungi (plant growth promoting fungi, PGPF) strains stimulating plant growth are used [2-4]. Microbiological preparations are living cells of microorganisms selected for useful properties and either in the culture liquid or adsorbed on a neutral carrier.

PGPBs of the genus *Pseudomonas* synthesize metabolites that have a growth-stimulating and antifungal effect on plants [5-9]. When using these biological products, an increase in the biological and economic yield of a number of crops has been shown [7, 8]. Of interest are preparations created on the basis of joint cultures of two or more PGPBs, in particular, a microbiological product based on *Pseudomonas aureofaciens* and *Azotobacter vinelandii* [10-13]. These types of bacteria are intensively used as growth regulators of cultivated plants, and also stimulate their resistance to pathogens [14]. However, under the conditions of an unstable climate and the systematic negative impact of external factors, the problem of reducing yields because of stressful impacts remains relevant. In this regard, it is fundamentally important to look for new approaches to create biological preparations that also have a stress-modulating effect.

Thus, attention is drawn to the possibility of using representatives of another taxonomic group of microorganisms, fungi, in the creation of biological preparations. Few studies have shown that certain yeast strains produce phytohormones [15]. It is known that biologically active substances with hormonal activity can not only regulate the growth and development of plants, but also participate in protection against abiotic factors [16-18]. The fact that the ability to synthesize phytohormones was found in yeast allows us to consider them as a potential component of biological preparations for plant growing practice.

Fungi and bacteria co-exist in the rhizosphere, there is constant interaction between them. Microorganisms allocated to the PGPF and PGPB groups can affect the growth and development of plants [19-23]. However, in modern literature, we have not come across information on their joint action on plants. There is evidence that representatives of the genus *Pseudomonas* improved the growth of the basidiomycete *Agaricus bisporus*, but the mechanism of this interaction is not known [24].

In recent years, biological preparations containing bacteria and fungi (Biostop insecticide, Sporobacterin fungicide) have entered the market of agrochemicals and pesticides [25]. The main difficulties in the production of such preparations are associated with the different demands of the components on the composition of the nutrient medium, aeration, and cultivation modes. In bacteria, the optimal growth temperature is lower and the duration of cultivation is shorter compared to fungi. In addition, fungi require free amino nitrogen for biosynthesis [26, 27]. The study of the conditions of co-cultivation of PGPB and PGPF is necessary for the development of fundamental principles for the subsequent creation of complex biological preparations that affect agricultural crops.

In this work, we have established for the first time the positive effect of

Saccharomyces cerevisiae Y-4317 on the growth of *Pseudomonas chlororaphis* subsp. *auerofaciens* and revealed the optimal cultivation regimes and the composition of the nutrient medium for the joint growth of microorganisms of various taxonomic groups (PGPB and PGPF). The high efficiency of using beet molasses for the joint cultivation of *P. chlororaphis* and *S. cerevisiae* has been shown. The stimulating effect of the obtained cultural liquid (CL) on the germination and germination energy of seeds of cultivated cereals was revealed.

The aim of this work is to study the possibility of co-cultivation of the bacterium Pseudomonas chlororaphis subsp. auerofaciens B-5326 and yeast *Saccharomyces cerevisiae* Y-4317 and optimize their cultivation parameters to obtain a culture fluid that stimulates seed germination and initial growth of cereal plants.

Materials and methods. Lyophilisated strains of the bacterium *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326 and yeast *Saccharomyces cerevisia*e Y-4317 were obtained in ampoules from the All-Russian Collection of Industrial Microorganisms of the Research Center Kurchatov Institute — GosNIIgenetika (Moscow). The strains were not genetically modified.

Seeds of corn (*Zea mays* L.) hybrid Delitop, wheat (*Triticum aestivum* L.) variety Mironovskaya 808, barley (*Hordeum vulgare* L.) variety Skipert were used.

To restore the viability of the culture, an ampoule with a dried strain of microorganisms was sterilely opened and suspended in a liquid nutrient medium (0.2-0.4 ml) was added, DMEM/F12 Merck (Sigma-Aldrich, USA) for *P. chlororaphis*, Malt extract (Pronadisa, Spain) for *S. cerevisiae*. The contents were sterilely transferred with a Pasteur pipette into Petri dishes on agar nutrient media of the same composition. Next, the strains were grown separately for 48 h in a thermostat TS-1/20 SPU (Smolenskoye SKTB SPU, Russia) at 28 °C.

When preparing inoculums, a single colony with a characteristic morphology of the strain was selected from each dish, transferred to 250 ml flasks, and cultivated on an SPH-2102 shaker-incubator (BIORUS, Belarus) at 28 °C and 130 rpm. The medium for growing *P. chlororaphis* contained 10 g/l of glucose and 0.5 g/l of $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, KH_2PO_4. The medium for growing *S. cerevisiae* is a synthetic modified Reader's medium: 10 g/l glucose, 0.5 g/l peptone, 1 g/l yeast extract, 0.5 g/l $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, KH_2NO_4 each. After 48 h, the titers of *P. chlororaphis* and *S. cerevisiae* were 10^7 and 10^5 CFU/ml, respectively.

For co-culture of two strains of microorganisms, 15 ml of the obtained starter culture was taken, transferred to 500 ml flasks, and grown for 48 h at 28 °C and 130 rpm on an SPH-2102 shaker-incubator. The composition of the medium for the co-culture of microorganisms was as follows: 0.5 g/l peptone, 1 g/l yeast extract, 0.5 g/l $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, KH_2PO_4, carbon source varied.

To determine the most acceptable composition, three variants of nutrient media were used, differing in the source of carbon: 1 — glucose (10 g/l), 2 — fructose (10 g/l), 3 — beet molasses (15 g/l). Variants with variation of some other components of nutrient media were considered (no significant differences were found with the main recipe). Subsequently, the influence of temperature (from 20 to 32 °C) on the co-culture of *P. chlororaphis* and *S. cerevisiae* on a shaker-incubator was determined, and the number of viable microorganisms (by the number of colony-forming units, CFU) in dynamics (from 0 to 48 h and from 48 to 72 h) was also estimated.

Co-culture of *P. chlororaphis* and *S. cerevisiae* was also carried out in a BIORUS GJ laboratory fermenter (BIORUS, Republic of Belarus), in which the

air supply rate through the filters was varied (from 1 to 6 l/h). Cultivation lasted 20 h at 30 °C. The medium was similar to that used in the previous experiment, with molasses as the main carbon source.

The amount of biomass obtained was estimated by drying the filters to constant weight in a UF75 oven (Memmert GmbH + Co. KG, Germany) for 1-2 h at 105 °C, separating microorganism cells from the culture liquid (CL volume 10 ml, with 3-fold washing with distilled water), and determining the dry weight on an Analytical Balance ME54T/A00 analytical balance (Mettler Toledo, USA) to a constant value (±0.1 mg) [28].

The viability of the cells of each microorganism under study in the culture liquid was determined by inoculation on an agar universal medium containing 10 g/l glucose, 0.5 g/l peptone, 1 g/l yeast extract, and 0.5 g/l $NaNO_3$, $(NH_4)_2SO_4$, NH_4NO_3, and KH_2PO_4. Samples of the culture fluid were taken with a 5 ml microbiological pipette. The number of microorganisms was determined by the method of serial 10-fold dilutions, counting the number of colonies of each species, the indication was carried out according to the morphological description of colonies of yeast and bacteria of the studied genera [29-31]. The microobjects were visualized using a microscope with a Fixed Microscope Adapter (Levenhuk, USA).

Seeds of agricultural plants were treated by spraying with cultural liquid in different dilutions (1:200, 1:150, 1:100, 1:50, 1:25), after 12 h, the seeds were germinated in water in Petri dishes (20 or 50 seeds per dish) at 25 °C in a thermostat TS-1/20 SPU. Seeds treated with water served as control. Germination energy and germination were determined by conventional methods after 3 and 7 days, respectively [32].

All experiments were carried out at least three times, each including 5 flasks or Petri dishes with the same material grown under the same type of conditions. When determining cell viability, each variant was analyzed in 10 replications. Seed germination was carried out in 5 repetitions (Petri dishes) for each variant of the experiment.

Statistical processing of the results was carried out according to standard methods [33] in the Microsoft Excel 2007 program. The data in tables and graphs are given as arithmetic mean values with standard errors (M±SEM). Differences in sample means were assessed by Student's t-test at $p \leq 0.05$ using Microsoft Excel 2007 and Statistica v.12 (StatSoft, Inc., USA).

Results. After 48 h of separate cultivation of *P. chlororaphis* subsp. *aureofaciens* B-5326 and *S. cerevisiae* Y-4317, their abundance reached 2×10^8 and 7×10^6 CFU/ml, respectively. Next, we studied the possibility of co-growth of two cultures in a liquid nutrient medium. Note that we did not find relevant data on such cultivation and its conditions in the available literature.

To optimize the co-culture of two strains of microorganisms, we studied the effect of the composition of the nutrient medium on changes in their biomass and abundance. Glucose was used as the main source of carbon, while fructose and beet molasses serbed as alternatives.

On media with glucose and fructose, a relatively weak accumulation of total biomass was observed (Fig. 1, A). The maximum value of the total biomass during the co-culture of the studied strains was recorded on the medium containing molasses, 20.4 g/l, or 17-22% higher than on the media with glucose and fructose.

During co-culture, the abundance of *P. chlororaphis* and *S. cerevisiae* did not fall below 6×10^8 and 3×10^6 CFU/ml, respectively (see Fig. 1, B). The CFU values for *S. cerevisiae* and *P. chlororaphis* did not change significantly on all the

studied media. The studied strains were able to grow together on molasses without a significant decrease in cell viability (see Fig. 1, B). Therefore, in all subsequent experiments, we used a nutrient medium containing molasses as a carbon source.

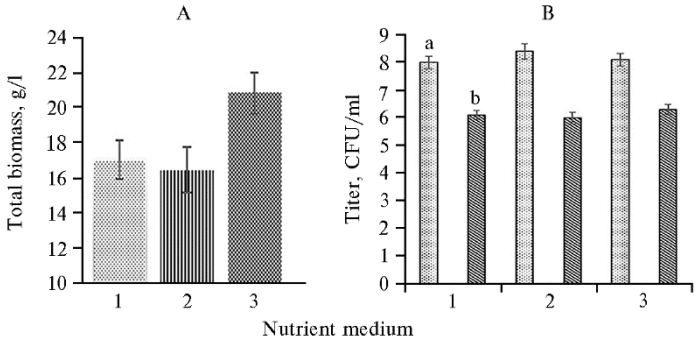

FIg. 1. Total biomass (A) **and the cell number** (B) **in co-culture of *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326** (a) **and yeasts *Saccharomyces cerevisiae* Y-4317** (b) **depending on liquid medium composition:** 1 — glucose (10 g/l), 2 — fructose (10 g/l), 3 — beet molasses (15 g/l) (N = 10, M±SEM).

The successful use of beet molasses for the co-culture of strains of microorganisms can be explained by the fact that, along with other components, it contains about 1% raffinose. On the one hand, raffinose can inhibit the growth and biofilm formation of *Pseudomonas* bacteria [34]; on the other hand, it also affects bacterial phenotypes, colony morphology, matrix formation, and colony motility [35]. The use of beet molasses for the co-culture of *P. chlororaphis* subsp. *aureofaciens* B-5326 and *S. cerevisiae* Y-4317 can reduce the economic load in the commercial production of the created biologicals.

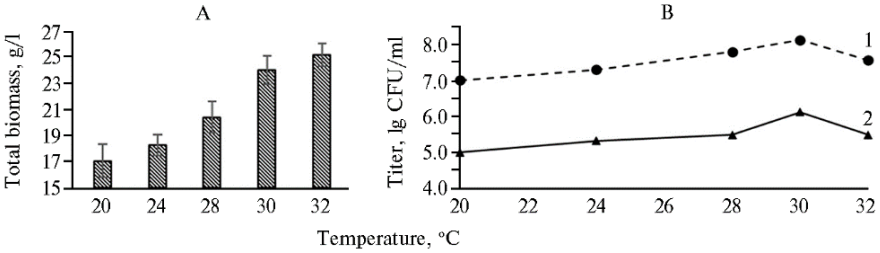

Fig. 2. Total biomass (A) **and the cell number** (Б) **in co-culture of *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326** (1) **and yeasts *Saccharomyces cerevisiae* Y-4317** (2) **on beet molasses** (15 g/l) **medium depending on temperature** (N = 5, M±SEM). Markers on Fig. 2, indicate the ±SEM values.

The temperature regime during the co-culture of bacteria and yeast affected the change in the total biomass of these microorganisms (Fig. 2, A). At temperatures of 20 and 24 °C, the total biomass turned out to be minimal (we did not record any statistically significant differences between these options). At 28 °C, the total biomass was 20.5 g/l, at 30 °C it was 24.3 g/l. A further increase in temperature (up to 32 °C) did not significantly increase the total biomass of bacteria and fungi compared to that at 30 °C.

The temperature regime also influenced the viability of bacteria and fungi (see Fig. 2, B). From 20 °C, the CFU values of *P. chlororaphis* and *S. cerevisiae* increased, and the maximum was recorded at 30 °C (10^8 and 10^6 CFU/ml, respectively). With a further increase in temperature (up to 32 °C), the number of colonies decreased significantly, to 7.5×10^7 and 5.4×10^5 CFU/ml for *P. chlororaphis* and *S. cerevisiae*. Therefore, in the joint cultivation of microorganisms of

different taxonomic groups, the optimum temperature is 30 °C.

Yeast by type of nutrition are facultative anaerobes; the presence of oxygen inhibits alcoholic fermentation and they actively accumulate biomass [36]. Therefore, the task of the next stage of the study was to select the optimal aeration regime for the joint cultivation of yeast and bacteria in a bioreactor (Table 1). We have found that the optimal aeration regimen is 4 l/h. It should be noted that the presence of oxygen in the medium had a greater effect on the viability of yeast, since the number of viable *P. chlororaphis* cells under all aeration modes did not fall below 2×10^8 CFU/ml. The data obtained at this stage are comparable with the results of previous experiments, when cultivation was carried out on a shaker-incubator in flasks with cotton-gauze stoppers. From this, we can conclude that the studied microorganisms are not very demanding on aeration regimes and there is no obvious competition between them.

1. The number of viable cells (CFU/ml) **of *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326 and *Saccharomyces cerevisiae* Y-4317 in co-culture** (a BIORUS GJ bioreactor, BIORUS, Republic of Belarus) **in a medium with beet molasses** (15 g/l)**, depending on the aeration regime** ($N = 5$, $M\pm$SEM)

Aeration, l/h	*P. chlororaphis*, $\times10^8$	*S. cerevisiae*, $\times10^6$
1	4.2±0.31	0.8±0.05
2	2.1±0.18	2.4±0.09
3	3.6±0.24	3.6±0.18
4	5.9±0.36	5.9±0.12
5	3.2±0.17	2.1±0.26
6	2.4±0.08	4.2±0.16

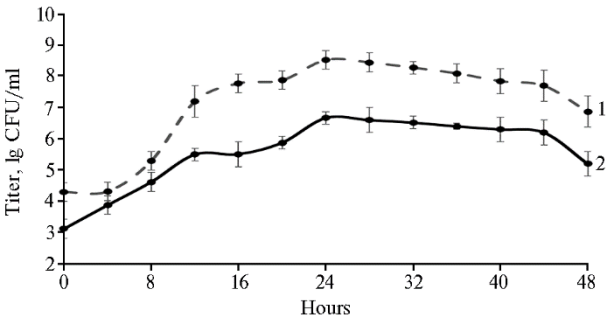

Fig. 3. The number of *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326 (1) **and *Saccharomyces cerevisiae* Y-4317** (2) **in co-culter in a medium with beet molasses** (15 g/l) **at 30 °C** ($N = 5$, $M\pm$SEM)**.**

Further, we studied the dynamics of changes in culture titers (CFU value) in the co-culture of *P. chlororaphis* and *S. cerevisiae* in a medium with molasses at 30 °C (Fig. 3). In the first 8 h, the CFU value in yeast increased much faster and more significantly than in bacteria, and the titers of *P. chlororaphis* and *S. cerevisiae* were 6×10^4 and 3.8×10^4 CFU/ml, respectively. Obviously, growth during the logarithmic phase in the first hours of cultivation in yeast was more intense than in *Pseudomonas*. After 12 h, the growth curve of *S. cerevisiae* entered the stationary phase, after which the number of yeasts slightly increased. After 24 h, it began to decrease. *Pseudomonas* showed the most active growth in the period from 8 to 24 h of culture, which correlates with literature data [37]. The maximum number of *P. chlororaphis* cells was recorded after 24 h of co-culture with yeast (8.4×10^8 CFU/ml). In the period from 36 to 48 h, the number of bacterial cells gradually decreased and the titer of *P. chlororaphis* decreased by almost 100 times (to 8.8×10^6 CFU/ml). The elongation of the logarithmic growth phase of *P. chlororaphis* observed in the co-culture of yeast and bacteria can be explained by the inclusion of molasses containing complex carbohydrates in the substrate composition, the use of which increases the duration of exponential cell growth. The subsequent slowdown in growth may be due to competition for nutrients [38].

In order to test this assumption, we extended the co-cultivation of microorganisms up to 72 h, while taking into account the growth of P. chlororaphis in pure culture (Fig. 4). At the same time, paradoxically, it turned out that in the mixed culture with S. cerevisiae yeast, there was no further decrease in the bacterial titer, while in the pure culture of Pseudomonas, after 72 h, the titer of viable cells decreased to the limiting minimum (5×104 CFU/ml).

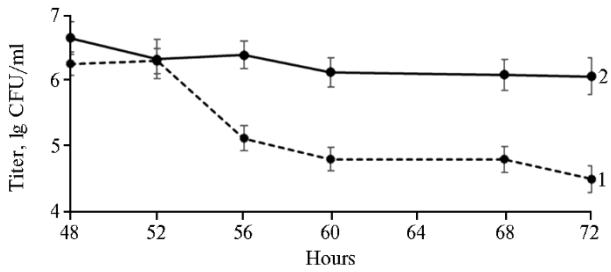

Fig. 4. ***Pseudomonas chlororaphis*** **subsp.** ***aureofaciens*** **B-5326 growth in pure culture** (1) **and co-culture with** ***Saccharomyces cerevisiae*** **Y-4317** (2) **for 72 h in a medium with beet molasses** (15 g/l) **at 30 °C** ($N = 5$, M±SEM).

Thus, a positive effect of S. cerevisiae on the viability of P. chlororaphis h was shown, since after 72 of co-cultivation the bacterial titer was 1×10^6 CFU/ml, which is approximately two orders of magnitude higher than in pure bacterial culture. J.D. Romano et al. [39] described similar effects of fungal metabolites on the viability of bacteria during the interaction of *Pseudomonas* and *Saccharomyces.*

It is known that the mechanism of interaction between bacteria and fungi is based on the ability of yeast to synthesize gluconate from glucose. Gluconate, in turn, increases the viability of bacteria of the genus *Pseudomonus* [40]. This can partly explain the revealed positive effect of *S. cerevisiae* on the growth of *P. chlororaphis.* In addition, in recent years, data have appeared that yeast are active producers of the auxin indolyl-3-acetic acid (possibly, other phytohormones as well). Approximately 90% of all known yeast species are capable of synthesizing auxins: yeasts of the genus *Cyberlindnera*, *Rhodotorula graminis*, *Rhodosporidium fluviale*, *Rhodosporidium paludigenum*, *Aureobasidium pullulans*, *Saccharomyces cerevisiae* [41-43]. In terms of the activity of synthesis of hormonal compounds, yeast is practically not inferior to bacteria and algae. It has been shown that yeast culture liquid can influence the growth and development of plants [44]. It can be assumed that it was the production of phytohormones by the yeast *S. cerevisiae* in co-culture that determined the active growth and high viability of *P. chlororaphi*s.

We preliminary evaluated the effectiveness of the complex bio-preparation by determining the germination of seeds of cereal plants (barley, wheat, corn) after treatment with the culture liquid, obtained by co-culturing yeast and *Pseudomonas*, in four dilutions (from 1:200 to 1:25) (Table 2). In the control, the germination energy and seed germination were quite high and ranged from 79 to 89%.

2. Germination energy and germination (%) **of treated cereal seeds at different dilutions of the culture fluid from co-culture of** ***Pseudomonas chlororaphis*** **subsp.** ***aureofaciens*** **B-5326 and** ***Saccharomyces cerevisiae*** **Y-4317** (M±SEM)

Parameter	Dilutions					Control (water)
	1:200	1:150	1:100	1:50	1:25	
Barley (*Hordeum vulgare* L.) cv. Skipetr						
Germination energy	90±2.1	88±2.5	91±3.1	89±2.8	87±2.5	85±2.4
Germination	96±2.9*	92±3.4	95±3.8	92±2.2	89±3.2	87±3.0
Wheat (*Triticum aestivum* L.) cv. Mironovskaya 808						
Germination energy	93±3.2	91±2.9	95±3.7	89±2.6	85±2.8	86±3.2
Germination	94±3.6	95±3.1	96±4.1	92±2.8	90±3.1	89±2.7
Corn (*Zea mays* L.) hybrid Delitop						
Germination energy	89±2.9*	87±3.6	86±4.1	80±3.8	81±2.9	79±3.1
Germination	96±4.6*	89±3.6	90±2.8	85±3.0	88±3.3	83±3.4

* Differences from control are statistically significant at $p \le 0.05$.

Pre-sowing treatment with a biological product stimulated the germination of seeds of all studied cereal crops. The efficiency of treatment depended on the concentration (dilution) of the culture liquid. Germination of barley seeds statistically significantly ($p = 0.05$) increased by 9% compared to the control when using CL at a dilution of 1:200. A clear trend towards an increase in the germination energy and germination of barley seeds was noted at all dilutions (differences from the control are not significant). Similar relationships were found for wheat, where the highest germination rates were observed for dilutions of 1:200-1:100, but the differences with the control were not significant.

In the experiment with corn seeds, the lowest concentrations of the biological product also turned out to be the most effective. When using a dilution of 1:200, the germination energy significantly ($p = 0.05$) increased by 10%, and germination by 13% compared to the control. Dilutions of 1:150 and 1:100 showed a tendency to stimulate seed germination (differences from control are not significant). Higher doses of the biological product (dilutions 1:50 and 1:25) slightly increased the germination energy and the germination rate.

An increase in germination energy and high seed germination are controlled by phytohormones which activate cell division and elongation and increase stress resistance [16, 17].

Thus, we have shown the possibility of co-cultivation of the bacterium *Pseudomonas chlororaphis* subsp. *aureofaciens* B-5326 and yeast *Saccharomyces cerevisiae* Y-431, which will make it possible to create a biological product based on microorganisms of different taxonomic groups. Beet molasses (waste of sugar production) fully satisfies the needs of the studied microorganisms in basic nutrients. The optimal conditions for co-culture were selected, i.e., a temperature of 30 °C, aeration mode 4 l/h, culture duration 24 h. A positive effect of *S. cerevisiae* on the viability of *P. chlororaphis* was revealed during long-term (72 h) co-culture. Therefore, in the presence of *S. cerevisiae*, the titer of *P. chlororaphis* was 10^6 CFU/ml, in pure culture it was 10^4 CFU/ml. Pre-sowing treatment of seeds with a preparation obtained in co-culture of these microorganisms stimulated the vigor of germination and germination rate in barley, wheat and corn. The complex preparation showed the highest efficiency in relation to corn seeds. The maximum increase in the energy of seed germination and the germination rate for all the studied crops was revealed at a dilution of the preparaion 1:200.

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BIOLOGICAL EFFICIENCY OF THE EXTRACT OF *Haplophyllum perforatum* AGAINST *Tuta absoluta* AND ITS INFLUENCE ON THE PHYSIOLOGICAL PROPERTIES OF TOMATO PLANTS

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Abstract

The tomato leaf miner *Tuta absoluta* (Meyrick) (*Lepidoptera: Gelechiidae*), whose natural range includes the countries of South America, has become widespread in Africa and Europe. Since 2015, the pest has been registered in the Republic of Uzbekistan. It is known to develop resistance to many chemical plant protection products. In addition, pesticides can significantly change the intensity of plant metabolic processes, in particular photosynthesis, which reduces the productivity and quality of the crop. In this regard, plant substances are of interest, the use of which is promising in plant protection. The present work, for the first time, shows the biological effectiveness of the application of the extract of the plant *Haplophyllum perforatum* against the larvae of the tomato leaf miner in the field. The extract had a positive effect on the content of chlorophylls in tomato leaves and increased the total leaf area of the plant. The aim of our work was to study the biological effectiveness of extract of the plant *Haplophyllum perforatum* and its complex with the growth regulator Uchkun against larvae *Tuta absoluta*, as well as their effect on the physiological parameters of tomato. The field experiment was carried out on the area farming facilities "Super Garden" (Tashkent region, Kibray district). Tomato plants of the TMK-22 variety were sprayed with an extract of *H. perforatum* and its composition with growth regulator Uchkun, which was developed on the basis of polyprenols isolated from cotton leaves. We also used the insecticide of natural origin Proclaim, the active substance of which is 5% emamectin benzoate (Syngenta Crop Protection AG). The seedlings were sown on April 24, 2020. The placement of the plots is randomized, in four repetitions. Spraying was carried out in the phase of the beginning of flowering of tomatoes in the presence of pest numbers not lower than the economic threshold of harmfulness. The design of the experiments was as follows: control (option without treatment), standard Proclaim, 5 % (W.S.G-water-soluble granule, 0.4 kg/ha), extract of *H. perforatum*, 1.0 % (W.E-water emulsion, 0.4 kg/ha), composition — *H. perforatum* extract, 1.0 % + Uchkun, 0.0001 % (W.S.E, 0.4 kg/ha). The biological effectiveness of the extracts was evaluated by reducing the number of pest larvae. For 1 day before treatment, on days 3, 7 and 14 after treatment, larvae of the 1st-2nd and 3rd-4th in stars were counted. The content of chlorophylls was estimated by the spectrophotometric method. During the growing season, biometric indicators were recorded: leaf surface area, plant height, number of leaves, flowers and fruits. The total leaf surface area was determined by the gravimetric method. We have revealed high toxicity of the *H. perforatum* extract and its composition with the Uchkun preparation against the tomato leaf miner. The greatest decrease in the number of pest larvae occurred on day 7 after spraying tomatoes. Against an increase in the number in the control, the effectiveness of the extract against larvae of the 1^{st}-2^{nd} instar was 87.1 %, of the 3^{rd}-4^{th} instar — 77.5 %. For the treatment with the composition (84.1 and 70.0 %) and the insecticide (87.0 and 70.0 %), the efficiency was almost comparable. Treatment of plants with the studied solutions contributed to an increase in the content of photosynthetic pigments in damaged leaves. After exposure to the extract, the content of chlorophyll a was higher than the control by 1.1 times, chlorophyll b — by 1.8 times, their sum — by 1.5 times, after treatment with the composition, the amount of chlorophyll a and b exceeded the control by 2.2 and 2.1 times, their sum — by 1.7 times. An increase in leaf surface area was also observed: after treatment with *H. perforatum* extract, the indicators were higher

than the control by 75.0 %, with the composition — by 58.3 %, the number of leaves increased by 85.1 and 89.9 %, respectively. In the variant with the use of the extract, the number of flowers was 8.3 pcs/plant, fruits — 3.8 pcs/plant, when treated with the composition — 8.9 and 4.1 pcs/plant, with Proclaim — 7.8 and 3.5 pcs/plant, in the control — only 2.2 and 1.2 pcs/plant. Thus, we have shown the possibility of using the plant extract of *H. perforatum* against the tomato leaf miner. It has been established that treatment with a composition of *H. perforatum* extract with a growth regulator improves the physiological and biochemical parameters of tomato plants.

Keywords: *Haplophyllum perforatum*, extract, *Tuta absoluta*, biological effectiveness, photosynthetic pigments, leaf area, biometric parameters

The tomato (*Lycopersicon esculentum* Mill., family *Solanaceae*) is one of the most widely cultivated vegetables in the world. More than half of world production (56.71%) is concentrated in four countries. China remains the world's leading tomato producer (31.81%), followed by India (10.39%), the US (7.36%) and Turkey (7.12%). The growth of tomato production in the period from 2005 to 2016 was 29.08% with an average annual growth rate of 3.14% [1]. In the Republic of Uzbekistan, approx. 40% area is allocated for the cultivation of tomatoes from the crops of other vegetable crops. In a large volume, products are exported abroad [2].

In recent years, the mass distribution of the tomato leaf miner *Tuta absoluta* (Meyrick) (*Lepidoptera*: *Gelechiidae*) has been observed in the republic, which leads to significant losses in tomato crops. This species is included in the EPPO European and Mediterranean Plant Protection Organization list of quarantine pests [3]. After the invasion of Spain in 2006, it quickly spread throughout Afro-Eurasia and became a major threat to global tomato production [4]. The emergence of *T. absoluta* in the republic has been confirmed in greenhouses and open fields since 2015 [5].

Mining-type damage caused by *T. absoluta* larvae in the mesophyll of leaves, young shoots and fruits sharply reduces their photosynthetic ability, which leads to a reduction in the number of ripe fruits formed, their size and quality. In addition, due to damage, secondary pathogenic microorganisms, including putrefactive ones (saprophytic fungi and bacteria), penetrate into plant tissues, which leads to shedding of unripe fruits and a sharp decrease in the quality of harvested fruits, their commercial value and a general drop in yield [6].

T. absoluta has been reported to develop resistance to chemicals [7, 8], so while there is a need to improve plant protection against this pest, the use of synthetic insecticidal compounds needs to be reduced [9].

Agricultural intensification, especially in exporting developing countries, is leading to the widespread use of pesticides, which affects soil quality, populations of non-target organisms and human health [10, 11]. Pesticides can significantly change the intensity of plant metabolic processes, in particular photosynthesis, which leads to a decrease in productivity and crop quality [12-14]. There are plant species that have a toxic effect on insect pests that can be used to obtain biological products [15, 16]. Previously, we have established a high insecticidal activity of the extract of *Haplophyllum perforatum* A. Juss [17, 18].

It is known that plant growth regulators are used to eliminate the negative impact of various stress factors. So, the biostimulator Uchkun, developed at the Yunusova Institute of Chemistry of Plant Substances of the Academy of Sciences of the Republic of Uzbekistan, has a stress-protective activity when cultivating plants in conditions of salinity and water deficiency [19-21].

In this work, for the first time, the biological effectiveness of the extract of the plant *Haplophyllum perforatum* against the larvae of the tomato leafminer in the field is shown. The extract had a positive effect on the content of chlorophylls in tomato leaves and increased the total area of the leaf surface.

The aim of our work was to study the biological effectiveness of the plant extract of *Haplophyllum perforatum* and its complex with the growth regulator Uchkun against *Tuta absoluta* larvae, as well as their effect on the physiological parameters of tomato.

Materials and methods. The field experiment was carried out at the site of the Super Garden farm (Tashkent region, Kibray district). Tomato plants of the TMK-22 variety were sprayed with an extract of *H. perforatum* and its composition with growth regulator Uchkun, which was developed on the basis of polyprenols isolated from cotton leaves [19]. We also used the natural insecticide Proclaim (Proclaim®), the active ingredient of which is 5% emamectin benzoate (Syngenta Crop Protection AG, Switzerland) [22].

For experiments, tomato plants of the TMK-22 variety were planted on plots by the nesting method [23]. The soil of the site is medium loamy gray. The seedlings were sown on April 24, 2020. Placement of plots was randomized, with a 4-fold repetition. The plants were sprayed with a knapsack pneumatic sprayer, the consumption of working solutions was 3 l/100 m^2. Spraying was carried out at the beginning of flowering of tomatoes with the pest population not lower than the economic threshold of harmfulness. During the experiments, the temperature was 30-34 °C, air humidity 23-28%, daylight for 15 h.

The scheme of experiments was as follows: control (without treatment), standard Proclaim, 5% (WSG — water-soluble granule, 0.4 kg/ha), extract of *H. perforatum*, 1.0% (WE — water emulsion, 0.4 kg/ha), and a composition of 1.0% *H. perforatum* extract + 0.0001% Uchkun (WE, 0.4 kg/ha).

The biological effectiveness of the extracts was evaluated by reducing the number of pest larvae according to the formula C.F. Henderson and E.W. Tilton (W.S. Abbot formula adjusted for control) [24]:

$$C = \frac{n\text{K (prior treatment)} \times n\text{B (after treatment)}}{n\text{K (after treatment)} \times n\text{B (prior treatment)}} \times 100\ \%,$$

where n is the number of pest larvae, B is the experiment, K is the control.

One day before treatment, on days 3, 7, and 14 after treatment, larvae of 1-2 and 3-4 instars were counted in accordance with the guidelines of V.I. Dolzhenko [25] and the working program drawn up before the treatment of plants with preparations.

Chlorophyll content was assessed spectrophotometrically using a V-5000 spectrophotometer (Metash Instruments Co., Ltd., China) [8]. During the growing season, biometric indicators were taken into account: leaf surface area, plant height, number of leaves, flowers, and fruits. The total leaf surface area was determined by the gravimetric method [26].

Mathematical processing of the obtained data and calculation of statistical parameters were carried out using the Microsoft Excel 2016 software package. Average values of the indicators (M) and standard errors of the means (±SEM) are presented. To compare data, combined on the same trait, we used a one-dimensional one-way analysis. When evaluating the ratio of intergroup variability, Student's t-test was used to assess the null hypothesis of the equality of means for the treatments for a significance level of $p < 0.05$.

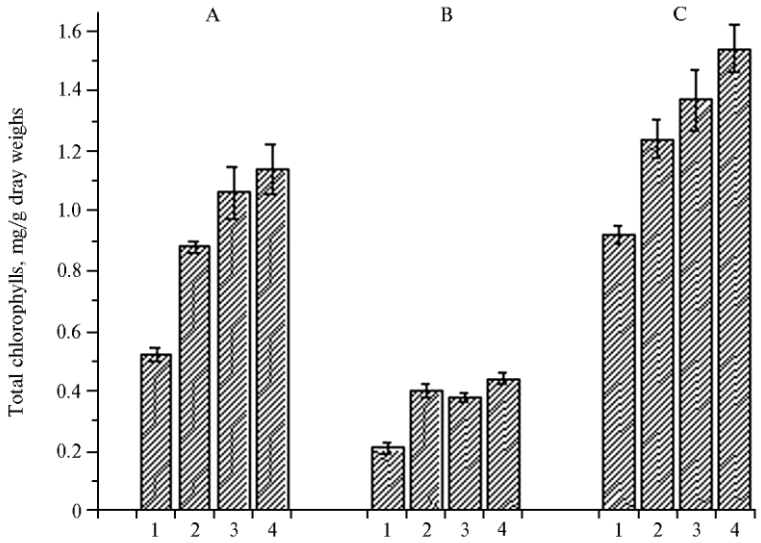
Results. The biological effectiveness of the extract of *H. perforatum* on the day 3 against the larvae of the tomato miner moth *T. absoluta* of the 1st-2nd age was 81.0%, of the 3rd-4th age 83.6%. The effectiveness of the composition was somewhat lower, 73.3 and 69.6%, respectively, of the insecticide Proclaim 78.0 and 76.0% (Table 1).

1. Biological efficiency of the *Haplophyllum perforatum* A. Juss extract and its composition with growth regulator Uchkun against *Tuta absoluta* (Meyrick) larvae of 1-2 and 3-4 instars on tomato *Lycopersicon esculentum* Mill. cv. TMK-22 (n = 4, M±SEM; Tashkent region, Kibray district, 2020)

Treatment	Prior treatment			Days after **extract of**								
				3-и			7-е			14-е		
	1-2 instars	3-4 instars	total	1-2 instars	3-4 instars	total	1-2 instars	3-4 instars	total	1-2 instars	3-4 instars	total
				The number of larvae per plant								
Control (without treatment)	5.7±0.3	6.5±0.5	12.2±0.6	9.7±1.2	8.8±0.6	18.5±0.7	12.7±0.8	10.7±0.8	23.4±1.3	17.7±1.3	14.0±0.5	31.7±1.5
Proclaim (standard), 0.4 kg/ha	6.5±0.9	4.0±0.2	10.5±0.9	2.4±0.5	1.3±0.2	3.7±0.4	1.9±0.5	1.8±0.3	3.9±0.5	3.2±0.5	2.9±0.8	6.1±0.9
Extract, 0.4 kg/ha	6.2±0.9	5.4±0.6	11.6±0.5	2.0±0.4	1.2±0.0	3.2±0.3	1.8±0.2	2.5±0.4	3.8±0.3	3.4±0.5	3.0±0.6	6.4±0.1
Composition, 0,4 kg/ha	5.5±0.6	3.4±0.5	8.9±0.9	2.5±0.6	1.4±0.5	3.9±0.5	2.0±0.3	1.7±0.3	3.7±0.6	2.9±0.5	3.4±0.6	6.3±1.0
				Biological efficiency, %								
Control (without treatment)												
Proclaim (standard), 0.4 kg/ha				78.0	76.0	77.0	87.0	72.6	81.0	84.1	66.3	77.6
Extract, 0.4 kg/ha				81.0	83.6	82.0	87.1	71.9	83.0	82.3	74.2	78.7
Composition, 0,4 kg/ha				73.3	69.6	71.1	84.1	70.0	78.3	83.0	53.6	73.0

We observed the greatest decrease in the number of pest larvae on day 7. With an increase in the number in the control, the effectiveness of the extract against larvae of the 1st-2nd age was 87.1%, of 3-4th age 77.5%. With the use of the composition and the insecticide, the efficiency was practically comparable (see Table 1). On day 14 after spraying, the biological effectiveness of the extract remained (82.3 and 74.2%), the biological effectiveness of the composition against larvae of the 1st-2nd age remained high (83.0%), of the 3rd-4th age decreased (53.6%).

Treatment of tomato plants with *H. perforatum* extract contributed to a significant increase in the amount of photosynthetic pigments: 7 days after treatment, the content of chlorophyll a in the leaves was 1.1 times higher (1.06 mg/ml, $p < 0.05$) than in the control, chlorophyll b was 1.8 times higher (0.38 mg/ml, $p < 0.05$), their sum was 1.5 times higher (1.37 mg/ml, $p < 0.05$) (Fig.).



The content of chlorophyll a (A)**, chlorophyll b** (B) **and total chlorophyll** (C) **in the leaves of tomato *Lycopersicon esculentum* Mill. cv. TMK-22 after treatments:** 1 — control (without treatment), 2 — Proclaim (standard), 05 %, 3 — *Haplophyllum perforatum* A. Juss extract, 1.0 %, 4 — composition of the *H. perforatum* extract, 1.0 % + Uchkun, 0.0001 % ($n = 4$, $M \pm$SEM; Tashkent region, Kibrai district, 2020).

When using the composition, the amount of photosynthetic pigments was significantly higher. The content of chlorophyll a (1.14 mg/ml) and chlorophyll b (0.44 mg/ml) exceeded that in the control variant by 2.2 and 2.1 times, respectively ($p < 0.05$), their sum (1.54 mg/ml) by 1.7 times. The high content of photosynthetic pigments in the experiment with the use of the composition may be due to the synergistic effect of the biologically active substances of the Uchkun preparation in combination with the substances of the *H. perforatum* extract. Due to the decrease in the number of the pest after treatment with the insecticide Proclaim, an increased content of chlorophyll was also observed compared to the control, for chlorophyll a (0.88 mg/ml) by 1.7 times ($p < 0.05$), for chlorophyll b (0.40 mg/ml) by 1.9 times, for their sum (1.24 mg/ml) by 1.3 times ($p < 0.05$) (see Fig.).

Also, due to the decrease in the number of larvae of *T. absoluta*, the growth rate of the leaf surface area in the budding phase increased after spraying the plants

with the extract of *H. perforatum* (105 cm^2 per plant) and the composition (95 cm^2/plant), while in the control, it was 60 cm^2 per plant (Table 2). The most intensive growth rates occurred at the beginning of fruit formation, with the use of the extract 850 cm^2 per plant, with the composition 855.3 cm^2 per plant. In these options, these values significantly exceeded the control (440 cm^2 per plant) and standard (780 cm^2 per plant). The decrease in the leaf surface area before the mass ripening of fruits occurred mainly due to the death of the main part of the leaves of the lower tier (see Table 2).

2. Total leaf area (cm^2) **in tomato *Lycopersicon esculentum* Mill. cv. TMK-2 plants treated with an extract of *Haplophyllum perforatum* A. Juss. and its composition with growth regulator Uchkun** (n = 4, M±SEM; Tashkent region, Kibrai district, 2020)

Treatment	Stage of growth				
	intendive growth	budding	blossoming	fruit formation	ripening
Control (without treatment)	45.3±3.0	60.0±2.5	99.0±1.5	440.3±3.2	310.3±0.5
Proclaim (standard), 0.4 kg/ha	49.0±2.0	89.0±1.0	165.0±3.7	780.0±3.2	615±3.0
Extract, 0.4 kg/ha	55.3±1.5	105.0±2.5	255.0±3.0	850.0±3.7	650.3±1.5
Composition, 0,4 kg/ha	50.0±1.0	95±1.0	230±2.5	855.3±2.5	640.0±2.6

Spraying plants with *H. perforatum* extract and its composition with a growth regulator had a significant effect on the biometric parameters of tomato plants. When treated with the extract, the height of plants was 1.7 times ($p < 0.05$) higher than in the control; with the composition, it was 1.8 times higher ($p < 0.05$) (Table 3). In terms of the number of leaves, these variants exceeded the control one by 1.8 and 1.9 times, respectively ($p < 0.05$), and the values were comparable to the results of insecticide application. By reducing the negative impact of pests on plant development, the number of flowers and fruits increased in the experimental variants (see Table 3).

3. Biometric parameters in tomato *Lycopersicon esculentum* Mill. cv. TMK-2 plants when treated with an extract of *Haplophyllum perforatum* A. Juss. and its composition with growth regulator Uchkun (n = 4, M±SEM; Tashkent region, Kibrai district, 2020)

Treatment	Plant height, см	The number of leaves	The number of flowers	The number of fruits
Control (without treatment)	28.5±0.1	31.6±0.1	2.2±0.1	1.2±0.1
Proclaim (standard), 0.4 kg/ha	35.6±0.1	58.7±0.0	7.8±0.1	3.5±0.0
Extract, 0.4 kg/ha	49.5±0.1	58.5±0.1	8.3±0.0	3.8±0.1
Composition, 0,4 kg/ha	50.2±0.0	60.0±0.1	8.9±0.1	4.1±0.1

The resistance of the tomato miner moth to chemicals occurs worldwide. In Brazil, Chile and Argentina, the effectiveness of Vertimec, as well as some organophosphorus compounds and pyrethroids, against the pest has decreased [27]. Successful pest control requires multiple pesticide treatments, which speeds up the selection of the most resistant individuals in the pest population. Preparations based on carbamates (Lannat) and pyrethroids (Decis) can cause rapid death of adults and caterpillars [28]. Currently, effective agents include Tracer (spinosad), Pirate (chlorfenapyr), Aktara (thiamethoxam), Emperor (chlorpyrifos, cypermethrin), Koragen (chlorantraniliprole) [29, 30]. In Spain, LIDA Plant Research's new organic products, Ecothrin® and Acaridoil®, are used to control a wide range of pests, including *Tuta absoluta*. This unique pyrethrin-based product is produced in microcapsules for greater persistence and greater effectiveness in pest control. Acaridoyl is a natural product based on oleic acids from olive oil [31].

An example of an effective pesticide based on azadirachtin from neem seeds is NimAtzal T/S. The biological, as a contact and at the same time systemic insecticide, has a good effect on the tomato mining moth. The biological effectiveness of azadirachtin (active ingredient) against *Tuta absoluta* was 80.0% [32-35].

In this work, we found that the biological effectiveness of the extract of the plant *Haplophyllum perforatum* against the tomato leaf miner in open ground conditions reached 87.1%. To a greater extent, the extract was toxic against larvae of the 1st-2nd age. Similar data were obtained with Fame (flubendiamide), Divipan, Talstar EC (bifentrin) and Tracer, which are effective against young larvae up to 5 mm in size [31].

It is known that plant growth regulators can influence all processes of growth and development. We have shown the effectiveness of using the composition of the extract of *Haplophyllum perforatum* with growth regulator Uchkun: spraying tomato plants with this composition contributed to an increase in the content of photosynthetic pigments, leaf surface area and the number of fruit elements.

Thus, out findings indicte the high toxicity of the *Haplophyllum perforatum* extract and its complex with the growth regulator Uchkun against the tomato leaf miner *Tuta absoluta*. On day 7 after spraying the tomatoes with the extract, the biological effectiveness against larvae of the 1st-2nd age was 87.1%, of the 3d-4th age 77.5%, for composition, it ccounted for 84.1 and 70.0%, respectively. In damaged leaves, the content of chlorophyll a after exposure to the extract was 3.8% higher than the control, of chlorophyll b by 80.9% higher, their total amount by 48.9% higher, after treatment with the complex, the values exceeded by 119.2, 109.5 and 67.3%, respectively. With reducing the leaf damage, an increase in leaf area occurres, after treatment with *H. perforatum* extract, it was by 75.0% higher than the control, after treatment with the composition by 58.3% higher, the number of leaves increased by 85.1 and 89.9%, respectively. With the use of the extract, there was 8.3 flowers per plant and 3.8 fruits per plant, when treated with the composition, these values were 8.9 flowers and 4.1 fruits per plant, respectively, for Proclaim, 7.8 flowers and 3.5 fruits per plant, while in control only 2.2 flowers and 1.2 fruits per plant.

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EFFECTS OF *Litsea cubeba* (Lour.) Pers. ESSENTIAL OIL AND ITS MAIN COMPONENT TO THE DEVELOPMENT OF THE GREENHOUSE WHITEFLY *Trialeurodes vaporariorum* Westw.

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Abstract

Trialeurodes vaporariorum Westw. (*Hemiptera*, *Aleyrodidae*) causes significant economic damage to vegetable and ornamental crops due to phloem sap feeding and the transmission of viral diseases. High reproductive potential of the phytophage and multiple treatments with chemicals lead to the emergence of resistance to various insecticides. Thereof, it becomes necessary to search for new effective environmentally safe plant protection products. Plant essential oils are of considerable interest in this regard. In this work, for the first time, we obtained information about the effectiveness of *Litsea cubeba* essential oil and its main component, citral, as fumigants and repellents for controlling the number of greenhouse whiteflies. As the problem of reducing the insecticidal load is especially acute in greenhouses, the aim of our study was to examine mechanisms of action of *L. cubeba* essential oil and citral on *T. vaporariorum* — one of the most harmful phytophages for greenhouse crops. Obtaining information on the effectiveness of the tested samples will serve as the basis for the development of a new protective tactic against the greenhouse whitefly. The whiteflies were lab-reared on bean (*Phaseolus vulgaris* L.) plants at 24±1 °C and a 16 h light period. The essential oil of *L. cubeba* and citral were obtained from the Crop Research Institute (Prague, Czech Republic). For testing, 1 % solutions of *L. cubeba* essential oil or citral were prepared by dissolving 100 μl of the substance in 900 μl of ethanol, followed by the addition of 9 ml of water with stirring. Concentrations of 0.5, 0.25, and 0.125 % were obtained by sequential dilution with water. The phytotoxicity of *L. cubeba* essential oil and citral was pre-assessed. The pest preimaginal stages were treated to assess the effect of the essential oil on egg hatching and the larvae development. The influence of *L. cubeba* essential oil and citral on the choice of plants by *T. vaporariorum* for feeding and oviposition under free choice was also investigated. When studying the fumigation effect, the number of live, dead individuals and laid eggs was assessed. The experimental data were analyzed with one-way analysis of variance (one-way ANOVA), the mean values were compared using the Tukey's HSD test. Differences between the means were considered significant at $p \le 0.05$. When the *T. vaporariorum* eggs were treated with solutions of *L. cubeba* essential oil, none of the tested concentrations affected the vital parameters of the phytophage during the entire preimaginal period of development. A similar pattern occurred after the treatment of larvae. The obtained results show the absence of both direct toxic effects and aftereffects during treatment at the embryonic and larval stages of whiteflies. When whitefly adults were kept on plants treated with 0.25 % concentration of *L. cubeba* oil, the number of laid eggs significantly decreases (by 25 % compared to the control). Oil volatiles at 0.25 % concentration had a repellent effect and reduced the offspring numbers. The preference index was –18.7, and the number of laid eggs decreased by almost 40 %. The fumigation effect of the *L. cubeba* oil on the greenhouse whitefly was most noticeable. *L. cubeba* oil (9.0 and 6.0 μl/l) caused the 90 % death of adults and a decrease in the number of eggs by 98.2 and 93.8 %, respectively, compared to control. Citral had no repellent effect but its fumigation activity was not inferior to that of essential oil. The maximum used citral concentration of 6.0 μl/l led to 85.9 % mortality of adults and a decrease in the number of eggs by more than 90 %. Our findings suggest prospects of the *L. cubeba* oil application as a fumigant and repellant aainst *T. vaporariorum* in greenhouses.

Keywords: essential oil plants, citral, *Trialeurodes vaporariorum*, whitefly, toxicity, fumigation, repellent effect

Greenhouse whitefly *Trialeurodes vaporariorum* Westw. (*Hemiptera*, *Aleyrodidae*) can affect 859 plant species from 469 genera and 121 families [1]. Suppression of plant development and a decrease in yield resulted from phloem sap feeding and the sooty fungus infection developed on the honeydew that are secreted by the whitefly. *T. vaporariorum* actively transmits plant viral diseases [2], therefore, even with a small number of pests, frequent use of insecticides is necessary, which provokes the development of whitefly resistance [3].

In the search for new effective means of protection, environmentally low-hazardous substances are preferable. Vegetable essential oil that have various effects on phytophages, from direct toxicity to the regulation of behavior and development of arthropods, are of considerable interest [4, 5]. There are approx. 3,000 ethereal plants in the world that produce and accumulate essential oils, but only 200 species, containing a sufficient amount of the product of the required quality are of commercial importance. Among the ether-bearing plants cultivated in Russia, crops grown for grain and flower-herbaceous raw materials predominate (fruits of coriander, anise, fennel, cumin, dill, flowers and green mass of lavender, wormwood, hyssop, sage, rose, mint, oregano). However, only 6-8% of the world's essential oils derive from the Russian Federation.

Currently, many facts confirm the biological activity of plant essential oils against whiteflies [6, 7]. Vegetable oil from *Litsea cubeba* (Lour.) Pers. (family *Lauraceae*) with insecticidal properties are promising [8-10]. The natural ranges of this deciduous shrub or a small tree plant are southern China, Japan, Southeast Asia, the mountainous regions of Taiwan, Thailand, northeast India, Korea, Vietnam and Indonesia. Despite the dependence of the chemical composition of the oil on the location of *L. cubeba* and the plant parts used [11], 59 of its components were identified. Citral is the main component of this oil, regardless of the growing zone [12].

This work, for the first time, submits information on the effectiveness of the *L. cubeba* essential oil and citral as fumigants and repellents to control the greenhouse whitefly population.

The aim of our work was to reveal mechanisms of action of *Litsea cubeba* essential oil and its main component, citral, on the whitefly *Trialeurodes vaporariorum*.

Materials and methods. The whitefly was grown under laboratory conditions on bean plants (*Phasŭolus vulgaris* L.) at 24±1 °C and a 16 h light period. The essential oil of *L. cubeba* and citral were obtained from the Crop Research Institute (Prague, Czech Republic).

For tests, 1% solutions of *L. cubeba* essential oil or citral were prepared by dissolving 100 μl of the substance in 900 μl of ethanol, followed by the addition of 9 ml of water with stirring. Concentrations 0.5, 0.25 and 0.125% were obtained by sequential dilution with water.

Before starting the experiments, the phytotoxicity of *L. cubeb*a essential oils and citral was evaluated to determine the maximum possible concentration. Bean plants were sprayed with solutions of substances and observed for 7 days.

The exact number of whiteflies in each replication (live and dead) was counted. The abundance of the daughter generation was calculated per one original individual.

When studying the effect of *L. cubeba* essential oil on the embryonic stage of *T. vaporariorum*, bean plants grown individually in 200 ml cups were placed in cages with whitefly adults for colonization. After 1 day, adults were removed. After counting the eggs laid, the plants were treated with a 0.25% oil solution until the drops closed, the control plants were treated with water. The effect was assessed by the number of hatched larvae, formed puparia and hatched adults.

To evaluate the effect of the essential oil on the larvae, the plants were colonized as in the previous experiment, but after the adults were removed, the plants were placed in a clean box for 9 days. Appeared larvae were counted before treatment with 0.25% oil solution (in control with water). Further counts were carried out a described above.

Under conditions of forced maintenance of *T. vaporariorum* imago, bean plants in the experiment were treated with 0.25% oil solution, in the control with water. Plants were indicidually placed in 10 l cylinders, 30 whitefly adults were released into the cylinders without separation by se. The cylinders were covered with mill gas to prevent the escape of the phytophage and normal ventilation. After 1 day, the number of adults and eggs laid on the plants was counted. Further counts were carried out as in the test with egg treatment.

To study the effect of the *L. cubeba* essential oil and citral on the choice of plants for feeding and oviposition by *T. vaporariorum*, 2 experimental and 2 control plants were placed in Plexiglas cages (60×60×60 cm) with ventilation holes, and 60 adult whiteflies were relized to each cage. After 1 day, the number of phytophages on plants and the number of eggs laid were counted. The influence of the test samples on the attraction of adults was assessed by the preference index (PI): PI = (Xc – Xt)/Xtot, where Xc is the number of individuals on the control plant, Xt is the number of individuals on the test plant, and Xtot is the total number of attracted individuals.

The attractiveness of plants for the development of offspring was determined by the decrease in the number of eggs (%) = [(Xc – Xt)/Xc] × 100, where Xc is the number in the control, Xt is the number in the test [13].

To assess the fumigation effect of the *L. cubeba* essential oil and citral on *T. vaporariorum*, the essential oil or citral was diluted in ethanol to a certain concentration and applied to filter paper (dispenser), 10 μl per repetition. The dosages were 9.0. 6.0. 4.5. 3.0 and 2.25 μl/l air. In the control, 10 μl of ethanol was applied to the dispenser. After the solvent had evaporated (in 2 min), the dispenser was attached to the inside of the lid of a 265 ml plastic container; a bean leaf was placed on the bottom, the petiole of which was in an Eppendorf tube with water. After the release of phytophage adults (30 individuals) into the container, it was tightly closed with a lid. After 1 day, live and dead individuals and laid eggs were counted. There were 10 repetitions in each treatment. Citral was evaluated at concentrations used for *L. cubeba* essential oil.

Mortality was calculated by the O. Schneider-Orelli formula [14]. The effect of test samples on the number of eggs was calculated by the W.S. Abbot formula [13].

Statistical processing was carried out using the MicroCal Origin program, version 3.01 (https://microcal-origin.software.informer.com/). Mean values (*M*) and standard errors of means (±SEM) were calculated. Experimental data were analyzed using one-way analysis of variance (one-way ANOVA), mean values were compared using Tukey's HSD test. Differences between the means were considered significant at $p \leq 0.05$.

Results. The evaluation of the phytotoxicity of the tested samples showed that the maximum concentration that did not adversely affect the bean plants was 0.25%.

When whitefly eggs were treated with *L. cubeba* essential oil during the entire preimaginal period, none of the tested concentrations affected the vital parameters of the phytophage. Death at the studied stages did not statistically differ from the control. A similar pattern occurred after the treatment of larvae. The average total death for the entire period of observation (before emergence of

adults) did not exceed 13% and did not differ significantly from that in the control (Table 1).

When adults of the whitefly were kept on plants treated with *L. cubeba* oil, a statistically significant decrease (by 25%) in the number of eggs laid occurred compared to the control at a concentration of 0.25% ($F = 4.55915$, $p = 0.04674$). The total death did not differ significantly over the treatments ($F = 3.66306$, $p = 0.07167$) (see Table 1).

1. Stages of ontogeny of *Trialeurodes vaporariorum* Westw. under the influence of the *Litsea cubeba* (Lour.) Pers. essential oil (0.25% solution, *M*±SEM; a lab test)

Treatmen	Original numer of individuals	Numer of laid eges per 1 imago	Laid egg decrease, %	Numer of dead individuals.			Average dead individuals
				embryonic stage	larvae stage	puparia	
Embryonic stage:							
test	254			3.30±1.62	2.30±1.21	1.30±0.78	6.90±2.17
control	270			2.30±1.01	1.90±0.89	3.00±0.85	7.20±1.72
Larvae stage:							
test	225				8.06±0.85	4.35±1.13	12.4±1.04
control	218				5.91±1.05	5.34±1.40	11.2±1.75
Imafo stage:							
test	301	0.68±0.07*	25.3	7.30±1.56	6.50±0.92	4.20±1.47	17.9±1.39
control	282	0.91±0.08		4.90±1.22	5.00±0.68	4.80±1.22	14.7±0.91

* Differences from control are statistically sifnifnicant at $p \le 0.05$ (see the "Materials and methods" section.

At 0.25% concentration of essential oil, the phytophage gave preference to control plants for feeding and laying eggs, the PI was 18.7, and the number of eggs laid decreased by almost 40%. After treatment of plants with 0.125% essential oil, the repellent effect was almost completely absent and all parameters did not significantly differ from the control ($F = 0.88411$, $p = 0.35953$ for the distribution of imagoes, $F = 0.37043$, $p = 0.55037$ for the number of eggs). Citral had no effect on the whitefly behavior (Table 2).

2. Free choice of bean (*Phasŭolus vulgŏris* L.) plants by greenhouse whitefly *Trialeurodes vaporariorum* Westw. for feeding and oviposition depending on the concentration of the *Litsea cubeba* (Lour.) Pers. essential oil and citral (*M*±SEM; a lab test)

Concemtration, %	Imago distribution on plnts, number of individuals		Preference index	Number of eggs		Decrease in egg number, %
	test	control		test	control	
Essential oil						
0,25	13.9±1.48*	20.3±1.26	–18.7	10.5±1.92*	17.1±2.18	38.6
0,125	15.8±2.01	18.5±2.06	–7.9	10.3±1.78	11.7±1.46	12.0
Citral						
0,25	15.3±1.75	19.7±2.12	–12.6	8.5±1.28	10.9±1.54	22.0
0,125	16.6±2.57	17.7±3.60	–3.2	8.8±1.22	9.2±1.27	4.3

* Differences from control are statistically sifnifnicant at $p \le 0.05$ (see the "Materials and methods" section.

3. Fumigation action of the *Litsea cubeba* (Lour.) Pers. essential oil and citral on the viability of the greenhouse whitefly *Trialeurodes vaporariorum* Westw. adults and the abundance of daughter generation (*M*±SEM; a lab test)

Dosage, μl/l	Total number of individuals	Imago death rate, %	Death rate including control, %	Egg number per I imago	A decreas in egg number, %
Essential oil					
9.0	290	97.1±1.22*	97.1	0.02±0.006*	98.2
6.0	275	91.3±2.93*	90.3	0.03±0.011*	93.8
4.5	288	54.2±7.53*	51.5	0.06±0.019*	90.6
3.0	284	3.0±1.57	0.6	0.53±0.039	7.0
Citral					
6.0	287	86.0±5.33*	85.8	0.03±0.014*	94.3
4.5	272	73.9±3.77*	72.7	0.07±0.029*	87.5
3.0	267	10.1±2.87	6.9	0.31±0.072*	57.8
2.25	290	7.0±2.35	6.3	0.35±0.056*	45.9

* Differences from control are statistically sifnifnicant at $p \le 0.05$ (see the "Materials and methods" section.

When studying the fumigation effect of test samples on *T. vaporariorum*, *L. cubeba* oil (9.0 and 6.0 μl/l) caused the death of 90% of adults and a 98.2 and 93.8% decrease, respectively. in the number of eggs compared to the control. At a concentration of 4.5 μl/l, about half of the tested insects died, while the number of eggs in the experiment decreased by 90.6%. After another 1.5-fold reducing the dosage, the effects were completely leveled (Table 3). The use of citral at the maximum concentration (6.0 μl/l) with this mode of exposure led to 85.9% death of adults and a decrease in the number of eggs by more than 90%. A sharp decrease in toxicity, similar to *L. cubeba* oil, was found at 3.0 μl/l, and the negative effect on fertility remained (see Table 3).

The scientific literature provides sufficient information on the mechanisms of action of the essential oil of L. cubeba and citral on arthropods. Thus, *L. cubeba* essential oil is characterized by pronounced contact toxicity for some species of *Coleoptera*, e.g., adults of *Lasioderma serricorne* (LD_{50} 27.33 μg/cm^2) and *Liposcelis bostrychophila* (LD_{50} 71.56 μg/cm^2), *Tenebrio molitor* larvae and beetles (LD_{50} 21.2 μg/cm^2), *Sitophilus zeamais* [8, 9]. For *Trichoplusia ni* caterpillars, *L. cubeba* oil showed moderate toxicity (LD_{50} 112.5 μg per larva) [10].

In our experiments, the essential oil of *L. cubeba* (0.25%), when 1-day-old eggs and 1-2-day-old whitefly larvae were treated, did not have a negative effect. Observation of the treated individuals before the emergence of adults did not reveal any differences in mortality between the experiment and control. However, when assessing the effect of oil (0.25%) on the phytophagous imago and their offspring, a decrease in the fertility of the whitefly on the treated plants was shown, and further development during the preimaginal period did not differ in the experiment and control.

Repellent property of the *L. cubeba* essential oil was clearly demonstrated on the beetles *Sitophilus zeamais* and *Tribolium castaneum* [15], mosquitoes *Aedes albopictus* [16], termites [17], and ants *Monomorium pharaonis* [18]. Citral acts as repellent against the mosquito *Aedes albopictus* [19] and the beetle *Lasioderma serricorne* [20].

Our expeiments on the behavioral responses of the whitefly also also revealed a decrease in the attractiveness of plants treated with the 0.25% essential oil of *L. cubeba* for both feeding and oviposition. At the 0.125%f dosage, the revealed effects leveled out. Citral, even at the maximum concentration of 0.25%, did not cause significant changes in the behavior of the phytophage compared to the control. It is possible that higher concentrations of oil and citral would also have had an effect on the greenhouse whitefly *T. vaporariorum*, but the phytotoxicity did not allow an increase in the dosage for treatment. The phytotoxicity in oils was reported earlier [21, 22].

The essential oil of *L. cubeba* and citral showed the greatest efficiency in fumigation, both having a direct toxic effect on adults and reducing the abundance of the daughter generation. *L. cubeba essential* oil has fumigation properties against several harmful arthropods. The examples are the beetles *Lasioderma serricorne* and *Liposcelis bostrychophila* (LD_{50} 22.97 and 0.73 mg/l, respectively) [8], the ants *Solenopsis invicta* (more than 90% death at a dosage of 5.33 μl/cm^3) [23], the larvae of the gall midge *Camptomyia corticalis*, a pest of shiitake mushrooms (LC_{50} 3.46 mg/cm^3) [24], and tobacco whitefly *B. tabaci* (100% mortality at 2.4 μl/cm^3) [25].

Fumigation properties was also described for citral against the cabbage moth *Plutella xylostella* (LC_{50} for adults 1.65 mg/l, for larvae of the 1st age 0.35 mg/l, for eggs 4.28 mg/l) [26], beetles *Tenebrio molitor* [9], ants *Solenopsis invicta* (more than 90% death at a dosage of 5.33 μl/cm^3) [23]. We have previously

identified similar properties of *L. cubeba* against the dangerous quarantine pest *Frankliniella occidentalis* which is often present together with the whitefly on the same crops in greenhouses [27, 28]. The concentrations we studied were significantly lower than those reported in the literature, and the death rates of the whitefly were similar to those reported. The toxicity of the tested samples was comparable at the same concentrations, but, unlike *L. cubeba* essential oil, citral reduced the number of eggs even at lower dosages.

A comparison of the whitefly behavioral response and viability under the influence of two volatile products, the essential oil and citral, did not reveal a common pattern in the effects of a single substance (citral) or the multicomponent oil of *L. cubeba* on fecundity of the phytophage. Some authors suggested that the biological activity of vegetable essential oils is due to the synergistic effect of the compounds that make up the composition [23, 29]. Therefore, it is not always possible to expect that a single substance, even if it is significantly dominant in an essential oil, will be more active than the original product itself. The delayed emergence of pest resistance to essential oils and various mechanism of their action may be due to the multicomponent nature of these bioactive substances.

Thus, at the initial stages of embryonic and larval development of greenhouse whitefly *Trialeurodes vaporariorum*, the *Litsea cubeba* essential oil at a concentration of 0.25% did not have either a direct toxic effect or an aftereffect. Contact of adults with plants treated with essential oil at the same concentration caused a 25.3% decrease in the number of eggs laid. Oil volatiles of the oil at the 0.25% concentration had repellent effects (the preference index accounted for 18.7) and reduced the offspring abundance (38.6% reduction in egg count). In 0.25% citral, these properties were less pronounced. The oil was the most effective against the greenhouse whitefly during fumigation. At a dosage of 4.5 μl/l, more than 50% of whitefly adults died and the number of eggs decreased by 90.6%. The same properties were characteristic of citral (4.5 μl/l) with the estimated values of 72.7 and 87.5%, respectively. The fumigation and repellent effect of *L. cubeba* essential oil and citral on the whitefly that we have revealed indicates their ability to reduce the abundance of the phytophage. Our findings prove that *L. cubeba* oil is promising as a fumigant and repellent against *T. vaporariorum* in greenhouses where the phytophage develops year-round in 10-16 generations, regardless of weather conditions, and there are strict phytosanitary requirements for the applied protective preparations. Further studies in greenhouses will substantiate the effectiveness of *L. cubeba* essential oil in more details. The mode of applications will also depend on the test sample properties. Fumigation activity can prevent the spread of whiteflies during the transportation of plant materials and crops, while repellent action can reduce plant colonization by phytophages.

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