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CONTENTS

Agrophysical Research Institute: from basic physics towards practical plant growing (1932-2017)

AGROPHYSICAL, GENETIC AND BREEDING ASPECTS OF AGROBIOCENOTIC CONTROL IN AGRONOMY
(towards 85 Anniversary of Agrophysical Research Institute, Russia)

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A b s t r a c t

This overview dedicated to 85 anniversary of Agrophysical Research Institute gives a retrospective of agrophysics formation as a specific research field covering investigation of physical, agronomical and biological factors to control agroecological systems. The article describes achievement in physics, mathematics, biology and pedology that ensured transition from a descriptive agronomy to the agronomy based on evaluation of the factors essential for plant grow and development, on plant productivity calculation, and on managing productivity of crops by special agrotechnologies. The development of IT communication and precision agricultural technics equipped with detectors of global targeting system, specific sensors and software, as well as the use of geographic information systems led to a new conception of plant yield control, i.e. a precision agriculture. Our researches are focused on computer-aided design and realization of precise agrotechnologies in field conditions. Further progress in plant growing seems to be due to modern genetic and breeding allowing to improve precision agriculture both for populations and individual plants influenced by different ecogeographic conditions. Recent approaches in genetics can speed up breeding new varieties for specific use in precision agriculture. In two experiments which differed only in temperature and illumination regimes under invariability of other parameters, 99 QTL determining 30 agronomically important traits have been identified in spring soft wheat. According to QTL mapping and ANOVA, changes in the temperature and illumination regimes did not influence 21 of 30 studied traits, which remained stable in manifestation. Only nine traits varied under tested conditions, indicating their manifestation to depend on these environmental factors. Elucidation of the QTL effects allows further analysis of the identified correlations and interaction between QTL and environment under natural conditions. In turn, these data allow to provide expression of certain genetic determinants which control physiological basis for economically valuable traits in specific ecogeographic conditions.

Keywords: agrophysics, physics of soil, pedology, precision agriculture, factors of plant growth, physiology and biochemistry of productivity, breeding and genetic analysis

At the end of the 19th century, the founders of Russian agrophysics were outstanding plant growers, soil scientists, agronomists and climatologists K.A. Timiryazev, V.R. Williams, V.V. Dokuchaev, P.A. Kostychev, A.A. Izmailsky, A.G. Doyarenko, A.I. Voeikov and N.I. Vavilov; they first defines agrophysics as the study of physical factors in plant life [1]. A.F. Ioffe started agrophysical research in 1930-1931 at the Physical-Technical Institute of the USSR Academy of Sciences, which coincided in time with founding research institutes by N.I. Vavilov, the president of the Soviet Academy of Agricultural Sciences, and corresponded to his ideas about the role of climatic and physical factors in formation of the properties and productivity of species. N.I. Vavilov supported the proposal of

A.F. Ioffe. By the decision of the Board of the People's Commissariat of Agriculture of the USSR (January 5, 1932) and the Presidium of the Academy of Agricultural Sciences No. 89 (January 7, 1932), the Physico-Agronomical Institute (FAI), now the Agrophysical Research Institute (API), was founded.

Scientific principles and retrospective. According to A.F. Ioffe, the first director of the API, agrophysical studies must transform agronomy from descriptive discipline into the practices and technologies of managing production process in the fields based on measurements and calculations. The problems he has formulated [2] are so fundamental that they remain strategic milestones of modern agrophysics. It was determined that it is necessary to group the factors affecting agroecological systems into five blocks: light, thermal, moisture, atmospheric and soil in correlation with plant cenosis. Modern trends include studying (i) the fundamental functioning patterns of agroecological systems; (ii) the development of bases, methods and tools, including information ones, for studying physical, physicochemical, biological, and biophysical processes in soil—plant—active layer of the atmosphere and managing the productivity of agroecological systems (sustainable farming and crop production under natural and regulated conditions); (iii) creation of simulation mathematical models of processes and technical means of collecting information on the state of plants and their habitats under continuous stochastic variability [1].

Among those worked at the API in the 1930s, there were N.A. Maksimov, the founder of ecological plant physiology, physicists D.L. Talmud and P.P. Kobeko, biophysicist G.M. Frank, soil scientists F.E. Kolyasev and P.V. Vershinin, and plant physiologist V.P. Malchevsky.

In the API laboratory of light physiology established by N.A. Maksimov the energy and regulatory function of light was studied using electric lighting. V.P. Malchevsky showed the influence of illumination on anatomy, biochemical composition, early ripening and productivity in more than 50 plant species and possibility of harvesting several times per year. D.A. Fedorov and V.A. Carfunkel substantiated the use of acetyl cellulose layer instead of glass coatings in greenhouses. B.S. Moshkov, who headed the laboratory of light physiology and light culture from 1945 to 1988, established a scientific school to study the physiological mechanisms of photoperiodism (B.S. Moshkov, V.I. Razumov and M.Kh. Chailakhyan). B.S. Moshkov also discovered the physiological role of leaf as an organ that determines photoperiodicity, and for the first time proved that these reactions are involved in crop precocity, productivity and resistance to various effects. The study of artificial alternation of light and darkness, long-day and short-day photoperiods, the interruption of darkness by light of different spectral composition, and the replacement of darkness by infrared radiation made it possible to simulate the transition of long-day and short-day plants from growth to reproduction with regard to the role of phytochrome, biological clock, biochemical and other changes. Attention was paid to the localization of the perception of photoperiodic signals, the induction of vegetative and seed reproduction. The idea of a comprehensive function of the photoperiodic reaction turned out to be extremely fruitful [3]. In the 1980s, B.S. Moshkov proposed the concept of a juvenile period the feature of which is the independence of its duration from illumination and temperature. The practical result of light-physiological studies is the development of methods for controlling early maturity and productivity in light culture, greenhouses and open ground.

Biophysical and radiobiological approaches were used in the development of an apparatus for determining the resistance of plants to stresses by the intensity of thermally induced chemiluminescence. Electrophysiological analysis of plant tissue viability and molecular genetic evaluation of plant and seed po-

tential productivity were developed. X-ray express diagnostics of sufficiently/insufficiently filled and damaged seeds, radioactive irradiation of seeds [4], magnetic and electromagnetic treatment for increasing sowing quality and yielding were used (G.R. Rick, N.F. Batygin, M.V. Arkhipov, V.N. Savin, V.F. Nikolenko, E.E. Gak). V.G. Karmanov proposed non-invasive methods for the dynamic study of water movement in conducting vessels of stems, water entering the roots, respiration and photosynthesis rates, electrophysiological quantitative characteristics of organs with changing illumination, temperature, moisture supply, and mineral nutrition. This resulted in development of a system of plants phytomonitoring and management of environmental conditions involving a plant as a sensor (V.G. Karmanov, O.O. Lyalin, S.S. Radchenko, S.N. Meleshchenko). Several generations of the first domestic climatic chambers and phytotrons were equipped with devices for regulation of temperature, humidity, intensity and spectrum of the light flux, gas composition of air, etc. Various types of phytotrons were created for research, breeding and agrotechnological tasks.

Bio-cybernetic approaches developed at the API from the mid-1960s. The first computer center in the system of agricultural institutions was organized at the API, which served as a prototype of similar structures in the system of the Ministry of Agriculture of the USSR and Academy of Agricultural Sciences [5].

Resource-saving ways of growing crops on artificial root-inhabited environments, proposed by E.I. Ermakov, laid the foundations of an industrial environmentally safe intensive technology for year-round vegetables growing [1].

The soil with high porosity, large pore sizes and lower density more actively regulates physical and biological processes than microaggregate or structureless soils [6]. P.V. Vershinin, I.A. Romanov, O.A. Agafonov and T.N. Danilova showed that the use of surface active substances and artificial structure-forming agents, including polymeric ones, makes it possible to increase the moisture content of the root layer, the availability of soil moisture for plants, the water resistance of soil aggregates, enhance their hydrophilicity and mechanical strength, improve the water-physical characteristics of solonetzic soils and the illuvial horizon of podzolic soils, and other important soil characteristics. The theory of minimal soil cultivation was further developed in the 1960s-1980s by I.B. Revut and A.V. Sudakov who showed that it is possible to obtain sufficiently high yields while preserving soil, resources and energy consumption.

In soil hydrophysics, a quantitative theory of moisture flow, methods for estimating rate of its evaporation, condensation, transpiration, their regulation and water supply of plants were developed (S.V. Nerpin, B.N. Michurin, N.F. Bondarenko, A.M. Globus) [7]. Mechanisms of heat transfer, the role of mineralogical composition, density and soil moisture in the intensity of heat transfer were revealed in thermophysics [8]. Methods for studying the radiation and heat balance of the active surface have been developed, and methods for its regulation have been outlined. Creation of a heat balance analyzer for research and automation of irrigation (the computer version of the device uses a modern element base) was an important result. The devices for recording parameters of the surface air layer were proposed to control field microclimate (M.A. Kaganov, B.L. Shinderov, Yu.L. Rozenstock) [9].

Dynamic and basic models of agroecosystem productivity, the mathematical theory of population dynamics on genetic, age and sex structure, control of cultured unicellular organisms [11] were a result of the theory of energy and mass transfer processes in the soil−plant−atmosphere system [10].

In the following years, the agrotechnical methods of plant growing in the North [12], the use of translucent polymer materials for greenhouses [13], "physical fertilizers" [14], the technology of using semiconductor materials in measuring instruments, energy devices and refrigerators were developed [15]. Recently, new

research areas appear, such as biological restoration of soils polluted with poisonous organic substances [16, 17], development of theory of plant ontogeny calculation based on computational analogues [18, 19]. The liming of acidic soils [20] is associated with the optimization of mineral nutrition and the physical state of arable horizon. Creation of automated control systems for technological processes in plant growing and agriculture also remains among the main task.

Precision farming system. The knowledge of the climate factors enabling crop production in different zones, the methods for monitoring characteristics of plants and their habitats, and mathematical modeling allow optimized practice when growing plants in large areas. Professor A.F. Ioffe, the founder and first director of the API, was in fact the first in the world who suggested the concept of an "electronic agronomist" in the middle of the 20th century [2] and defined precision farming (PF) as a new cropping paradigm. Realization of this concept became possible due to modern IT-technologies. In PF, a field is considered as a set of homogeneous (quasi-homogeneous) areas with different indicators of fertility and/or the state of sowings. If the differences are agronomically significant, the treatments are appropriately differentiated. The local adjustment of the technological impact is controlled by the onboard computers of agricultural units. Precision increases with the use of geostatistical approaches, the GLONASS system, remote sensing of the Earth and other methods for estimating the intrafield variability.

Computer design and comparison of the efficiency technologies for different intensities in model experiments [21-23] became the basis of domestic PF systems. In 2005-2015, production tests confirmed their prospects for ensuring high (not less than 5-6 t/ha) yields of grain with an increased protein content, regardless of soil and climatic conditions. At the same time, the costs of agrochemicals and fertilizers decreased by 25-30 % with 35-60 % lower load on the environment, a 1.5-1.7-fold increase in the payback of fertilizers and plant protection products, and the economic effect amounted to 840-1460 rubles/ha [24].

PF necessitates intelligent systems to maintain agrotechnologies, including improved measuring complexes and software to predict and monitor soil parameters, weeds, diseases, pests, etc., and to regulate the consequences of the techniques used. This, in turn, requires further in-depth genetic study of yield formation, interactions of the genotype and the environment, and the varietal features at the modern level.

QTL analysis in agrophysical research and improvement of precision farming. Analysis of quantitative trait loci (QTL), which allows us to speed up the selection of targeted varieties for PF systems is one of the biological approaches that can expand the possibilities of PF at the level of both populations (i.e. for specific ecogeographic conditions) and a single plant.

Using QTL analysis, identified QTL may be mapped, cloned and introduced into desirable genotypes by traditional hybridization [25]. The locus determining fruit size of tomato plants is a classic example of QTL identification and the first case of cloning a chromosome fragment that determines a quantitative economically valuable trait in this species [26]. The identification and molecular genetic mapping of QTL for morphological and economically valuable traits in *Triticum aestivum* L. [27, 28] and *Brassica rapa* L. [29, 30] were carried out from 2005 to 2016 on experimental fields in different geographical locations (Leningrad, Moscow, Samara and Kirov regions, Republics of Dagestan and Adygea). QTL mapping performed for the first time at the API in 2012-2013 in agroecobiopolygon, made it possible to identify for the first time yje chromosome loci in spring soft wheat that determined the manifestation of economically valuable traits under controlled conditions.

The chambers of the agroecobiopolygon are protected against external impacts and equipped with the system of microclimate control and regulation, allowing year-round growing plants of different heights (the latter is especially important for agricultural crops), and the devices for remote and contact diagnosis of the physiological and morphobiological state of objects when growing in vegetation vessels or peat-filled shelves [31, 32]. In controlled conditions (preset contrast regimes for the investigated factors with the invariability of the remaining parameters), E.I. Ermakov et al. [31] and G.G. Panova et al. [32] revealed the inheritance of sprouting to stalk-shooting period and stalk-shooting to earing period in wheat at year-round cultivation, and clarified a number of theoretical positions on selection of genotypes by transgressive traits. A methodology was developed for creating wheat lines with parameters corresponding to zonal soil and climatic conditions [31, 32], and in the same way a sort of daikon Peterburgskii (http://reestr.gossort.com/reg/cultivar/14807) was obtained.

The precise QTL identification and localization under controlled environment is based on the character of genotype-environment interaction, regardless of uncontrolled external environmental variables stochastically affecting the manifestation of the studied, primarily quantitative, traits. It is important both for studying plant physiology and genetics and for the breeding practice [25, 33]. Breeders can use QTL analysis data only when the results are reproducible, which was observed for some detected QTL [25, 34]. If the QTL manifestation will depend on environmental conditions, then, for example, in the PF system [21-23], the breeder will be able to correct growing conditions of the plants in such a way that the necessary trait appears. It has been established experimentally [25] that such a dependence of QTL does not always appear. In this case, the QTL position in the linkage group is remained the same regardless of year conditions and geographic location, although the LOD (logarithm of odds) may vary. Mapping QTL in API agroecobiopolygon made it possible for the first time to establish loci that affect the traits under study, primarily productivity [34]. Similar work is needed both in pre-breeding study of genetic determinants to implement PF programs [21-23] and, generally, in genetic and breeding research [35, 36].

Often, low LOD are characteristic of stable QTL which contribution to trait variability typically makes 10-20 %. Probably, stable manifestation of productivity traits is associated with these QTL. In contrast, in QTL, determining variability under certain conditions, the LOD values are often high. QTL, controlling plant size and productivity are mainly located in several linkage groups. Thus, in two experiments under controlled conditions of agroecobiopolygon [34] which differed in the illumination regime (40±0.5 and 50±0.5 W/m^2 PAR) and temperature (24-25/19-20 °C and 28-29/23-24 °C for day and night, respectively), 99 QTL were identified which determined 30 different agronomically significant traits. Based on QTL analysis and single-factor variance analysis, it was found that only 9 out of 30 estimated traits depended on the variable factors. Both used methods of statistical analysis yielded complementary results. In each of the statistical approaches, the maximum likelihood criterion was used, and the statistical significance of the results was determined. The reliability of the relationship between the identified QTL and the polymorphism on one or another characteristic was evaluated using the threshold value of the likelihood ratio for logarithm of odds (LOD-score). The QTL analysis revealed the block structure of the *T. aestivum* genome. The percentage of phenotypic variability due to each of the identified QTL was determined, and it was shown which of the parents introduced one or another QTL allele. The molecular markers genetically linked with the identified QTL were established.

A detailed understanding of the QTL position effects (taking into ac-

count their stability in a specific ecological zone) creates prerequisites for analyzing the observed correlations between the appearance of some QTL and light-temperature regimes, and for establishing the QTL−environment interaction under natural conditions to ensure the expression of genetic determinants of valuable traits in specific ecological and geographical conditions. In addition, QTL for morphological and phenological traits, first identified in different soil and climatic conditions of Russia in the mapping populations of *T. aestivum* and *B. rapa*, can be used to establish the genetic nature of quantitative traits in higher plants and to find out genotype−environment interaction mechanisms in order to develop marker-assisted selection (MAS) methods [25, 33]. Chinese cabbage varieties Yuna, MEGGI, VITAVIR (http://reestr.gossort.com/reg/main/516) and turnip Palitra (http://reestr.gossort.com/reg/cultivar/21036) derived with these technologies, which accelerate selection 2-3 times, are a successful application of this approach.

Thus, the carried out agrophysical research is aimed at the introduction of physical and mathematical methods in agronomy, agriculture, crop production, and productivity management and the scientific provision of precise farming as one of the most actively developed areas of the agro-industrial complex. The development of genetic selection methods extends the possibilities of modern agrophysics and precision agricultural technologies. Here, prospects are associated with the accelerated breeding technologies to derive varieties of different eco-geographical adaptation.

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CROP MODELS AS RESEARCH AND INTERPRETATIVE TOOLS

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Mechanistic (eco-physiological), or process-oriented, approach to simulation modeling of the production process of plants assumes considering the essences of processes and cause-effect relationships in the agroecosystem with a description of their dynamics based on physically interpreted dependencies (as opposed to logically interpreted dependencies at the empirical approach) (A. Di Paola et al., 2016; R.A. Poluektov, 2010). The analysis of the possible use of dynamic simulation models of agro-ecosystems in the mechanistic nature of applied and theoretical research of agricultural biology is presented. The current practice of the development and usage of these models shows their highest suitability for research purposes in comparison to the potential usefulness and relevance to the practical problems of agronomy. Specific examples of model applications demonstrate the possibility of computer-based model experiments to get nontrivial results, which are not directly incorporated into the logic of the model algorithms (V. Badenko et al., 2014; S. Medvedev et al., 2015). The role of simulation model as a tool of obtaining new knowledge and interpretation of the empirically observed phenomena has been showed. To demonstrate the potentials of simulation models for agricultural biology, some results of authors' studies have been reviewed, including analyze of the appearance of a non-monotonic response function of crop yield on the doses of nitrogen fertilizer, the results of computer experiments on interpretation of the effect of the time delay during management of nitrogen feeding «on the leaf», and the joint impact of combined water and nitrogen stresses. Based on analysis of recent publications, conclusions of perspectives of models application to accelerate the plant breeding process were justified. It is concluded, i) further «biologization» of existing models is a prerequisite for a successful development of the dynamic crop growth modeling, and ii) it is necessary to increase the level of scientific validity of model approaches, which are used to describe the biotic processes in the soil—plant—atmosphere system.

Keywords: agro-ecosystems simulation model, crop production process, mechanistic approach, ideotype, plant breeding, breeding, G×E×M-oriented models

Imitation modeling of the plant production process is an independent scientific field with its own methodology, history [1, 2] and qualitatively different approaches to creation and use of computer models. In foreign literature, these approaches are traditionally designated as mechanistic, or process-oriented and empirical ones [3]. The empirical (functional) approach is characterized by broad application of heuristic description of key processes using regression ratios, allometry equations, multiple stress functions, etc. Such formal description at the logical level may perfectly reflect the properties of a real system in the "entry-exit" terms within a relatively narrow class and a limited range of affecting factors but is almost not associated with the essence of physical, chemical and biological effects in the soil—plant—atmosphere system [4]. On the contrary, the mechanistic (eco-physiological) approach implies considering the essence of processes and cause-to-effect relationships in the agroecosystem with description

of their dynamics based on the physically interpreted dependencies (in contrast to the logically interpreted dependencies at the empirical approach). The mathematical apparatus of the theoretical models are a system of differential or difference equations, as a rule, describing the balance of matter and energy for each of the considered spatial and functional compartments. The number of factors and processes, which should be taken into the account for providing a complex, stable and non-contradictory model of yield formation in the sowing within the fully mechanistic approach, severely hinders achievement of such an aim. Therefore, currently basic models of production process [5], which are a synthesis of process-oriented and empirical descriptions, pretend to be scientifically justified.

When the theory and methodology of imitation modeling appeared in agroecology, an issue was raised about the applicability of computer models. Five aims were designated [6], in accordance with which a hypothetical ideal model of the plant production process can be a communication means for specialists, a means of cognition, learning and purposed planning of experiments and an instrument of making decisions to control complex systems. The first two roles are rather related to purely theoretical studies, and the latter three ones are closer to practical agronomy. For a long time, there was an unspoken certainty among specialists that good process-oriented model can be successfully used for achievement of all five aims. However, at the end of the 20th century, doubts were expressed [7], and in the last 20 years, a directly opposite impression has formed. Many publications repeatedly state that there is no universal mathematical model to be equally applied both in theoretical studies and in practical calculations and predictions for wide spatial and time scale, and it is impossible to provide such a model in principle. Therefore, a correct strategy is not to constantly improve some single model platform in order to obtain a universal calculation tool but to create specifically narrowly oriented solutions for each specific problem (ad hoc modeling) [8].

In the world practice, the usefulness of most provided complex basic models for the agricultural production tasks (a support system for technological solutions, operative prediction of yield, mass calculations of potential productivity) appeared to be quite low [9-11]. Planning and controlling authorities use proven simple empirical models (agrometeorological predictions) of the expected yield of the main crops, and the involvement of satellite images and other remote probing data apparently completely resolves an issue of using such a complex instrument as process-oriented imitation models. In precise agriculture, their use also did not develop quite well: the number and variety of factors of inter-field heterogeneity significantly overcomes the functional possibilities of even the most advanced modern models. Moreover, the real variability degree of the determining parameters formally tuned in the most models (water stress, nitrogen nutrition, etc.) is often below the sensitivity threshold of the model algorithms; therefore, statistical models are most often used here [11]. Thus, for agronomical practice most process-oriented models appeared to be no better than their empiric analogues.

A popular application for imitation models could be theoretical science, specifically theoretical agricultural biology. Until recently, most biologists (if bioinformatics and molecular level genome studies are not referred to) were skeptical of computer experiments as a means of cognition and interpretation of observed events. They doubt a possibility of obtaining principally new knowledge from a model, which has been charged only with known facts and regularities. However, for process-oriented models it is only partly correct. At investigation of models that are indeed science intensive, new phenomena may be found or

original explanation for events observed in nature can be found, and such models themselves may serve as an intrinsically valuable scientific instrument and effective replacement of a field or lab experiment. We would confirm this statement below by examples of some mini-discoveries and explanatory hypotheses obtained by us in computer experiments with the models we have developed. First of all, emergent (i.e. not clearly charged to a model) unexpected effects exhibited as a result of and not clearly following from sufficiently simple starting conditions with visible physical interpretation should be related to these. Recently, speeding up of breeding has also become a perspective and actively developed application of dynamic models.

An imitation model as an instrument of hypothesizing and phenomenon interpretation. *Model interpretation of abnormal productivity functions.* One of the most known classic problems of agrochemistry discussed for more than 150 years but still important remains the determination and formalization of crop productivity response to external factors, in particular, to the dosages of introduced fertilizers [5]. Most often smooth unimodal curves or saturation curves are used for approximation of this dependence, which is completely compliant with the effects of increasing dosages of ecological factors (Liebig's and Shelford's laws). At the same time, in open sources one can often encounter a mention that the processing of obtained experimental data has led to identification of a more complex and, importantly, non-monotonous productivity function (further herein only the effect of nitrogen fertilizers would be referred to). In the area of the average values of the affecting factor, there is a trend to an intermediate plateau of the response function or even an intermediate breakdown occurs, and with increased dosages, yield increase is again observed, according to the expected standard trend [12-16].
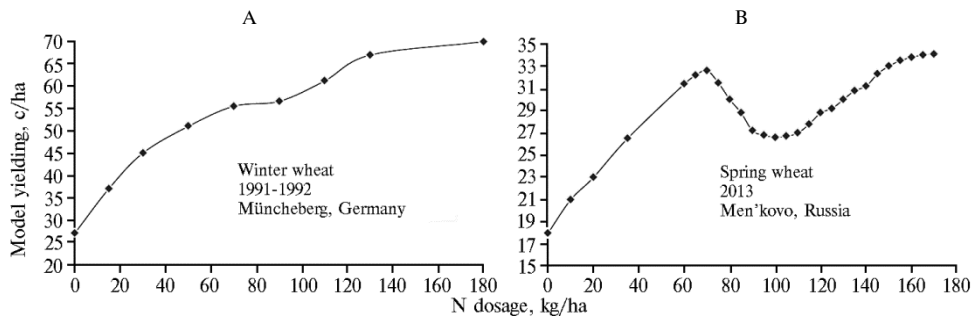
Fig. 1. Model functions of wheat productivity depending on nitrogen fertilizer dosages with "intermediate plateau" effect: A — AGROSIM model (Leibniz Centre for Agricultural Landscape Research, Germany), B — AGROTOOL (Agrophysical Institute, Russia).

It is not very easy to unambiguously and simply explain the intermediate plateau or intermediate breakdown effect. In the joint study initiated by the authors, an attempt was made to use the calculations according to alternative dynamic models of the production process for spring wheat, AGROTOOL [17], and winter wheat, AGROSIM [5], in order to identify the described effect and to give a model interpretation to it. It was successful for both models (Fig. 1); the explanation of non-monotonousness of the obtained productivity function appeared to be different, although the required conditions of reproducibility for the described case for both models were similar (the presence of drought, i.e. a stress period of plant development preceding flowering) [17]. A principal factor in the AGROSIM model developed by specialists of Leibniz Centre for Agricultural Landscape Research (ZALF, Germany), was a partial translocation of structural biomass of roots and sprout in order to provide for energy to support

breathing. Such a phenomenon is observed in the model for medium dosages of pre-sowing fertilizer administration only, because at lower dosages the low value of structural biomass accumulated to the beginning of stress period does not form critical requests for breathing support while at large dosages these effects are covered by the present spare carbohydrate pool. In the AGROTOOL model, the same effect unclearly follows from the formalization of the carbon-nitrogen relationships in the plant realized therein [18].

In accordance with the proposed modeling method [6], the mechanism controlling the distribution of primary assimilates between sprouts and roots (growth functions) is an adaptive algorithm of balanced, i.e. most effective, growth of organs generating N and C, the main substrates for structural compound synthesis during vegetative ontogenesis in a double-stream system with spare metabolite pools.

Timing and microdosing effects in controlling the nitrogen nutrients. The AGROTOOL-based calculations in order to determine optimal timings and dosages of nitrogen nutrients for intensive Daria spring wheat variety in the field tests (Men'kovskii branch of the Agrophysical Institute) gave non-trivial and unexpected results. The calculation experiments in the number of cases demonstrated an unusually high sensitivity of the model to timings of the non-root application of nutrients. Thus, it appeared to be that at certain conditions a 3- to 5- day delay in the treatment, when compared to the reference date, can reduce yield by up to 60 % from the maximum possible level, whereas at "guessing" of the nutrition timing, the model yield sharply increased compared to the control variant even at relatively insignificant (small) dosages of the active compound. Such results could have been ascribed to model imperfection but such behavior is quite well compliant with the relatively widely known and published data of real field experiments [10, 11, 19] confirming significant effects of nutrition timings on the final yield and a possibility to considerably affect it by small fertilizer dosages (microdosing).

The development of a model, i.e. a possibility to trace the growth and development of a model plant "in the tuning mode" allowed for understanding the reasons of the described effects on the algorithmic level. Thus, the performed studies have led to a conclusion that the timing effect (e.g. a drastic reduction in the final productivity at selection of an unfortunate nutrition date) is indeed reproduced in the model at certain combination of several specific conditions (in particular, at insignificant but necessary nitrogen deficit in the soil during the entire vegetation growth). The detailed analysis has shown that the obtained results may be interpreted and naturally follow from the simple assumptions that are comprised in the sub-model of carbon-nitrogen interaction [6, 18]. Thereby, nitrogen nutrition prior to flowering has not only a quantitative but also qualitative effect, regularly affecting growth, which can be both substantially positive and drastically negative.

Combined effect of water and nitrogen stress. It is well known to specialists of practical crop research that at poor moisture supply, i.e. under drought conditions, the introduction of significant dosages of mineral fertilizers may lead to the reverse effect and negatively affect the final productivity of the sowing. This fact may be reproduced in computer experiments with mechanistic agroecosystem models, even if they do not comprise any special non-linear dependencies and mechanisms of combined effect of water and nitrogen stress, and also description of the salt status of the plant is completely ignored.

Thus, the said regularity was obtained at the processing of results of model calculations when analyzing and comparing the sensitivity of two alternative models, AGROTOOL [17] and MONICA [20] to the simultaneous and separate effect

of drought and nitrogen deficit. In order to do this, a multi-factor computer experiment has been planned and carried out in the APEX system, a special control shell for automation of multi-variant calculations and the agricultural crop production models [4].
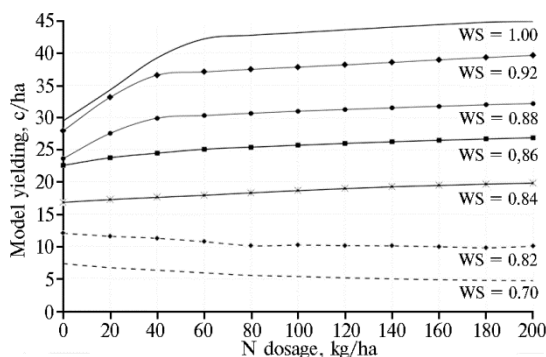
Fug. 2. **Effect of combined influence of water and nitrogen stress on spring wheat (calculation data according to the AGROTOOL model):** WS — water supply.

The control of the water stress parameters was carried out using the module of imitation of the automatic watering system realized in the AGROTOOL model [18]. The example of the obtained results (Fig. 2) demonstrates that the plots of the response function (the dependence of the economic crop on the pre-sowing dosage of nitrogen fertilizers for different conditional moisture supply) appear to be not similar, not equidistant, and, moreover, they are principally different. Thus, under strong water stress the final productivity of the sowing does not grow but falls with an increase in the dosage of the pre-sowing nitrogen introduction. A detailed study of the model algorithms allows explaining the observed effect within the scope of the main used hypothesis on the double-stream C-N control in the plant and the mechanisms of calculating the root penetration depth comprised in the model depending on the moisture content in the soil profile.

Perspectives of using the imitation models in information selection provision. One can hardly recite a more conservative field of agricultural biology than selection. The existing gap between the flow-balance approach to describing physiological processes in plants accepted in all classical agroecosystem models and breeding methods based on the analysis of the quantitative traits at the genetic level appears to be especially terminal. Nevertheless, as traditional agricultural genetics encounters a necessity of an adequate response to modern challenges, complex mathematical models of the production process begin to be seriously considered as a genetic investigation tool. Most of such challenges are associated with the observed global changes, firstly, with the climate change [21]. Already almost no one argues the expectance of a significant increase in the instability if future crop yields due to the growth in the frequency and intensity of extreme effects (such as temperature stress and droughts). A response may be to provide principally novel crop varieties, firstly grain crops [22]. One of unexpected but powerful means of accelerating traditional selection may be the instrumentation of imitation modeling of agroecosystems [23]. These studies are associated with the ideotype term [24-25] or an ideal variety, as accepted in the Russian literature [26]. An ideotype is a theoretical future variety capable of giving theoretically possible yield in accordance with the bioclimatic potential (essentially, a potential aim of selection).

The qualitative assessments of the perspectives of the ideal variety for scientific forecast of the plant state and its separate features at growing under the present conditions have proven to be working [27, 28]; however, all advantages of such an approach can be disclosed only in combination with application of imitation agrosystem models. Thereby, genotypic adaptation, which implies introduction of new traits in the variety, as expected, would be one of the most important future strategies at climate change, and the modeling of agroecosystems may serve as basis for assessment of biophysical potential of crops provided

by such adaptation [29]. As a whole, one can refer to two trends which were formed in modern studies of agroecosystem model application in agricultural ecology and selection. These are the determination of zones beneficial for already existing varieties taking into the account the significant dynamics due to the climate changes, and the justification of requirements to traits of new varieties adapted to the expected environmental conditions.

The first direction is illustrated by the papers [30, 31], which, in particular, show that certain barley genotypes may be promising for the predicted climate change. The authors have proposed the most beneficial zones for specific ideotypes having a combination of several key genetic traits (phenology, leave growth, photosynthesis, drought resistance and grain formation). R.P. Rötter et al. [29] have analyzed the studies reflecting the second trend. Their review describes the main limitations and limits of using imitation modeling for supporting selection of agricultural crops and also outlines examples of assessment and formation of grain varieties for expected conditions using modeling. The agroecosystem models are used for identification of traits required for future varieties [32]. In the Western scientific community, the use of imitation models for acceleration of selection process has already become practical. Based on the advanced biologization of existing models, works have been started for their adaptation for novel application fields. In Russia, this trend should also be activated.

Therefore, despite certain objective limitations for practical agronomy, the imitation agroecosystem model can and should be viewed as a real tool for formalization of knowledge about processes and effects determining growth and development of cultivated plants. Moreover, the production process models help investigators in the agricultural biology to accelerate a transition from passive accumulation of plant description data to the active control of knowledge about processes and effects in agroecosystems [33-36]. The international symposium "Crop Modeling for Agriculture and Food Security under Global Change" (Berlin, 2016) has clearly outlined achievements, set specific goals and determined perspectives of using the imitation modeling in agricultural biology. It was noted that models can be a driving force of progress in improving agricultural crops, being a unique instrument of analysis and determination of beneficial genotypes (G, genotype) and management methods (M, management) for specific soil and climatic conditions (E, environment) taking into the account the resources present [37]. Only modeling allows for effectively analyzing the full spectrum of the G × M × E combinations and provides a rational basis for development and testing of novel wheat ideotypes optimized for target agricultural landscapes and future climatic conditions. The next generation models should comprise the so-called genetic coefficients for modeling differences between hybrids (gene-based crop model) [38]. Genomic forecasting using agricultural crop models is capable of performing better than statistical methods using only genetic data [39]. The main direction of model development is associated with improvement in the G × M × E interaction.

Thus, it is expected that substantial biologization would mainly determine the development of mechanistic models of the production process of agricultural crops. Moreover, we believe that within the scope of this direction results of any field experiments should be used only for identification of models, not for building them. Thereby, in the first place one should use hypotheses about the essence and driving force of biological processes in plant. Due to realization of such ideal plan, the model becomes an instrument of producing new knowledge for studying the agroecosystem response to all possible effects including those which still cannot be reproduced in a field experiment (e.g. associated

with the climate change).

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MODEL-BASED ASSESSMENT OF SPATIAL DISTRIBUTION
OF STOMATAL CONDUCTANCE IN FORAGE HERB ECOSYSTEMS

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A b s t r a c t

Stomatal conductance is an important factor which controls carbon and water exchange. By changing stomatal width, a canopy simultaneously controls both the carbon dioxide supply and water loss during transpiration. Stomatal conductance is a parameter of photosynthesis and can help to estimate canopy growth and development in ecosystems. Therefore, it is a necessary component of transpiration models. The aim of this study was to validate a stomatal conductance model using radiometric measurements of energy balance parameters for vegetated surfaces: vegetated surface temperature, sensible and latent heat flux. Considering atmospheric surface layer stability, the crop was assumed to be a «big-leaf», with stomatal conductance influenced by environmental factors. External conditions not only control stomata width, but also directly affect the transpiration processes. We have tested the stomatal conductance model by J.M. Blonquist et al. (2009) based on radiometric canopy temperature and energy balance components such as latent and sensible heat fluxes. The applicability of the model for estimating stomatal conductance using automated ground-based measurements and remote sensing was first shown. Observations were carried out at two locations with forage herbs (60°5′6″N, 30°25′27″E and 60°5′16″N, 30°24′32″E) at Bugry in the Leningradskaya Province (on May 15 and 31, 2016, respectively). Model inputs, such as air temperature and humidity, atmospheric pressure, wind speed, radiometric temperature and net radiation of vegetated surface were measured with automatic mobile field agrometeorological equipment AMFAE (Agrophysical Research Institute), with measurements taken every 90 seconds. Ground observations were carried out simultaneously with LandSat-8 satellite data surveys. LandSat-8 is an American Earth observation satellite, it contains two instruments: OLI (Operational Land Imager) has 5 visible bands and 4 near infrared bands, TIRS (Thermal InfraRed Sensor) has 2 longwave infrared bands. LandSat-8 data is freely available on the US Geological Survey. Atmospheric correction of satellite imagery was made using the 6S (Second simulation of the satellite signal in the solar spectrum) open source model with publicly available data of aerosol optical depth at 550 nm provided by the MODIS system and the global digital elevation model ASTER GDEM (data is freely available on the US Geological Survey). Components of the energy balance including net radiation, soil heat flux, sensible and latent heat flux were calculated with the SEBAL (Surface Energy Balance Algorithm for Land) model by W.G.M. Bastiaanssen (1998) using the ground observation meteorological data from AMFAE. Obtained maps of net radiation and sensible and latent heat fluxes were used to estimate the spatial distribution of stomatal conductance over the forage herbs. For stomatal conductance calculations the LandSat-8 data for pixel values representing dense vegetation (NDVI > 0.7) were used. As a result of the study, maps of forage herbs stomatal conductance were obtained depending on the canopy temperature and the components of the energy balance with a stratification of the atmosphere boundary layer.

Keywords: stomatal conductance, stomatal resistance, transpiration, energy balance equation, vegetation surface temperature, LandSat-8, automatic mobile field agrometeorological equipment — AMFAE

Plants can change an aperture of stomatal pores quickly to optimize environmentally dependent water loss (transpiration) and CO_2 absorption through stomata. The stomatal conductance varies under the influence of numerous fac-

tors. The transpiration rate is regulated by the stomatal conductance that may be simulated for a leaf or a crop canopy. The state-of-the-art physiological equipment (diffusion porometers, gas analyzers) [1] enables to measure the transpiration and stomatal conductance on a leaf surface. However, it is impossible to estimate the values under study by direct measurements.

As an alternative, models reflecting the stomatal conductance dependence on environmental factors were proposed. Input data here is meteorological and actinometric measurements, and aerodynamic parameters of the underlying surface. Mathematical description of the stomatal conductance has been widely used in recent decades. The review of G. Damour et al. [1] considers 35 similar models. Some models are the most popular in current studies [2-4].

Quantification of H_2O and CO_2 flows in a biogeocenosis under the influence of climatic factors requires both experimental and model-based research methods to be developed. Simulation approaches is a main mechanism for studying the energy and mass exchange and taking into account the spatial heterogeneity and biological peculiarities of plant development during the growing season [5, 6]. A great number of approaches were elaborated to describe flows of H_2O and CO_2 in the soil—vegetation—surface air system. It was shown that stomatal resistance for water vapor and CO_2 diffusion depends on a large number of parameters which describe a plant habitat (e.g. solar radiation, air temperature, surface air conditions, wind velocity, CO_2 concentration, air humidity and soil moisture deficit, etc.) and status [7-11].

Stomatal regulation of diffusion resistance may be considered as a cybernetic system with different feedbacks [7]. One of them is activated when CO_2 partial pressure in intercellular spaces changed; the other one provides a rapid reaction of a stomatal apparatus to relative air humidity fluctuations. Both feedbacks are manifested within a few minutes and control the stomatal conductance at optimal water conditions.

There are several information levels in simulating the water and carbon exchange of plants. The first three levels do not take into account the stomatal regulation, and in this the mass exchange is calculated based on the water potential of soil or determined based on data on water potential of a leaf, solar radiation and other environmental factors. At the fourth level, special models capable of simulating the stomatal apparatus reaction to fluctuating environmental factors are used. By analogy with the Ohm's law, it has been proposed to use resistance (r_s) instead of stomatal conductance (g_s) (7). In the model described by G.S. Campbell et al. (12), the stomatal conductance, heat and water fluxes above the vegetated surface are measured in molar units. The stomatal conductance to resistance relationship is nonlinear and depends on air temperature and atmospheric pressure [13]. Stomatal resistance (r_s, $s \cdot m^{-1}$) is expressed through stomatal conductance (g_s) and molecular air density (ρ_{mol}, $mole \cdot m^{-2} \cdot s^{-1}$) as follows:

$$r_s = \frac{\rho_{mol}}{g_s} \ .$$
(1)

The molecular air density depends on the atmospheric pressure (P) and ambient air temperature (T) [12]:

$$\rho_{mol} = 44.6 \frac{P}{101,3} \frac{273,15}{T} \ .$$
(2)

Water vapor fluxes near the vegetated surface by means of turbulent and molecular exchanges. A water vapor flow from a stomatal cavity is defined by the molecular diffusion and, in accordance with the Fick's law, is proportional to

the local gradient of water vapor density.

In this study, we for the first time have shown the use of a stomatal conductance calculation model [13] based on satellite data on the surface temperature distribution and visible and near infrared reflectance in conjunction with parameters obtained in simultaneous ground truth measurements using the predeveloped automatic mobile field agrometeorological equipment (AMFAE) [5]. As a result, thematic maps of the stomatal conductance spatial distribution on the fields studied have been compiled. In addition, the stomatal conductance at a single point was calculated based on the AMFAE measurements.

The aim of the study was to test techniques for automatic monitoring of stomatal conductance and its spatial distribution based on spaceborne remote sensing data combined with ground-based meteorological measurements with the use of mathematical simulation.

Technique. The stomatal conductance was calculated for forage herbs grown on the fields near Bugry village (Leningrad Region, 60°5′6″N, 30°25′27″E) as of May 15, 2016 and May 31, 2016.

To estimate the stomatal conductance, the model of J.M. Blonquist et al. [13] was selected. It includes automatic ground-based vegetation temperature measurements by infrared thermometers in conjunction with the calculation of radiation balance and components of heat balance. The equation of heat balance above the vegetated surface is as follows:

$$R_{nc} = H_c + \lambda E_c + A_n ,\qquad(3])$$

where R_{nc} is the radiation balance above the vegetated surface, $W \cdot m^{-2}$; H_c and λE_c are sensible and latent heat fluxes, respectively, $W \cdot m^{-2}$; A_n is carbon dioxide flow, $W \cdot m^{-2}$. The carbon dioxide flow in the heat balance is generally ignored (except for cases when its determination constitutes the aim of a study).

Remote sensing data was obtained from American LandSat-8 (freely available on U.S. Geological Survey Site: https://www.usgs.gov/). The satellite is equipped with special instruments: OLI (Operational Land Imager) takes visible (5 channels) and near infrared (4 channels) images, TIRS (Thermal InfraRed Sensor) takes far infrared (thermal) images (2 channels) (14).

The brightness temperature (satellite images) was converted into the true one using an algorithm [15] based on the comparison of data obtained from two LandSat-8 heat channels and ground surface classification by underlying surface types.

Information about the spatial distribution of radiation balance, total evaporation and turbulent heat flux taking into account the surface air stratification was obtained with the SEBAL (Surface Energy Balance Algorithm for Land) model [16, 17] based on the heat balance equation and visible, near infrared (IR) and thermal data of satellite sensing.

Atmospheric correction of the visible and near IR satellite imagery was made using the 6S open source model (Second simulation of the satellite signal in the solar spectrum) [18-20] that described the electromagnetic radiation transmission through the atmosphere. The atmospheric condition parameters required for the calculations (aerosol optical thickness of the atmosphere at λ = 550 nm) are freely provided in the MODIS system [21] and the global digital elevation model ASTER GDEM [22] allowing for extracting local data (U.S. Geological Survey).

Additional ground-based measurements of the wind velocity at a single point (required for the SEBAL model), as well as the air temperature and humidity, atmospheric pressure (required to insert in the spatial stomatal conductance distribution model) [13] were made simultaneously with satellite observations using the automatic mobile field agrometeorological equipment (AMFAE) (designed at

Agrophysical Research Institute) [5] fitted with air temperature HEL-705-U-1-12-C2, relative air humidity HIH-4602-C and atmospheric pressure MPX4115AP sensors (Honeywell International, Inc., USA), as well as wind velocity sensor Windgeschwindigkeitssensor (Hydrometeorologische Instrumente und Messanlagen, Germany) (with meteorological ground truth measurements taken every 90 seconds).

Being a ground-based meteorological station for ground truth measurements, the AMFAE was also used as an independent instrument for automatic monitoring of the stomatal conductance and components of the heat balance at a single point. The AMFAE has an infrared temperature sensor Optris CT LT (Optris GmbH, Germany) and balance meter Peleng SF-08 (OAO Peleng, Republic of Belarus) to determine the vegetated surface temperature and radiation balance based on which the stomatal conductance at a single point was calculated.

The stomatal conductance g_s was calculated by the formula of J.M. Blonquist et al. [13]:

$$g_s = \frac{g_V P\,[(R_{nc} - A_n) - g_H C_p (T_c - T_a)]}{g_V \lambda (e_{sc} - e_A) - P[(R_{nc} - A_n) - g_H C_p (T_c - T_a)]}\,, \tag{4}$$

where T_c is radiometric temperature of the vegetated surface, ˚C; T_a is air temperature, ˚C; C_p is specific heat capacity at constant pressure, $J \cdot kg^{-1} \cdot K^{-1}$; λ is latent heat of vaporization, $MJ \cdot kg^{-1}$; e_{sc} is water-vapor saturation pressure at the vegetation temperature T_c, kPa; e_a is partial water vapor pressure, kPa; P is atmospheric pressure, kPa; g_H is heat conductivity in the surface air, $mole \cdot m^{-2} \cdot s^{-1}$; g_V is water vapor conductivity in the surface air, $mole \cdot m^{-2} \cdot s^{-1}$.

The heat conductivity (g_H) in the surface air where flows were not affected by molecular viscosity were calculated taking into account the underlying surface roughness [5]. The lower and upper levels of the layer depend on roughness parameters for a flux of momentum (z_m), heat flux (z_h) and water vapor flux (z_v) over the displacement layer (d) [23]:

$$g_H = \frac{u \rho_{mol} k^2}{[\ln(z_u - d/z_m) - \Psi_m][\ln(z_{Ta} - d/z_h) - \Psi_h]}\,. \tag{5}$$

The roughness parameters may be defined as $d = 0.65 h_c$, $z_m = 0.123 h_c$, $z_h\ 0.1 z_m$, $z_v \approx z_h$; z_u and z_{Ta} are levels of air velocity and temperature measurements, respectively; h_c is canopy height; k is Karman constant; u is wind current velocity at the level of z_u; Ψ_m, Ψ_h are universal functions for the flux of momentum and heat flux [23-25].

The water vapor conductivity in the canopy layer g_V is calculated similarly to g_H by formula (5) using the corresponding parameters.

Results. The stomatal conductance g_s values obtained in the first determination (on May 15) based on the model [13] using the automatic mobile field agrometeorological equipment (AMFAE) when measuring the vegetated surface temperature and radiation balance at a single point generally were lower than those obtained in the second determination (on May 31) (Fig. 1).

The observed result can be explained by that, on May 31, the vegetated surface temperature under similar meteorological conditions was lower than the air temperature, there was an oasis effect, and the turbulent heat flux was directed towards the vegetated surface. When measuring on May 15, the surface temperature exceeded the air temperature and, therefore, the radiation balance reflected not only transpiration and photosynthesis, but also the turbulent heat flux from the vegetated surface.

Having processed the remote sensing and ground truth monitoring data after the atmospheric correction of LandSat-8 satellite imagery using the 6S model [18-20], we have obtained the visible (5 channels) and near IR (4 channels) reflectance values which were used to calculate the radiation and aerodynamic characteristics of vegetation by the SEBAL model. Then, we have calculated the true surface temperature using the algorithm proposed by C. Du et al. [15] in order to insert it in the formula for calculating the stomatal conductance by the model applied [13].

The satellite image classification by types of the underlying surface, NDVI (Normalized Difference Vegetation Index), and ground-based measurements of wind velocity made it possible to calculate the spatial distribution of roughness parameters for the heat and momentum fluxes which were required for the turbulent heat flux estimation. To assess the spatial distribution of the stomatal conductance, the thematic maps have been compiled for all the heat balance components calculated by the SEBAL model taking into account ground-based meteorological measurements using the AMFAE system.
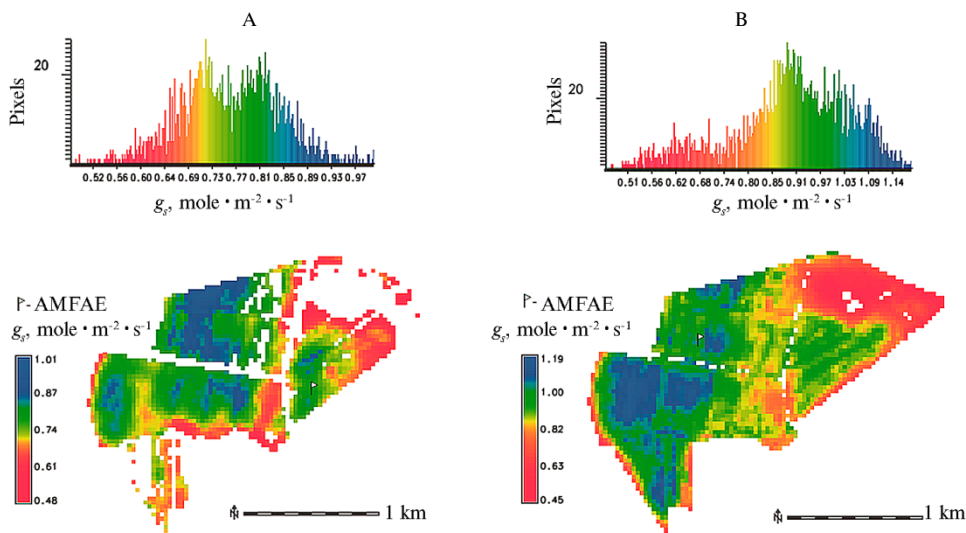
Fig. 2. Spatial distribution of the stomatal conductance (g_s) in forage herbs when estimating by the model of J.M. Blonquist et al. [13] in conjunction with the SEBAL model [17] and LandSat-8 (USA) images [14]: A and B — on May 15 and May 31, 2016, respectively; upper line — bargraph of conductance values, lower line — pixel characteristics of images (Bugry village, Leningrad Oblast, 2016). A flag designates the place of ground-based registration of data by the automatic mobile field agrometeorological equipment (AMFAE) [5] (May 15 — 60°5′6″N,

30°25′27″E; May 31 — 60°5′16″N, 30°24′32″E). Scale is 1 km.

As a result, based on the satellite data (the satellite transit time is about 12^{04}) and results of ground-based measurements, thematic maps and bargraphs of the stomatal conductance g_s have been constructed (Fig. 2). On average, the stomatal conductance values (like in determining at a single point) on May 15 were lower than those on May 31. In the high stomatal conductance areas, g_s values were approximately twice as much as those in the low stomatal conductance areas.

Thus, it was shown that the tested model could be applied for the automatic monitoring of spatial distribution of the stomatal conductance in plants using data of radiometric measurement of the vegetated surface temperature, model calculation of components of the underlying surface heat balance obtained by the remote sensing in conjunction with simultaneous ground-based measurements of air temperature and humidity, atmospheric pressure and wind current velocity. To improve the stomatal conductance accuracy, the highly sensitive measurement of the true temperature of the canopy taking into account its radiation capacity is required, and the use of the high-precision atmospheric correction of the satellite channels is also necessary to obtain true ground parameters not affected by atmosphere.

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BIOLOGICAL PECULIARITIES IN THE RESPONSIVENESS OF VEGETABLE CROP ROTATION TO PRECISION FERTILIZATION

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A b s t r a c t

Spatial and temporal variability of growing conditions which affects the production process management is characteristic of agrophytocenosis. Spatial heterogeneity of soil essential properties is widely reported. A precision fertilization should be effective tool to control crop productivity. The highest potential of such fertilization could be expected for vegetable crops in the favorable soil and climatic conditions of the Nechernozemie of North-West Russia. In a microvegetation stationary two-factor experiment, plastic bottom-less pots of 1 m^2 area were used to artificially form the upper part of the soil profile (A$_{arable}$ 0-22 cm and A$_2$B 22-40 cm horizons) simulating natural lithogenic mosaics of agro sod-podzolic sandy, sandy loam, light loam and medium loam soils subjected to weak and good cultivation. Their minimum, maximum, and average parameters for the 0-22 cm horizon were as follows: pH$_{KCl}$ of 4.34-6.35 and 5.40, humus content (by Tyurin) of 0.92-2.50 and 1.72 %, labile phosphorus and potassium (according to Kirsanov) of 125-550 and 390 mg/kg and 22-400 and 209 mg/kg, respectively. The vegetable crop rotation included black radish (*Raphanus sativus* L.)—potato (*Solanum tuberosum* L.)—beetroot (*Beta vulgaris* L.)—cabbage (*Brassica oleracea* L.)—carrot (*Daucus sativus* L.). For a comparison, we used different system of fertilization, i.e. control (no fertilizers); zonal system (ZS); precision fertilization 1 (PF-1); precision fertilization 2 (PF-2). In the ZS providing for a uniform application of the fertilizers based on the average soil properties, we used lime (4.5 t/ha + N$_{95}$P$_{20}$K$_{125}$) for black radish; manure (45 t/ha) + N$_{100}$P$_{30}$K$_{90}$ for potatoes; N$_{130}$P$_{50}$K$_{150}$ for beetroot; lime (2.1 t/ha) + manure (50 t/ha) + N$_{120}$P$_{10}$K$_{90}$ for cabbage; and N$_{100}$P$_{40}$K$_{130}$ for carrot. In the PF-1, two months before the radish was sown a precision soil cultivation has been performed using lime at 0-20 and 6.6 t/ha, peat at 0-900 and 390 t/ha; phosphorite flour at 0-750 and 94 kg/ha; potassium sulfate at 0-1710 and 407 kg/ha (as min-max and average). Further application of organic and mineral fertilizers before sowing (planting) was uniform, i.e. N$_{70}$K$_{60}$ for black radish; manure (45 t/ha) + N$_{80}$K$_{100}$ for potatoes; N$_{100}$P$_{30}$K$_{130}$ for beetroot; manure (50 t/ha) + N$_{100}$P$_{10}$K$_{70}$ for carrot; N$_{100}$P$_{10}$K$_{120}$ for white cabbage. In PF-2 providing average doses of all fertilizers equal to these in ZS, but differentiated for each pot based on actual soil parameters, we used lime (0-12 t/ha) + N$_{70-120}$P$_{0-90}$K$_{60-200}$ for black radish; manure (30-65 t/ha) + N$_{80-110}$P$_{0-110}$K$_{70-150}$ for potato; N$_{90-170}$P$_{0-150}$K$_{80-240}$ for beetroot; lime (2.1 t/ha) + manure (30-70 t/ha) + N$_{110-135}$P$_{0-60}$ K$_{40-120}$ for cabbage; N$_{85-115}$P$_{10-90}$K$_{79-180}$ for carrot. The experiments were arranged in four replications. In a field experiment the precision fertilization provided an increase in the productivity of vegetable crop rotation of 22.3 and 43.5 t/ha in control and ZS, respectively, to 47.9-49.4 t/ha. PF-1 and PF-2 resulted in the *Cv* reduction from 32 % and 16 % in the control and ZS to 9 %, and in an increased natural profitability of fertilizers by 21-49 %. A responsiveness of vegetable crop rotation to precision fertilization depended on biological features, the specific farming techniques and soil conditions. A decreasing responsiveness was as follows: black radish > car-rot ≈ beet > potatoes > cabbage. A uniform application of high doses of organic fertilizers was the factor reducing precision fertilization effectiveness. Significant advantage of PF-1 compared to PF-2 was established only for black radish, beet and carrot. When designing precision fertilization technologies, one should take into account the following decrease in sensitivity of vegetable crops in crop rotation to optimized (reduced) doses of fertilizers in the well-cultivated parts of a field: cabbage > beet > carrot > radish black > potatoes. Due to differentiated doses of ameliorants and fertilizers and integrated optimization of soil properties, the precision fertilization eliminates the effect of soil heterogeneity in cultivation and granulometric composition on crop production and

allows to increase productivity and payback of natural fertilizers to 28-42 and 21-67 % in sand, 17-26 and 25-47 % in sandy loam, 30-31 and 49-55 % in a light loam, and 11-16 and 0-35 % in middle loam soils, when compared to ZS.

The optimization of production process in olericulture is mostly based on agrotechnologies [1-4]. The time-spatial alteration of growing conditions, which is characteristic of agrophytocenoses, is associated with the weather and climate dynamics [2], phytosanitary state [5, 6], terrain-dependent soil differentiation [7-11], initial soil heterogeneity [12-15], irregular application of fertilizers and ameliorants [15-17], and is recorded almost everywhere [18-21]. The absence of reliable estimates of soil heterogeneity is still regarded as an important reason of reduced fertilizer efficiency as it was when chemicals were intensively used (in Russia, this was happening in 1970s and 1980s) [1, 2]. The problem required new geoinformation approaches [22-26] and specially planned field experiments [22, 27]. Together with the experience in controlling plant nutrition, this allowed development of a concept of production process integrated control in agroecosystems [1-3, 28-29] with the view of improving effects of fertilizers, reducing non-productive losses of biogenic elements, and environment protection [30-33].

The instruments here are precise fertilizer regime using non-generalized data of geo-reference study of soil and sowings, information technologies and precision equipment [2, 22, 27]. The non-black soil belt with a contrast and complex soil layer, pronounced heterogeneity of agrophysical and agrochemical properties, soil and climate conditions beneficial for fertilizers is classified among regions perspective for use of precision systems [1-2, 15, 27]. One of the factors defining their performance is the biological features of cultivar and variety nutrition [2, 34-36]. Due to its undervaluation, the advantage of precise fertilizer regime may not manifest itself [33]. Such systems are especially important in olericulture crops different in requirements to soil conditions and nutrition [2, 35-37].

In the present study, we have shown for the first time that precise fertilizing allows carrying out effective vegetable farming despite strongly pronounced deviations in sod-podzolic soil fertility, with the response of different crops to such systems being individual.

The aim of the study was to experimentally assess specifics and parameters of the vegetable crop rotation in response to precise fertilizing on sol-podzolic soils with lithogenic mosaic typical for non-black soil belt.

Technique. In the stationary micro-field trials (the experimental station of the Agrophysical Research Institute, Prometheus collective farm, Gdov Province, Pskov Region, 2007-2011) in the polyethylene bottomless vessels of 1 m^2, the upper part of the soil profile was formed artificially (0-22 cm A_{arable} soil horizon and 22-40 cm A_2B soil horizon). For packing vessels, the sod-podzolic sandy, sandy loam, light loam and medium loam soils, of poor and good cultivation, which constitute the contrast soil structure simulating natural lithogenic mosaics [16] were sampled from the corresponding horizons. In the A_{arable} horizon, the min-max and average pH_{KCl} were 4.34-6.35 and 5.40, respectively, humus content (by Tyurin) was 0.92-2.50 and 1.72 %, mobile phosphorus and potassium (by Kirsanov) amounted 125-550 and 390 mg/kg and 22-400 and 209 mg/kg, respectively.

The crop rotations studied were black radish (*Raphanus sativus* L.) Zimnyaya kruglaya chernaya (Federal Research Center for Vegetable Growing, Russia)—potato (*Solanum tuberosum* L.) Nevskii (Leningrad Research Institute of Agriculture, Russia)—beetroot (*Beta vulgaris* L.) Bikores (Bejo Zaden B.V., Netherlands)—cabbage (*Brassica oleracea* L.) Kuisor F_1 (Syngenta Seeds B.V.,

Netherlands)—carrot (*Daucus sativus* L.) Narbonne F_1 (Bejo Zaden B.V., Netherlands). The sowing (seeding) patterns were 5×30 cm (black radish), 20×60 cm (potato), 5×30 cm (beetroot), 30×60 cm (cabbage), and 3×30 cm (carrot). Good phytosanitary conditions of the sowings were maintained by manual weeding, inter-row treatment, use of fungicides and insecticides.

The two-factor experiments comprised eight factor A variants of the soil including its type (poorly or well-cultured soil) and subtype according to the granulomertic content (sand, sod-podzolic sandy, sandy loam, light loam and medium loam), and also four factor B variants of the type of organomineral fertilizing (control — 0, no fertilizers; zonal fertilization system, ZS; precision fertilization system 1, PF-1; precision fertilization system 2, PF-2). In the ZS, fertilizers were introduced uniformly (based on the average soil properties) as follows: lime (4.5 t/ha) + $N_{95}P_{20}K_{125}$ for black radish (30 t/ha root-crop); manure (45 t/ha) + $N_{100}P_{30}K_{90}$ for potato (40 t/ha bulbs); $N_{130}P_{50}K_{150}$ for beetroot (30 t/ha root-crops); lime (2.1 t/ha) + manure (50 t/ha) + $N_{120}P_{10}K_{90}$ for cabbage (60 t/ha heads); $N_{100}P_{40}K_{130}$ for carrot (50 t/ha root-crops). In the PF-1, precise soil cultivation was carried out 2 weeks prior to sowing [16] using long-acting ameliorants and fertilizers: lime (on average 6.6 t/ha, min-max 0-20 t/ha), peat (390 t/ha, 0-900 t/ha), phosphorite flour (94 kg/ha of P, 0-750 kg/ha), potassium sulfate (407 kg/ha of K, 0-1710 kg/ha). Organic and mineral fertilizers were uniformly introduced prior to sowing (planting) correspondingly to the alteration in soil properties: $N_{70}K_{60}$ for black radish, manure (45 t/ha) + $N_{80}K_{100}$ for potato, $N_{100}P_{30}K_{130}$ for beetroot, manure (50 t/ha) + $N_{100}P_{10}K_{70}$ for cabbage, $N_{100}P_{10}K_{120}$ for carrot. In the PF-2, average doses of all fertilizers equal to those in the ZS were provided, but differentiated for each pot based on actual soil parameters: lime (0-12 t/ha) + $N_{70-120}P_{0-90}K_{60-200}$ for radish; manure (30-65 t/ha) + $N_{80-110}P_{0-110}K_{70-150}$ for potato; $N_{90-170}P_{0-150}K_{80-240}$ for beetroot; lime (2.1 t/ha) + manure (30-70 t/ha) + $N_{110-135}P_{0-60}K_{40-120}$ for cabbage; and $N_{85-115}P_{10-90}K_{79-180}$ for carrot. Lime dust, ammonium saltpeter, phosphorite flour, double superphosphate, potassium sulfate, potassium chloride, as to State Standards (Russia), were ameliorants, and peat (humidity 65 %, ash 24 %, $pH_{aq.}$ 6.1; N 1.05 %, P_2O_5 0.07 %, K_2O 0.04 %), pig manure, duff-semi-fusty (humidity 72-75 %, $pH_{aq.}$ 6.4-6.8; N 0.45-0.49 %, P_2O_5 0.15-0.20 %, K_2O 0.24-0.29 %) were local fertilizers.

The main and side product output was measured by weighing. The experiment was arranged in 4-fold repetition.

Responsiveness estimates were an average (*M*), minimum (m_{min}) and maximum (m_{max}) yields, the variation coefficient (*Cv*, %) and the natural payback of the fertilizer active substance. Results were processed by dispersion analysis (Statistica 7.0, StatSoft, Inc., USA). The reliability of the differences was estimated by *F* Fischer criterion.

Results. The studies have confirmed the high agronomic effect of differentiation in fertilizer dosages according to spatial soil heterogeneity. Yield variability in the control was 24 to 51 % (Table 1). In the ZS, when calculating optimal ameliorant and fertilizer dosages according to the weight-average agrochemical parameters (a group of the vessels), the crop productivity increased by 95 % compared to control, in the PF-2, with differentiated use of the identical fertilizer the value was 115 % higher compared to the control and 11 % higher than in FS. In the PF-1 with preliminary precision soil cultivation, the parameters were 122 and 14 %, respectively. As to fertilizer payback, the precision systems were superior to the zonal one (an increase by 49 % for the PF-1 and by 21 % for the PF-2). The costs of a precision soil culturing modeled for a more prolonged effect were not comprised in the calculation of payback of the PF-1.

All cultures positively but not identically responded to the fertilizer dosage differentiation. The yield gain in PF-1 and PF-2 increased by 69 and 36 % for radish, by 28 and 26 % for potato, by 39 and 20 % for beetroot, by 21 and 16 % for cabbage, and by 5 and 16 % for carrot with 1.9-2.6- and 1.4-1.9-, 1.8-2.4- and 1.3-1.8-; 1.6-1.8- and 1.1-1.3-; 2.2-2.7- and 1.0-1.2-; 2.5-4.3- and 1.4-2.4-fold decrease in spatial variability (Cv) as compared to the control and ZS, respectively, for the same crops.

1. Productivity of vegetable crop rotation and its spatial heterogeneity depending on the fertilizer regimes (field experiment, Pskov Region, 2007-2011)

Fertilizer regime	Yield, t/ha			Yield gain		Payback of 1 kg NPK (in grain units)
	M	m_{min}-m_{max}	Cv, %	t/ha	%	
Black radish						
Control-0	14.1	7.0-29.0	45			
ZS	21.8	15.0-41.0	33	7.7	55	5.1
PF-1	27.1	23.8-43.0	17	13.0	92	16.0
PF-2	24.6	18.5-42.0	24	10.5	74	7.0
LSD$_{05}$	1.30					
Potato						
Control-0	21.6	6,6-35,8	51			
ZS	53.3	28,6-74,2	37	31,7	147	15,7
PF-1	62.2	38,0-74,0	21	40,6	188	21,9
PF-2	61.5	30,6-75,4	28	39,9	185	19,8
LSD$_{05}$	2.60					
Beetroot						
Control-0	11.3	1,6-22,2	61			
ZS	24.2	8,0-35,8	42	12,9	114	4,5
PF-1	29.2	12,2-41,3	33	17,9	158	7,5
PF-2	26.8	12,8-40,3	38	15,5	137	5,6
LSD$_{05}$	1.60					
Cabbage						
Control-0	60.5	32,0-90,0	24			
ZS	105.1	87,0-123,0	11	44,6	74	13,3
PF-1	114.3	101,0-141,0	9	53,8	89	17,4
PF-2	112.4	95,0-137,0	11	51,9	86	15,2
LSD$_{05}$	4.40					
Carrot						
Control-0	23.4	11,9-35,2	30			
ZS	47.6	32,0-60,4	17	24,2	103	9,6
PF-1	58.5	41,4-69,8	12	35,1	150	15,4
PF-2	51.5	45,0-58,4	7	28,1	120	11,2
LSD$_{05}$	1.55					
Crop rotation (grain units)						
Control-0	22.3	12,8-32,3	32			
ZS	43.5	30,7-51,9	16	21,2	95	9,9
PF-1	49.4	39,6-53,8	9	27,1	122	14,8
PF-2	47.9	41,1-53,5	9	25,6	115	12,0
LSD$_{05}$	2.07					

N o t e. M — average value, Cv — variation coefficient. In payback calculation, 70 % and 30 % of the NPK manure costs were attributed to the first and the second crop, respectively. For description of the fertilizer regimes see the "*Technique*" section.

The relative yield gain ranked the crops in an obvious decreasing series according to the response to precision fertilizer regimes, i.e. radish > carrot ≈ beetroot > potato > cabbage. A different response was a consequence of differences both in the crop biology and in the fertilizing and agrotechnical methods. In particular, the treatment of potato and cabbage plants with high manure doses in the ZS led to the partial smoothing of the soil properties and, as a consequence, the PF-1 and PF-2 had less advantage. Thence, these crops, characterized by a high efficiency of nutrient consumption [14, 32-35, were the forth and the fifth in the series in terms of their response to differentiated use of ameliorants and fertilizers and at the maximum payback of fertilizers. As a result, combination of this factor to the biological features of edible root plants, which require soils with higher agrophysical properties, was a reliable advantage of the PF-1 over the PF-2.

The spatial heterogeneity is manifested both in poorly and well-cultured

soils [13, 16, 21]. Therefore, the differentiation in the fertilizer dosages is justified in the soils of any culturing. However, if the fertilizer regimes are based on the redistribution of ameliorants and fertilizer for the benefit of poorly cultured soils, it is not always possible to maintain yield on the well-cultured level (Table 2). Only radish, potato and carrot (the crops which are more flexible in requirements to soils) [14, 31, 33, 35] did not reduce the productivity at a reduced fertilizer and lime dosages (PF-2). Moreover, potato plants on the well-cultured soil appeared to be more sensitive to lower lime dosage (2.3 instead of 4.5 t/ha) when applied for the black radish, with a reliable increase in bulb yield (by 9 % compared to ZS). On the contrary, the productivity of cabbage and beetroot, more sensitive to nutrition condition, reliably decreased (by 6 to 9 %) with a reduction in fertilizer doses on the well-cultured part of the plot (PF-2). The same was recorded for carrot. Therefore, sensitivity of the studied crops to a reduced fertilizer doses on well-cultured soils decreased as follows: cabbage > beetroot > carrot > radish > potato. On well-cultured soil, PF-1 regime impacts the plants, except cabbage, more steadily and positively leading to 7 to 9 % increase in yield compared to ZS due to the after-effect of preliminary precise culturing.

2. Fertilizer regime efficiency depending on the crop of the crop rotation and soil conditions (field experiment, Pskov Region, 2007-2011)

Fertilizer regime (Factor B)	Efficiency under different soil conditions (Factor A)									
	poorly cultured soil					well-cultured soil				
	Y, t/ha	Cv, %	YG		P	Y, t/ha	Cv, %	YG		P
			t/ha	%				t/ha	%	
Black radish										
Control-0	9.7	22				19.0	32			
ZS	17.7	13	8.0	82	5.2	26.0	32	7.0	37	5.1
PF-1	26.3	15	16.6	171	20.5	27.9	26	8.9	47	11.7
PF-2	22.3	13	12.6	130	7.5	26.7	35	7.7	41	6.6
LSD$_{05}$ for A is 1.90; for B is 1.30; for AB is 3,80 т/га										
Potato										
Control-0	14.8	38				28.5	29			
ZS	47.6	27	32.8	222	16.3	59.0	15	30.5	107	14.9
PF-1	60.1	21	45.3	306	24.4	64.4	11	35.9	126	17.7
PF-2	60.0	26	45.2	305	19.9	63.0	12	34.5	121	19.6
LSD$_{05}$ for A is 3.70; for B is 2.60; for AB is 7.40 t/ha										
Beetroot										
Control-0	7.5	69				15.4	43			
ZS	19.8	47	12.3	164	4.2	30.3	29	14.9	97	5.4
PF-1	25.7	36	18.2	243	7.7	32.6	28	17.2	112	7.4
PF-2	26.0	28	18.5	247	5.5	28.4	30	13.0	84	6.4
LSD$_{05}$ for A is 2.30; for B is 1.60; for AB is 4.50 t/ha										
Cabbage										
Control-0	49.6	15				71.4	14			
ZS	98.0	7	48.4	98	14.5	112.4	9	41.0	57	12.3
PF-1	116.4	7	66.8	135	21.6	112.1	11	40.7	57	13.2
PF-2	122.7	6	73.1	147	18.1	102.1	7	30.7	43	11.2
LSD$_{05}$ for A is 6.20; for B is 4.40; for AB is 12.40 t/ha										
Carrot (2011 год)										
Control-0	17.4	22				29.4	12			
ZS	41.5	15	24.1	139	9.2	53.7	8	24.3	83	10.0
PF-1	58.4	16	41.0	236	16.0	58.6	6	29.2	99	14.8
PF-2	51.8	9	34.4	198	11.4	51.2	5	21.8	74	11.0
LSD$_{05}$ for A is 2.19; for B is 1.55; for AB is 4.38 t/ha										
Crop rotation (in grain units)										
Control-0	16.9	20				27.8	18			
ZS	38.9	15	22.0	130	10.2	48.2	10	20.4	73	9.6
PF-1	48.5	13	31.6	187	17.6	50.4	9	22.6	81	13.0
PF-2	48.9	11	32.0	189	12.6	47.0	8	19.2	69	11.3
LSD$_{05}$ for A is 2.94; for B is 2.07; for AB is 5.86 t/ha										

N o t e. Cv — variation coefficient; Y — yield, YG — yield gain, P — payback of 1 kg NPK (in grain units). For description of the fertilizer regimes see the "*Technique*" section.

The advantage of PF-1 and PF-2 was expectedly especially significant in poorly cultured soil with an increase in yield gain by 49 and 26 % for radish, 26 and 26 % for potato, 30 and 31 % for beetroot, 19 and 25 % for cabbage, 41

and 25 % for as compared to ZS. Despite the expectations, only for radish and carrot in the poorly cultured soil, the precise technology providing for complex optimization of agrophysical and agrochemical properties of all soil subtypes was more preferable than the PF-2. For potato, cabbage and beetroot under relatively beneficial weather and climate conditions, the optimization of soil properties due to the redistribution of ameliorants, organic and mineral fertilizers just prior to planting (sowing) (PF-2) appeared to be sufficient. As a result, in the crop rotation the reliable advantage of the PF-1 over the PF-2, with 18 % increase in productivity, was recorded only in well-cultured soils. In these regimes, the payback of fertilizers increased more significantly (from 15 to 40 %).

In our experiment, agrophysical heterogeneity of soils was predominantly associated with granulomertic composition. For potato, beetroot and carrot productivity the Cv values, which reflect the sensitivity to soil heterogeneity, in the control was 1.3 to 1.8 times higher in poorly cultured soil than in the well-cultured one, that for cabbage almost did not change, and that for radish decreased 1.5-fold. The pronounced distinction of the latter from the other crops is possibly associated with the peculiarities of its development in the second half of vegetation period after the soil remained semi-fallow for a long time. In this time, due to prolonged (up to mid-July) incubation, in the well-cultured sands, sandy loams, light and medium loam soils a pronounced heterogeneity in nitrogen regimen is formed due to microorganisms, which determines a high variability of yield in radish having a shortened period of active root nutrition. The precise fertilizing allowed for almost completely avoiding differences in productivity of the corps in poorly and well-cultured soils, reducing the Cv 1.5 to 1.8-fold and 2.0 to 2.3-fold, respectively.

Although the fertilizers provided a high absolute and relative yield gain for all soils, the effect of granulomertic composition was quite remarkable (Fig.). The complex effect of this factor on yield formation had a more fundamental character. Quite in compliance with biological features and requirements of the crops [14, 33-35], at high soil heterogeneity without fertilizing the yield of radish (19.6 t/ha) and cabbage (69.5 t/ha) was higher in the medium loam soil, the yield of potato (28.4 t/ha), beetroot (18.7 t/ha) and carrot (27.6 t/ha) was higher in light loam soil, and the minimum parameters were recorded for sand. Thus, the yields reflected the crop preferences to air and water conditions and nutrition depending on soil types as follows: medium loam > light loam > sandy loam > sand for radish and cabbage; light loam > medium loam > sandy loam > sand for potato; light loam > sandy loam > medium loam > sand for beetroot and carrot. As a whole, the productivity of crop rotation on sand substrate was minimal (17.1 t/ha g.u.) at a minimum absolute gain for ZS of 19.0 t/ha g.u. against 23.7 t/ha g.u. on other soils. The precise fertilizing have neutralized the effect of this factor, and the yield gain per rotation for PF-1 and PF-2 reached 26.2-27.5 and 24.4-26.4 t/ha g.u., respectively. Thereby, the PF-1 and PF-2 efficiency as compared to ZS was 42 and 28 % higher on sand, 26 and 17 % higher on sandy loam, 31 and 30 % higher on light loam, and 16 and 11 % higher on medium loam soil.

The variability in the yield and, correspondingly, in the crop rotation productivity also depends on soil granulomertic parameters. Thus, for radish and potato the maximum values of Cv were recorded in light and medium loam (42 to 45 and 34 to 71 %), for beetroot — in sandy loam and sand (54 to 75 %), for cabbage and carrot — in medium loam and sand (24 to 25 and 32 to 38 %) soils. Despite the commonly adopted conception of increased requirements of cabbage to agrophysical and agrochemical soil properties, its sensitivity to the granulomertic composition remained minimal. This was facilitated by normal watering

of the crop under the acutely arid vegetation period in 2010. For the crop rotation as a whole, the maximum Cv values were recorded for sandy and light loam soils in the control (25 to 34 %) with minimum for PF-1 and PF-2 (1 to 10 %) and intermediate values for ZS (8 to 15 %) (irrespectively of a granulomertic composition).
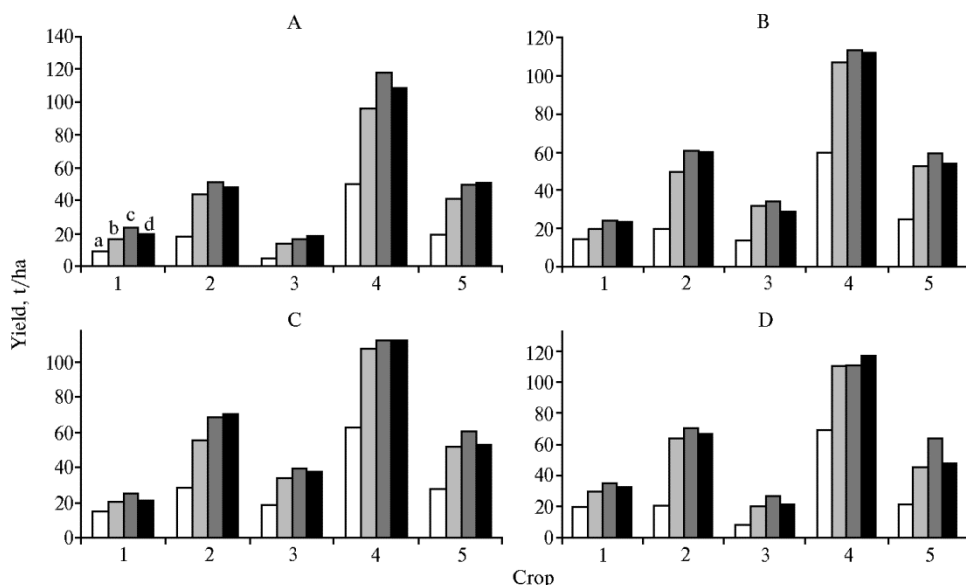
Crop yields in the vegetable crop rotation depending on soil types and fertilizing regime: A — sand, B — sandy loam, C — light loam, D — medium loam; 1, 2, 3, 4, 5 — black radish, potato, beetroot, cabbage, carrot, respectively; a, b, c, d — control-0, ZS, PF-1, PF-2 (for the description of fertilizing regimes see the "*Technique*" section) (field experiment, 2007-2011).

Thus, under high heterogeneity and lithogenic mosaic of sod-weakly podzolic soils, the precise and annually differentiated use of organic and mineral fertilizing (the PF-2 regime) or single precise use of ameliorant and subsequent uniform introduction of fertilizers (the PF-1 regime) provided an increase in the vegetable crop rotation productivity (in grain units) from 22.3 to 43.5 t/ha in control and in using zonal fertilization system (ZS) up to 47.9 to 49.4 t/ha (by 115-122 and 10-14 %, correspondingly). Thereby, the Cv values for the crop rotation productivity reduced from 32 and 16 % in the control and ZS to 9 %, and the natural payback of the fertilizers increased by 21 to 49 %. The crop response to the precise fertilizing decreased in the crop rotation as follows: radish > carrot ≈ beetroot > potato > cabbage. A reliable advantage of the PF-1 regime over the PF-2 has been established only in edible roots (radish, beetroot and carrot). The reduction in the payback of the precise fertilizing compared to zonal one was due to uniformly introduced high doses of organic fertilizers. When developing fertilizing systems for vegetable crop rotations, one should take into the account that the crop sensitivity to optimization (reduction) of fertilizer dosages in well-cultured parts of the field reduces as follows: cabbage > beetroot > carrot > radish > potato. The precise fertilizing, owing to differentiated dosages of ameliorants and fertilizers and complex optimization of soil properties, have eliminated the effect of soil different culturing and granulomertic composition. As a result, the increase in the productivity and natural payback of fertilizers compared to those in ZS was 28 to 42 and 21 to 67 % in sand, 17 to 26 and 25 to 47 % in sandy loam, 30 to 31 and 49 to 55 % in light loam, and 11 to 16 and 0 to 35 % in medium loam soil.

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SOIL ENZYMATIC ACTIVITY AND NITROUS OXIDE EMISSION FROM LIGHT-TEXTURED SPODOSOL AMENDED WITH BIOCHAR

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Soil amendment with biochar has been proposed as a tool to mitigate soil N_2O emissions by altering soil physical, chemical and biological properties. Little work has been focused on the effect of biochar on the soil enzymatic activity as the most sensitive indicator to diagnose the changes in soil quality. The 90-day laboratory experiment to study the effect of biochar on the dynamics of some soil enzymes and cumulative N_2O emissions from the loamy sand Spodosol was conducted with the aim to justify the sensitiveness of the enzymes to soil amendments with biochar. The experimental setup was a randomized block design with 9 replicates and included the soil control (K), the soil with biochar (BC), the soil with mineral fertilizer ($N_{16}P_{16}K_{16}$, N_{90}) and the soil with fertilizer and biochar mixture (N_{90} + BC). The hydrolases (urease) and oxidoreductases (catalase, peroxidase and polyphenol oxidase) were selected due to their high sensitivity to the changes in the soil quality and the significance in the nitrogen cycle. The results of the experiment have shown that the soil amendment with 10 t/ha of biochar significantly ($p < 0.05$) reduced (1.4-fold) cumulative N_2O emissions from the soil compared to the K and N_{90} treatments. The presence of biochar in the soil had no significant effect on the urease activity, while activity of the catalase, peroxidase and polyphenol oxidase was significantly ($p < 0.05$) higher (1.2 times in average) due to the changes in the physical properties of the soil (aeration in particular). There was high positive correlation between N_2O flux and catalase activity ($r = 0.85$ and 0.87 for BC and N_{90} + BC, respectively). Based on the calculation of the humification coefficient (polyphenol oxidase activity to peroxidase activity) revealed that soil mineralization increased by 10-13 % after soil amendment with BC, compared to the treatments without BC. The soil amendment with biochar significantly changed the red-ox soil conditions. That was reflected in the changes of enzymatic activity, N_2O emission and mineralization of humic substances. All the studied enzymes, except urease, were sensitive bio-indicators of soil conditions and could be used in the environment assessment methodology for land-use systems with biochar amendments.

Keywords: loamy sand Spodosol, biochar, mineral N-fertilizer, enzymatic activity, urease, catalase, peroxidase, polyphenol oxidase, nitrous oxide emission

Effects of various agricultural practices on the soil (tillage, application of fertilizers and ameliorants) and agricultural crops cause changes in microbiological and, consequently, enzymatic soil activity [1]. The intensity and trends of these biological processes as well as the integrated effect of soil and climatic factors influence the nitrous oxide (N_2O) emission which causes major climate changes [2].

It has been established that the application of biochar to soil alters physical and chemical conditions which affect the activities of microorganisms controlling C and N flows in the biosphere [3-8]. Obtained reliable data on the effectiveness of reduction in N_2O emission from sod-podzolic loamy sand soil us-

ing biochar raises the possibility of its application in light-textured soils, however, the justification of the practice requires clear understanding of occurring process mechanisms, particularly of the natural enzymatic complex properties. Special focus, in our opinion, should be on the catalyzing effect of hydrolases and oxidoreductases [9, 10]. Among the enzyme classes mentioned, there are ureases (EC 3.5.1.5), catalases (EC 1.11.1.6), peroxidases (EC 1.11.1.7) and polyphenol oxidases (EC 1.10.3.1). These enzymes have high sensitivity to various exposures and perform important functions in the organic matter transformation which the N cycle depends on [11-13]. The urease activity is associated with urea ammonification and nitrogen conversion into the plant-available form. Catalase belongs to oxidation-reduction enzymes. Polyphenol oxidase and peroxidase participate in multistage degradation and synthesis of aromatic organic compounds having an impact on humification [14]. Biological diagnosis of soils based on their enzymatic activity will enable to identify the nature and level of the man-caused impact on the soil cover and reveal mechanisms of interaction between the product applied and the soil.

We are the first to perform the coupled analysis of the biochar impact on N_2O formation in soil and activity of enzymes participating in the transformation of carbon- and nitrogen-containing substances, and show an essential role of oxidation-reduction enzymes in revealing the mechanisms of biochar action on soil.

The purpose of the model experiments conducted was to assess the biochar effect on N_2O emission and enzymatic activity of the sod-podzolic loamy sand soil.

Technique. Samples of the sod-podzolic loamy sand soil were taken from a plough layer (0-28 cm) before the field season of 2016 (biopolygon of Menkovskii Branch of the Agrophysical Institute, Gatchina Region, Leningrad Province), dried at 22 °C and sieved (through 2 mm openings). The biochar was produced by the fast anaerobic pyrolysis of soft wood of broad-leaved trees (alder, birch, aspen) at 550 °C. The biochar fraction with particles of <1 cm in size was applied to the soil.

The sieved soil (300 g) moistened up to 23 % of the minimum moisture capacity (MMC) were placed into each of 500 ml vegetation vessels with seal caps having rubber stoppers in their centers for air sampling using a syringe. The vessels were preincubated for the first 7 days to reach the balanced state of the microbial community and moisture content. Then, the soil was applied with biochar (B) at the dose of 0.42 g/vessel that was equivalent to 10 tons of biochar per 1 ha, ammonium nitrate phosphate fertilizer $N_{16}P_{16}K_{16}$ (N_{90}) at the dose of 0.23 g/vessel (90 kg N/ha) or a mixture of biochar and ammonium nitrate phosphate fertilizer (N_{90} + B). The substrates obtained were mixed uniformly. Soil without biochar and fertilizer was used as control (K) that was preincubated and mixed as well. The samples were compacted to 1.2 g/cm³ that corresponded to steady-state bulk density of the soil under study in the field conditions. The experiment included 9 replicates with randomized distribution of the samples. The vessels were placed into biological cabinets for incubation at the constant temperature of 28 °C. Throughout the experiment, the soil vessels were held open, they were closed for 30 min only for air sampling to analyze for N_2O. The soil moisture was maintained on the basis of the vessel weight at the initially set moisture level, the soil was moistened after the air sampling.

Upon biochar and mineral fertilizer application, air sampling was carried out every other day over the first 2 weeks, then 2-3 times a week, and once a week in 60 days. In total, the experiment lasted for 90 days.

N_2O concentration in the air samples was measured using Fractovap 4130 gas chromatograph (Carlo Erba Strumentazione SpA, Italy) equipped with

an electron capture detector.

The enzymatic activity dynamics (sampling every 10 days) was studied using standard procedures [13, 14]: the activity of catalase was identified by permanganatometry according to Johnson and Temple, the urease activity was determined photocolorimetrically using the Nessler's reagent, based on the amount of colored ammonia complexes formed during urea hydrolysis; and the activity of peroxidase and polyphenol oxidase was identified photocolorimetrically according to Galstyan technique (1968).

Microsoft Excel and Statistics 8.0 (Stat Soft Inc., USA) software were used to process data. The statistical analysis included calculations of mean (M) and standard deviations ($\pm SD$), and linear correlation coefficients (r) at $p \leq 0.05$. The significance of differences in the mean values was assessed by the one-way analysis of variance (ANOVA) and a posteriori analysis based on the Tukey's honestly significant difference test at $p \leq 0.05$ [15].

Results. The main agrochemical characteristics of the soil at the time of sampling were as follows: $C_{organic}$ — 2.4 %; N_{total} — 0.17 %; $N-NO_3$ — 11.2 mg/kg; $N-NH_4$ — 4.5 mg/kg; movable P_2O_5 — 299 mg/kg; movable K_2O — 97 mg/kg; pH_{KCl} 5.3; the biochar characteristics were: C_{total} — 87.4 %; N_{total} — 0.038 %, H — 28 mg/g, pH 7.6.

The cumulative emissions of N_2O calculated for 80 days have shown the significant ($p < 0.05$) differences among all the experiment treatments (Fig. 1). The application of $N_{16}P_{16}K_{16}$ (N_{90}) to the soil under study has increased the cumulative emission of N_2O twice as compared to the soil control, while the biochar application has decreased the cumulative emission 1.4 times both as compared to the soil control and as regard to the soil with the mineral fertilizer.
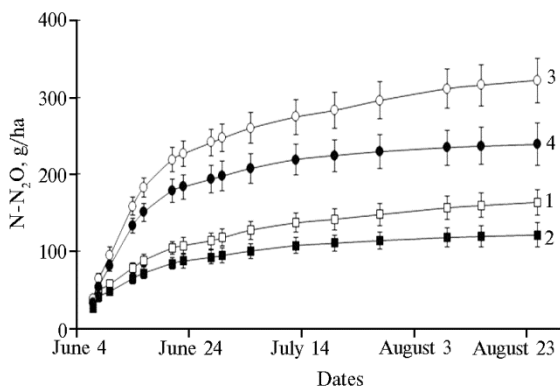
Fig. 1. Cumulative nitrous oxide emission from the sod-podzolic soil samples: soil control (1)**, when applying biochar** (2)**, fertilizer (ammonium nitrate phosphate fertilizer)** (3) **and the mixture of biochar and fertilizer** (4). The mean (M) and standard deviations ($\pm SD$) provided (laboratory experiment).

The obtained data concerning the biochar ability to reduce N_2O emission is consistent with other researchers' findings which showed that converting the biomass carbon pools during its pyrolysis into the aromatic form almost inaccessible for soil microorganisms reduced N_2O formation rate substantially. This is associated with changes in physical and chemical living conditions of the soil microorganisms responsible for generation of greenhouse gases. Some works show [4-7] that N_2O emission reduced considerably after the soil amendment with biochar, even when there were 78 % of water-saturated pores, i.e. under anaerobic conditions. The improvement of the air and water regime in soil and a decreased denitrification resulted in the reduction of cumulative N_2O emission from soils.

It has been also established earlier that biochar contributed to the increase in activity, group and functional diversity of the microbial community in soil [8, 16], stimulated the development of soil molds and aerobic cellulose-fermenting bacteria which consumed nitrogen intensively and inhibited the growth of nitrogen-fixing bacteria [17, 18].

The soil amendment with organic, mineral fertilizers and ameliorants

may change the activity of urease that defines important stages of transformations of nitrogen-containing substances in soil. The experiment results have indicated that, according to the enzyme enrichment assessment of soils [19], the soils studied were described as poor in urease activity (3 to 10 mg of N-NH$_4$/10 g of soil per day). The enzyme accumulation dynamics has shown the increase in activity during 30 incubation days with further decrease by the end of the experiment. The application of ammonium nitrate phosphate fertilizer has increased the urease activity significantly ($p < 0.05$) (1.6 times on average) as compared to the soil control, while the application of biochar caused no significant decrease in the urease activity as compared to both the soil control and N$_{90}$ (Fig. 2). Correlation coefficients (r) between N$_2$O emission and urease activity were low (at $p < 0.05$): 0.51 (K), 0.38 (B), 0.62 (N$_{90}$), and 0.67 (N$_{90}$ + B). In studies of H. Jin [20], it has been found that the soil amendment with biochar may cause decrease in the urease activity that is associated with pH change (the majority of biochars have neutral pH) and high adsorption capacity of biochar.
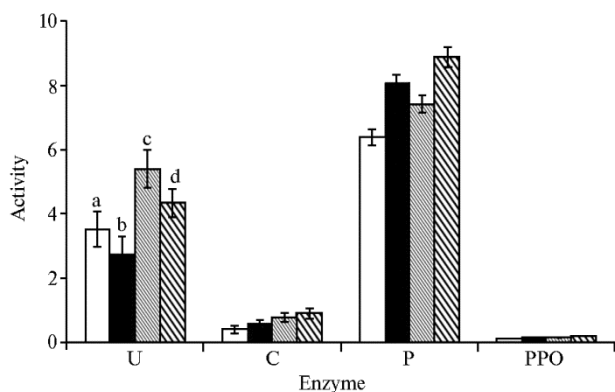
Fig. 2. Enzymatic activity of the sod-podzolic soil in the soil control (a), when applying biochar (b), fertilizer (ammonium nitrate phosphate fertilizer) (c) and mixture of biochar and fertilizer (d): U — urease, mg of N-NH$_4$/10 g of soil per day; C — catalase, mg of KMnO$_4$/g of soil per 20 min; P — peroxidase, mg of purpurogallin/100 g of soil: PPO — polyphenol oxidase, mg of purpurogallin/100 g of soil. The mean (M) and standard deviations ($\pm$SD) provided (laboratory experiment).

The catalase activity in our experiment decreased from the beginning to the end of observation in all treatments studied. The similar trend was also noted in other studies, for example M.A. Razmakhina [21] and E.V. Dadenko [22] reported that the catalase activity in laboratory conditions showed a general decrease trend by the end of the experiment and depended on the air conditions, moisture and bulk density of soils.

As for the enzyme enrichment [19], the soils studied in the experiment were described as extremely poor by their catalase activity (< 1 mg of KMnO$_4$/1 g per 20 min). The soil control contained the minimum amount of the enzyme that varied from 0.89 to 0.23 mg of KMnO$_4$/g for 20 min. The application of N$_{90}$ has increased the catalase activity significantly ($p < 0.001$) 1.4 times on average, and the application of biochar ($p < 0.05$) has also increased the enzyme activity 1.3 and 1.2 times as compared to the soil control and soil with ammonium nitrate phosphate fertilizer, respectively (refer to Fig. 2). The correlation coefficients between N$_2$O emission and catalase activity were high ($p < 0.05$): 0.89 (K), 0.85 (B), 0.84 (N$_{90}$), and 0.87 (N$_{90}$ + B).

The study results have shown that the peroxidase activity was on average 50 times higher than that of polyphenol oxidase, and the biochar application has increased the enzyme activities substantially ($p < 0.05$) as compared to those in the treatments without biochar. It is more likely to be associated with special aeration conditions provided owing to the porous structure of biochar (in contrast to the samples containing no biochar) [22-24]. However, the correlation coefficients between N$_2$O emission and peroxidase activity as well as polyphenol oxidase activity were low (at $p < 0.05$): –0.42 and 0.45 (K), –0.47 and 0.59 (B), –0.39 and 0.46 (N$_{90}$), –0.47 and 0.41 (N$_{90}$ + B), respectively.

The polyphenol oxidase activity to peroxidase activity ratio is generally thought to be a conditional coefficient of organic matter humification (C_{hum}), which can, to a certain extent, describe the formation trend of specific humic substance in soil (mineralization or immobilization) [22, 25]. The value of C_{hum} calculated by the ratio of the enzyme activity has appeared to be < 1 in all the experiment treatments, indicating that mineralization of humic substances dominated over their synthesis. The biochar application contributed to insignificant increase in the mineralization, 1.1 times on average, as compared to the soil control and soil with mineral fertilizers.

Thus, when applying biochar (10 t/ha) to the sod-podzolic loamy sand soil, N_2O emission was significantly ($p < 0.05$) lower (1.4 times on average) than in the control soil and soil with the mineral fertilizer. In this case, the urease activity decreased 1.2 times on average (with no considerable differences from the treatments without biochar). The activity of oxidoreductases under study increased significantly ($p < 0.05$), i.e. 1.3 times for catalase, 1.2 times for peroxidase and polyphenol oxidase, that was associated with changes in the physical properties of the soil caused by biochar amendment (first of all, aeration) and reflected changes in the oxidation-reduction conditions. The high positive correlation has been established between N_2O emission and catalase activity ($r = 0.85$ and $r = 0.87$ for biochar and the mixture of biochar and ammonium nitrate phosphate fertilizer, respectively). The ratio of polyphenol oxidase activity to that of peroxidase provided evidence of enhancing mineralization of humic substances in the presence of biochar. All the studied enzymes, except urease, have appeared to be sensitive bioindicators of soil conditions and can be used for environmental assessment of land-use systems.

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CHANGES IN BIOLOGICAL AND PHYSICAL PARAMETERS OF SOILS WITH DIFFERENT TEXTURE AFTER BIOCHAR APPLICATION

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A b s t r a c t

Maintaining a favorable microbial and physical state of soils using new management practices is one of the key directions of sustainable land-use. Application of biochar (BC) is one of the new ways to improve soil quality and sustainability by increasing carbon sequestration and making significant changes in soil properties. Slow and fast pyrolysis is used to produce BC from different feedstock. Technological conditions of pyrolysis and feedstock type have key impacts on BC properties and its interaction with soils. Many previous studies have revealed a positive effect of BC on soil properties and crop yields. Nevertheless, there are uncertainties in the understanding of BC effects on microbial processes of nitrogen and carbon transformation in soils. In the present study, two types of BC were used: BC1 and BC2 were produced by fast and slow pyrolysis of wooden feedstock, respectively. The aims of the study were, firstly, to assess differences in the effects of BC1 and BC2 on hydrophysical properties of sandy and clayey loam soils, and, secondly, to assess the effects of differences in soil texture on the degree of impacts of BCs on soil nitrification and denitrification. Samples of sandy and clayey loam gleyic Fluvisols (Slovakia) were used in the experiment. An amount of the applied BC on a hectare-scale was equal to 15 and 30 tones. Water retention of the soil samples was measured using a pressure-plate apparatus at water potentials from –0.1 to –300 kPa. Nitrification and denitrification rates were determined in laboratory conditions by measuring nitrous oxide accumulation in flasks in which the soils were incubated for 48 hours at 25 °C. Soil moisture content for nitrification and denitrification was equal to 48-55 % of the full saturation and 100 % saturation, respectively. The results indicate that BC1 and BC2 application increased water retention more for the sandy soil than for the clayey loam soil in the whole range of water potentials. Of the two biochars, BC2 had a greater influence on the water retention of both soils. Application of BC1 resulted in a significant decrease (p < 0.01) while application of BC2 produced only an insignificant decrease in nitrification rates in the clayey loam soil. BC1 and BC2 had no effect on nitrification rates in the sandy soil. Moreover, the application of the BCs at both rates contributed to a greater decrease in nitrification rates in the clayey loam soil than in the sandy soil.

Keywords: soils, texture, biochar, slow pyrolysis, fast pyrolysis, soil water retention, nitrification rate, denitrification rate

Intensive agrotechnologies may affect adversely the contents of mineral nitrogen and organic matter in soil due to leaching, atmospheric GHG emissions and mineralization. This results in the deterioration in soil and environment, therefore, various methods to improve or preserve the required quality of soil have been developed over recent years.

The application of biochar (BC, aromatic organic ameliorant) is one of the innovative methods to improve soil properties, carbon sequestration, and decrease nitrogen losses [1-5]. The biochar is produced from biomass of various

types (wood waste, plant residues, organic industrial waste) by slow (several minutes to hours) and fast (milliseconds to seconds) anaerobic pyrolysis at high temperatures (400-900 °C) [6-9]. Pyrolysis conditions and biomass type have the determining influence on the physical-chemical, physical, biochemical and microbiological properties of BC [8, 10-12]. Carbon content of BC can exceed 85 % and depends on the pyrolysis conditions and biomass type [9]. The organic matter of BC includes amorphous and crystalline aromatic structures and is therefore resistant to biotic and abiotic mineralization [13]. However, the BC produced by fast pyrolysis has more organic compounds, carboxyl and hydroxyl groups, macropores accessible for microorganisms than that produced by the slow biomass pyrolysis. After the fast biomass pyrolysis, the BC is subject to higher microbiological mineralization and has greater impact on the microbiological, physical-chemical, biochemical and physical properties of soils [7, 9, 11].

To date, numerous research of BC effects on soil properties have been performed. The BC application generally contributes to the increase in water retention capacity and porosity of soil [1-3, 14, 15], cation exchange capacity [16], and crop yields [17-19].

Despite successful results, there are uncertainties in the understanding of BC effects on microbial community and processes of nitrogen and carbon transformation in soils due to different properties of BC produced from biomass of various types and under different pyrolysis conditions. On the one hand, the research findings have shown that the BC application contributed to the increment in microbiological activity and carbon content of biomass of microorganisms owing to the increased content of accessible organic compounds in soils and improved physical and physical-chemical state of the latter [16, 17]. On the other hand, a number of experiments showed either a lack of significant effect of BC [20], or its adverse impact on the above-listed microbiological properties of soils [21]. The findings are explained by entry of insufficient amounts of available nutrients (nitrogen, phosphorus) into soils due to non-optimal doses of BC, and irreversible adsorption of available (non-aromatic) organic compounds by biochar [20].

Biochar is an advanced ameliorant for decreasing emissions of nitrous oxide (N_2O) from soils. According to the recent research findings, the BC application led to the significant reduction of N_2O emissions from soils [4, 6, 8, 17]. N_2O forms in soils as a result of microbiological nitrification and denitrification. The BC application contributed to change of soil conditions making them more favorable for nitrifiers due to enhancing aeration and increasing pH of soils as well as adsorption of nitrification inhibitors [1, 14, 22-24]. However, the adsorption of NH_4^+ on the BC surface or in its pores can result in the reduction of NH_4^+ availability for nitrifiers [22]. When comparing the effects of BC produced by the fast and slow pyrolysis, it is found that the mineral nitrogen immobilization by soil microorganisms was greater in the first instance [22]. The BC application was accompanied with the decrease in denitrification and N_2O emission both as a result of enhanced aeration and increased pH of soils and due to adsorption of available organic compounds and NO_3^- by biochar [1, 3, 9, 17].

Thus, the BC effects on soil properties should be studied further to standardize databases related to designs of experiments, technological conditions of biochar production and types of biomass used.

In this research, we have shown for the first time that the biochar produced by slow pyrolysis has greater ameliorative effect as compared to its analogue produced by fast pyrolysis as a by-product while generating electric and heat energy.

The purpose of the experiment was to compare the effects of biochar

produced by the fast and slow biomass pyrolysis on hydrophysical properties, nitrification and denitrification in soils having different granulometric textures.

Technique. The BC produced from wood residues in reactors of different types was used in the experiments. BC1 was produced by slow pyrolysis of biomass at 500 °C in Pyreg® (PYREG GmbH, Germany), a reactor used specifically for the BC production, and BC2 — by fast pyrolysis of biomass at 900 °C in Biomass CHP (Spanner Re2 GmbH, Germany), a reactor used to generate electric and heat energy. The specific surface of BC was measured by N_2 adsorption in NOVA analyzer (Quantachrome Instruments, USA).

Samples of gleyic alluvial sandy loam and clayey loam soil were taken in test areas of the experimental station of the Slovak University of Agriculture in Nitra. The water retention capacity with and without BC was measured using a pressure membrane apparatus (Soilmoisture Equipment Corp., USA) at water potentials of –0.1; –5, –20, –55, –100 and –300 kPa. The amount of the applied BC1 and BC2 on a hectare-scale was equal to 15 and 30 tones.

The nitrification intensity was studied in the laboratory experiment. The soil samples (weighing 15 g) moistened up to 48-55 % of the total moisture capacity were incubated in 100 ml glass flasks for 48 h at 25 °C. The amount of N_2O emitted was measured on a regular basis in the process of linear strengthening of N_2O up to the maximum concentration. The denitrification activity was assessed in the soil samples moistened up to the total moisture capacity. The samples were incubated in 100 ml glass flasks for 48 h at 25 °C with addition of acetylene (0.01 %) to prevent the transformation of N_2O into N_2 [25]. N_2O concentration in the flasks was measured on a regular basis until its maximum was reached. The intensity of nitrification and denitrification (based on N_2O concentration) was analyzed using GC-2010 Plus gas chromatograph (Shimadzu Corp., Japan) equipped with an electron capture detector.

Statistical processing of the results included the calculation of mean (*M*) and standard deviations (±SD). The significance of differences among mean values was assessed by the one-way analysis of variance (one-way ANOVA) at $p \leq 0.05$.

Results. The study of hydrophysical properties of soils has shown that the application of BC1 and BC2 contributed to the increase in their water retention capacity. The total water capacity of the initial sandy loam soil was statistically ($p < 0.001$) lesser (34.9±0.1 %) than that of the clayey loam soil (55.1±1.9 %). The moisture content (as a percentage of soil weight) in the sandy and clayey loam soil at water potentials ranged from –0.1 kPa to –300 kPa varied from 33.9±0.1 to 8.8±0.3 % and from 53.3±1.7 to 24.2±0,3 %, respectively. The application of BC2 (at doses of 15 and 30 t/ha) to the sandy loam soil has resulted in the significant ($p < 0.001$) increase in its total water capacity (up to 40.0±0.3 and 45.4±1.3 %, respectively). The water retention capacity of the sandy loam soil increased as well when BC2 was applied at doses of 15 and 30 t/ha: up to 37.8±0.4 and 42.4±1.4 % at the water potential of –0.1 kPa, and up to 17.7±0.8 and 22.9±1.3 %, respectively, at the water potential of –300 kPa. BC1 had lesser specific surface (123 m^2/g) than BC2 (258 m^2/g), therefore, its application at the doses of 15 and 30 t/ha to the sandy loam soil resulted in lesser but still significant ($p < 0.001$) increase in its water retention capacity: up to 39.4±0.5 and 43.2±0.7 % at the total water capacity, up to 37.0±0.4 and 39.7±0.6 % at the water potential of –0.1 kPa, and up to 12.3±0.7 and 15.2±1.3 %, respectively, at the water potential of –300 kPa.

The significant ($p < 0.01$) increase in the total water capacity of heavy loamy soil was observed only after BC1 and BC2 applied at a dose of 30 t/ha. The water retention capacity of the clayey loam soil over the range of water potentials under study increased significantly ($p < 0.05$) after both doses of BC1

and BC2 applied. Within the studied range of water potentials (from –0.1 to –300 kPa), the soil moisture at the ameliorant doses of 15 and 30 t/ha for BC2 varied from 56.9±1.0 to 30.5±0.3 % and from 62.0±1.5 to 36.7±0.8 %, respectively, and for BC1 — from 56.1±0.5 to 28.3±0.6 % and from 59.4±0.6 to 32.6±2.0 %, respectively. The obtained results show that, firstly, BC increases the water retention capacity to a greater extent for soils having light rather than heavy texture, and, secondly, BC2 when applied leads to the greater increase in the water retention capacity of soils than BC1.

The application of BC2 at the doses of 15 and 30 t/ha to the clayey loam soil has resulted in the significant ($p < 0.01$) decrease of the nitrification intensity, while the application of BC1 to the same soil had no significant effect on the nitrification intensity (based on N_2O production). In this case, the nitrification intensity in the clayey loam soil showed higher variability when applying BC1 than when using BC2 (Fig. A).
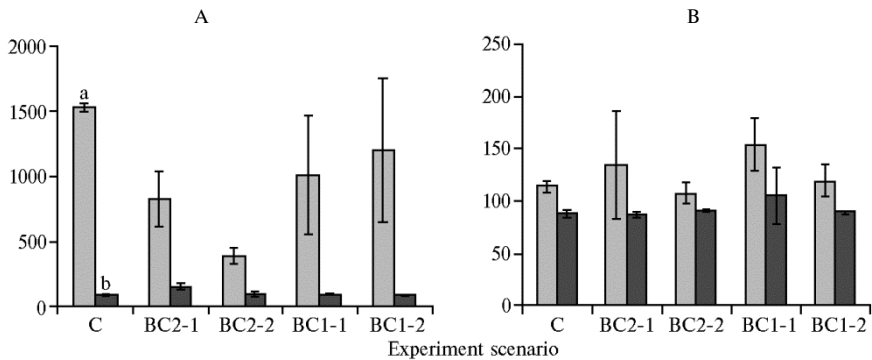
Intensity (M±SD, µg $N_2O \cdot kg^{-1} \cdot h^{-1}$) **of nitrification** (A) **and denitrification** (B) **in clayey** (a) **and sandy loam** (b) **soils upon applying different doses of biochar produced by slow** (BC2) **and fast** (BC1) **biomass pyrolysis:** C — control, BC2-1 — 15 t/ha, BC2-2 — 30 t/ha, BC1-1 — 15 t/ha, BC1-2 — 30 t/ha (amount per 1 ha, laboratory experiment). The significant decrease of the nitrification intensity in the clayey loam soil was observed when applying 15 and 30 t/ha of BC2 ($p < 0.01$), and the significant increase in the denitrification intensity in that soil occurred when applying 15 t/ha of BC1 ($p = 0.05$).

Generally, the effect of BC1 and BC2 on the nitrification in the sandy loam soil (as against clayey loam soil) was insignificant. The scenario with 15 t/ha of BC2 was an exception when the significant ($p < 0.05$) increase in the nitrification intensity from 96.3±9.9 to 156.3±22.0 rg $N_2O \cdot kg^{-1} \cdot h^{-1}$ was observed.

The decrease in the nitrification intensity or its inalterability after applying BC may be caused by several reasons: firstly, the reduction in NH_4^+ availability for nitrifiers as a result of adsorption of ammonium ions on the surface or in micropores of BC; secondly, the lack of sufficient mineral nitrogen accessible for microorganisms entering the soil together with BC; thirdly, N_2O association with functional groups and ions of metals (iron, copper) on the BC surface [14, 16].

The denitrification intensity in the presence of BC1 and BC2 mainly increased and was greater in the clayey loam soil than in the sandy loam one. However, the increase in the denitrification intensity in the clayey loam soil was significant ($p = 0.05$) only at the dose of 15 t/ha of BC1 (refer to Fig. B).

The BC application contributed to the higher increase in macroporosity (with pores > 50 rm in diameter) of sandy loam rather than clayey loam soils. The recent research findings have shown that the BC application resulted in the reduction in the number of aerated pores and air permeability of fine silty soils and the improvement of aeration of coarse sandy soils [1]. Hence, it follows that the BC application to clayey loam soil can lead to enhancing anaerobic conditions and, as a consequence, to more intensive denitrification in case of exces-

sive moistening. Our data has confirmed that, under anaerobic conditions, the denitrification intensity in the clayey loam soil (control and BC1 and BC2 scenarios) was greater than in the sandy loam soil (refer to Fig. B).

Thus, the comparison of effects of biochars produced by slow (BC2) and fast (BC1) pyrolysis has shown that the significant decrease of the nitrification intensity in the clayey loam soil resulted from the application of BC2. Generally, both types of BC have no significant impact on the nitrification intensity in the sandy loam soil. The use of BC1 and BC2 contributed to the increase in the denitrification intensity in the clayey loam soil. The application of BC1 and BC2 was accompanied with greater increase in water retention capacity for sandy loam rather than clayey loam soils. BC2 contributed to the increase in the water retention capacity of both types of soils to a greater extent than BC1. The application of BC as an ameliorant will probably be more effective for reduction of N_2O emissions as a result of denitrification in soils having light rather than heavy texture.

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Challenges and opportunities

Dedicated to the bright memory of professor Dr AA. Zhuchenko,
the initiator of organic agriculture in Russia

GENERAL POSITION OF ORGANIC AGRICULTURE IN WESTERN EUROPE: CONCEPT, PRACTICAL ASPECTS AND GLOBAL PROSPECTS

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A b s t r a c t

Here we present a deep analysis of agriculture in Western Europe, the provisions and principles of its ecological direction. It is noted that Germany and France are the leaders of the EU countries for the production of environmentally friendly products. Organic agriculture is viewed as a holistic ecosystem, where each change affects a complex of complex interrelations, which include both genetic species diversity of crops and livestock. In natural ecosystems, the synthesis, decomposition and consumption of nutrients with the participation of green plants (photosynthesis), insects, animals (herbivores and predators), and microorganisms are constantly occurring. The basis of ecological agriculture and organic farming is healthy soil and fertility soil. Fertile and biologically active soil will provide plants with a sufficient number of nutrients for optimal growth and development, minimize possible damage from diseases, pests and weeds. Improvement of soil ecosystems guarantees the best yields, it is a kind of circular model of long-term planning. The combination of plant growing and animal husbandry in the ecosystem is noted. It represents the alternation of processes of synthesis and decomposition, which is accompanied by an increase in the quality of life in soils, plants, animals, in the life of the entire agro-ecosystem. Organic fertilizers create favorable conditions for inclusion in the agro-ecosystem elements that accelerate life processes in the soil and thereby ensure the growth of plants. A special role is assigned to the rotation in ecological agriculture. It promotes the cultivation of legumes — the main suppliers of nitrogen in the agro-ecosystem. It is the most important means of protection against pests and diseases, and it interferes with a long monoculture. It is a mean of regulating weed vegetation. Inclusion of perennial forage crops in crop rotation helps a farmer prevent the extreme growth of each species of weeds to a dangerous level. The importance of manure and slurry for the ecosystem of the soil, as well as that of the straw litter for animals, was noted. Data on hunger and obesity in some countries are given. Integrated agriculture is also considered as an alternative to traditional agriculture. But solving environmental pollution problems by integrated agriculture is less effective. In the EU countries, financial support is provided to organic farming. By 2020, the world market for organic products is projected to be 200-250 billion dollars, with the potential of the Russian market of 700 billion rubles. That is, Russia can account for 10-15 % of the market. In Russia, there is a demand for organic food. Organic agriculture contributes not only to the production of healthy food, but also to the preservation of the habitat, biodiversity and respect for wild and agricultural animals.

Keywords: organic products, organic farming, agro-ecosystems, soil fertility, healthy soil, natural fertilizers, legumes, crop rotation, integrated agrotechnology, biodiversity

Agro-ecological farming systems, like organic-, permaculture and biodynamic-agriculture (or organobiological) agriculture, are mainly based on organic fertilizers (manure, composts, siderates), nitrogen fixation in legumes, crop rotation and integrated plant protection (by use of biomethods and varieties resistant

to pests, weeds and diseases), control of crop quality and optimal balance of nutrients along with a decrease in energy consumption per unit of final product. Resulted products are considered organic eco-friendly food [1-5]. In 2011, the world environmentally friendly products exceeded 45 bln €, including 21 bln € in the US and 21.5 bln € in Europe. The leaders of European market are Germany (6.6 bln €) and France (3.8 bln €). The countries with the highest consumption per capita of environmentally friendly products are Switzerland and Denmark (more than 160 € per year). The largest number of environmentally friendly food producers is located in India, Uganda, Mexico and Tanzania [6].

Organic farming: general principles. Plant growing, as a primary production system, is peculiar in its dependence on local natural conditions. Agriculture as a whole is an art of providing for necessary prerequisites to use the entire self-organizing bioecological potential of soil—plant—animal systems and to enhance the efficiency of naturally occurred processes. In fact, holistic living agro-ecosystems are here the essential mean and productive forces the basis of which is the soil.

The roots of plants are obviously hidden from our direct observation as they grow into the darkness of the soil. That plain fact hampers our immediate understanding of what they actually do, and how. Plant growth research in glassware is a helpful tool, but it creates a crucial artefact: in the laboratory as well as in our minds. We apply 'pure' chemicals that we presume to be plant nutrients, to find out which of them are needed. But using this presumption and its experimental setting, we can only find that plants indeed use (and thus 'need') soluble nutrients, which they absorb with sets of identical roots, in a uniform environment.

In this paper we choose for the concept wherein plants grow in soils that are seen as ecosystems or, in other words, in 'living' soils. Amelioration of the farms' soils is in that concept the prime goal of the farmer, with the overall objective to have ever better harvests by ongoing improvement of precisely those soils. These soils then again are seen within the farms biotope as a whole, in the farm's landscape, which includes all environmental compartments [2].

Soil is alpha and omega in agriculture. Plant growth depends on soil processes, and plant organic residues are decomposed in soil up to nutrients for other plants [7-10]. So soil health and fertility must be preserved and improved. Soil is a natural ecosystem with the greatest variety of micro and macro bionts [2, 7], i.e. fungi, algae, bacteria (including those decomposing mycelium and chitin), earthworms, beetles etc., which restore soil fertility. Soil biota also contributes to water retention in the biologically active plow layer, optimal growth and increase in plant resistance to diseases [8, 10]. This is a whole process that still is largely unknown in detail. Physicochemical properties (particle size and capillarity, etc.) are another important factor of soil quality [10]. The root systems multi-functionality invites us to provide them with a soil ecosystem wherein they can find what they need when they need it. And if the soil lacks structure, water reserves, bacteria, mycorrhiza, minerals or whatever, the challenge is to start growing crops that provide for what is missing. Apart from roots for feeding, plants have roots specialised in water uptake, in weathering rocks and in stabilising the plants' stems vertical uprising. And always, dying roots leave organic matter in the soils they have grown in: energy-food for the ecosystem. Healthy soil allows for uniform spread and deep penetration of plant roots. The more small rootlets and root hairs are in the plant root system, the healthier the soil and soil organisms are. Unbranched roots often indicate a weak biological activity of the soil due to densification and lack of air. In legumes, roots are indicative of soil air level. At enough aeration, in nodules, which appear on roots,

N$_2$-fixign bacteria can transform atmospheric nitrogen into fixed nitrogen usable by plants [9].

Measures to keep up natural balance in the agro-ecosystem should be consistent with the concept of organic agriculture, otherwise the result be the opposite. Some scientists assert that the introduction of mineral fertilizers reduces the activity of soil bacteria and nitrogen fixation in legumes [11, 12] and shifts ecological balance to increased plant sensitivity to diseases and pests. The latter, in turn, requires more chemicals for plant protection resulting in large-scale and long-term adverse impacts on environment and economy.

Biologically and economically sustainable agro-ecosystems must be based on ecological principles [12-15]. A commercial farm is considered as an integral ecosystem, where each change affects a complex of interrelationships. For example, a high content of soil nitrates can stimulate a strong development of weeds and cause nitrate pollution of groundwater. Cultivation of crops that consume nitrogen prevents its washing out and contributes to a better balance of nutrients thus avoiding the problem [13]. Genetic and species diversity is achieved through i) a wide range of crops in crop rotations, ii) preservation of natural environment areas as habitats of natural enemies of pests, and iii) adjustment of livestock population concordantly to capability of field crop cultivation. Crop and livestock diversity also contributes to sustainable farming when market fluctuations. In natural ecosystems, green plants, due to photosynthesis, insects, herbivorous animals and predators, and microorganisms can constantly synthetize, decompose and use nutrients. Agro-ecological farming cannot fully be the same as natural substance turnover but can simulate it. So, all products of vital activity of the agro-ecosystem are subject to utilization. Among other benefits, this makes it possible to minimize purchases for the needs of the farm.

No need to stress that organic agriculture basically goes for soil building. As soil fertility is cornerstone for ecological farming, an increase in yielding must be in compliance with careful use of natural resources (soil, water, air, forests, biodiversity). Organic fertilizers and residues, when involved in recycling, improve soil properties, and soil microfauna and flora promote the weathering of rocks and release important elements that are absorbed by plants and eventually incorporated into the ecosystem of the soil. Thus, crop production is associated with soil fertility restoration, food and feed production, and the maintenance of chemical element circulation in a soil—plant—animal system.

Main elements of commercial farming. Back in the XVIII-XX centuries, Russian and Soviet scientists noted [16, 17] that it is the linkage of plant growing and livestock that is crucial for optimal eco-farming. Manure and slurry compensate removal of N and other chemical elements with plant biomass [18], thus improving soil fertility and allowing to control over the agro-ecosystem. Therefore, the farm should cultivate both food and fodder crops, considering also which plants are dependent on organic fertilizers, and which are independent and perform the soil-improving function. For this, the soils are examined for suitability for certain cultivars. Legumes are the most important source of nitrogen and a factor in increasing soil fertility. The cultivation of nitrogen-fixing legumes in large areas also provides the livestock with high-protein fodder.

Hence, ruminants, eating post-harvest residues and giving valuable manure, are the main in animal husbandry under organic farming. In stall maintenance, biological features of breeds should be considered. Animal breeding necessitate special attention. Pigs and poultry livestock must be limited to not compete with other species and humans for fodder and territory. Manure can be produced both for own needs and for sale. Agrochemically, the function of animal husbandry is nitrogen recycling.

Crop rotation. Plants are the main sources of energy for the whole soil ecosystem. Flora along with soil microfauna contribute to the weathering of rocks and the release of minerals, which in turn are absorbed by plants and incorporated into soil ecosystems.

Nowadays, crop production is mainly estimated by quantitative indicators. This makes species composition of cultivated plants poorer, and low-field crop rotations (unlike multipole ones) do not provide a natural restoration of soil fertility [19-21], which is replenished by mineral fertilizers.

In eco-farming, crop rotations perform several functions [16, 22, 23]. The use of legumes provides the agro-ecosystem with nitrogen and fodder for livestock. Using different crops prevents the unrestricted reproduction of pests and pathogens, and also restrains the development of each species of weeds below a dangerous level due to the alternation of spring and winter crops (including late and early ripening varieties), crops that deplete and enrich the soils (some crops are more 'giving', others take more than the give), and the use of perennial seeded meadows.

The art of farming is to orchestrate all particular crops and animals in such a way, in space and time, that the overall effect benefits the soil fertility and human nutrition. Crop rotation (temporal variation) and mixed cropping (spatial variation) and mixed farming (plant and animal production) are tools thereto, which are particularly well developed in organic agriculture (agroecology, permaculture, biodynamics).

The soil in one rotation is equal in fertility. Crop rotation should be multifield, including at least two fields of perennial grasses (legumes and their mixtures with cereals), and involve different plant species. Legumes in the crop rotation are up to 25-33%. One year the fields are leaving fallow with annual forage grasses or used for fodder crops that provides weed control. The use of intermediate and subcultivated crops (beans) as often as possible is beneficial. Intermediate cultures as siderates inhibit the weed growth. In the Non-chernozem zone, the cultivation of intermediate crops has advantages [22-25]. Organic fertilizers are advisable to apply under the fallow culture and the crop used before sowing or planting tilled crops. Interval between cultures that consume soil nitrogen and legumes or crops treated with organic fertilizers should be no more than 2 years. Plants with a long early stage of development are included in the crop rotation after herbages that suppress weeds.

So for example a soil friendly crop rotation of $3/7$ in wheat (bread and fallow), $1/7$ in oil crops, $1/7$ in potatoes, beetroots and vegetables and $2/7$ in pulses and fodder grains would facilitate a European nutrition pattern on an on-farm fixed N ration [26].

Use of manure and slurry. These are the main organic fertilizers, but in uncontrolled application manure and slurry, like chemical fertilizers, adversely affect the environment [11, 18]. Critical processes in the nutrient cycling in the agro-ecosystem, especially nitrogen volatilization and leaching, attract special attention.

To avoid high nitrogen input to soil when using liquid excretions of animals and manure contamination, it is necessary to control the ratio of carbon and nitrogen in feeds (along with the amount of protein and exchange energy). In using straw as litters, its absorption capacity, porosity, ease replacement and good biodegradability is important (that is why the C: N ratio is important too). At storing, care should be taken to trap seepage slurry to use nitrogen and potassium in recycling (specific use). Storage should provide repeated absorption of nitrogen mineralized by microorganisms, and its inclusion in compostable sources of carbon and energy (straw, etc.). Straw of cereals and decompos-

able hollow stems of other plants, when added to solid and liquid manure, prevents nitrogen volatilization. Technical utilization of manure and slurry (filtration, drying, etc.) is energy-intensive, requires time and resources, including labor, and is not in line with the bioecological concept. In the Netherlands, cattle of a live weight of 490-500 kg produces on average about 12-15 tons of manure (litter with urine) per year, where the organic matter is 20-22 % and nitrogen is 0.7 -0.8 % [11, 26].

Organic agriculture and human health. A recent meta analysis [27] of 343 previous studies find organic milk and meat to contain ca 50 % more beneficial omega-3 fatty acids than non-organic. This also applies to organic dairy like butter, cream, cheese and yoghurt. Thus they appear to be nutritionally superior to the dairy from conventional agriculture. Further key findings are the follows. Organic meat had slightly lower concentrations of two saturated fats linked to heart disease; organic milk and dairy contains 40 % more conjugated linoleic acid (CLA) (CLA has been linked to a range of health benefits including reduced risk of cardiovascular disease, certain cancers and obesity, but evidence is mainly from animal studies) [27]. Organic milk and dairy contains slightly higher concentrations of iron, Vitamin E and some carotenoids. Organic milk contains less iodine than non-organic milk (an issue which organic farmers have started to address with mineral supplements).

Another survey states [28] that the benefits of organic products are higher levels of vitamin C, iron, magnesium, and phosphorous than nonorganic foods of the same type; higher levels of antioxidants, naturally occurring compounds associated with a reduced risk of chronic diseases and certain cancers; fewer toxic chemicals such as nitrates and pesticide residues; no genetically modified organisms (GMOs).

Organic food is especially recommended for vulnerable consumers, like children and pregnant woman,

Agro-ecology. In 2015 FAO's Deputy Director-General Maria Helena Semedo warns that agriculture is discovered as a big threat in the fight against climate change [29]. She calls upon governments to integrate this sector into their urgent climate policies. If fail to do so, for example because they see it as a threat for standing positions, she predicts ever more hungry people in the world. Agriculture and the good, carbon enriching use of soils, thus have made a strong debut to the series of measures against greenhouse gases, the sources of global warming. From the 186 countries that have already laid out voluntary plans to reduce their emissions, around 100 of them include measures related to the use of soils and agriculture. But M.H. Semedo warns that those measures must be effectively implemented, not stay paper on shelves.

A recent meta-analyses on the productivity of organic versus non-organic agriculture states that, with appropriate crop rotations and multi-cropping systems, organic (soil building) agriculture produces only 8-10% less as compared to non-organic (soil eroding) agriculture [30]. Moreover, the various types of organic agriculture all contribute, in one way or another, to a striking decrease of the externalised problems that conventional agriculture confronts society with, now and in the future. So for example, besides the soil erosion and land degradation, there is the loss of biodiversity, the eutrophication and oceanic dead zones, the pesticide effects on humans, environment and wildlife, the greenhouse gas emissions, and the regime shifts in hydrological cycling (drought). All this multiplies when forestry effects are included in (added to) those of agriculture.

Solving the global hunger problem — can organic agriculture feed the world? In today's world, there is a double problem — hunger and obesity as consequences of poverty. At present, the world production of

high-calorie products exceeds the needs of mankind, but social, political and economic factors limit its fair distribution, as well as the consumption of healthy food [31]. The latter stands for over 100 kg/pp in the NW countries and some 10 kg/pp in poor countries [32]. Only increasing yields will not solve these problems - it is obvious that it is necessary to reduce the difference in profitability between sustainable and traditional agriculture.

Therefore multiple food systems must be combined to improve resilience through enhanced diversity, and different forms of farming must be allowed to coexist, each fulfilling a different function. The example of Brazil shows how this can be fairly well and multi beneficially realised [33]. In opinion of B. Halweil (personal communications), looking at 77 studies from the temperate areas and tropics, the Michigan team found that greater use of nitrogen-fixing crops in the world's major agricultural regions could result in 58 million metric tons more nitrogen than the amount of synthetic nitrogen currently used every year. Research at the Rodale Institute in Pennsylvania showed that red clover used as a winter cover in an oat/wheat-corn-soy rotation, with no additional fertilizer inputs, achieved yields comparable to those in conventional control fields [33]. Even in arid and semi-arid tropical regions like East Africa, where water availability is limited between periods of crop production, drought-resistant green manures such as pigeon peas or groundnuts could be used to fix nitrogen [33]. In Kenya, farmers using leguminous tree crops have doubled or tripled corn yields as well as suppressing certain stubborn weeds and generating additional animal fodder [33].

Integrated agriculture as an alternative. Many farmers and some scientists do not agree that one can do without using chemicals. The concept of integrated agriculture is based on the fact that such measures should be used as rarely as possible and only in necessary cases, or biological preparations that are available to farmers should be applied [11, 34]. However, the widespread introduction of integrated agriculture will not solve the pressing problems of agricultural production. Chemicals for plant protection always violate the ecological balance (in recent years, the use of fungicides in the West has led to the widespread spread of the yellow dwarf barley virus, etc.), their use is associated with an increase in the toxicological and ecotoxicological risk to the environment and human health. Because of introduced mineral fertilizers, the content of nitrates in groundwater is constantly growing, and the soil is oversaturated with nitrogen and phosphorus. In ecological farming, when the livestock population correlates with the farm land area (no more than two animals per hectare), purchases of feed are greatly reduced and the introduction of mineral nitrogen fertilizers is prohibited, the problem intersects in its root. That is, addressing the problems of environmental pollution in the concept of integrated agriculture seems less effective.

Prospects for eco-agriculture. Because of the urgent need to eliminate overproduced food stuff in EU, for the first time in 1989, the program of ecological farming in the framework of extensification was financially supported, which increased the status of organic farming [35].

By 2020, the world market for organic products is projected to be 200-250 billion dollars, with the potential of the Russian market of 700 billion rubles, i.e. Russia can account for 10-15 % of the market [6]. In case of the development of organic farming, 1 million new highly paid workplaces will be created in the agroindustrial complex of Russia. Examples of regions for cultivation of ecologically clean products are Transbaikalia (the Baikal Lake protection law is effective there and any application of toxic chemicals is prohibited), and the Republic of Crimea. Because of extension of its territory and infrastructure problems, it is expedient to launch a closed cycle of organic production in the Far East [6]. Generally, organic farming shows its worth in areas where high farming

techniques are inconsistent. In Russia, there is a potentially high demand for organic food, and the current biotechnologies make it possible to move towards organic farming techniques for many crops without detriment to yields and increase in costs. Finally, this is not only healthy and nutrient-enriched food, but also the preservation of habitats, and caring attitude towards wild and agricultural animals.

So, traditional industrial agriculture provide large volumes of world production, but lead to land degradation, water resources and ecosystems, greenhouse gas emissions, and reduce biodiversity. At the same time, quality and nutritional value of products decreases, which causes obesity and an increase in food-related diseases. Precision technologies can improve some indicators of agriculture but do not provide long-term prospects. A fundamentally different model is based on the diversification of farms and agricultural landscapes, the stimulation of biodiversity and the interaction between different species. Such a model is part of the strategy for the formation of long-term fertility, healthy agro-ecosystems and the sustainable existence of mankind. Diversified agroecological systems are able to compete with industrial (traditional) agriculture for overall productivity (especially under environmental stress) and provide a greater variety of food nutrients that will improve the health of the population.

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Grain crops: genetics, improvement, agrobiotechnologies

ON HOW WE CAN NON-CANONICALLY INCREASE HEREDITARY DROUGHT RESISTANCE IN PLANTS (BY AN EXAMPLE OF CEREALS)

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A b s t r a c t

Improvement of hereditary drought resistance in crops is recently being among the main objectives for food security of humanity because of global warming and the growing costs for bakery and forage grain. Analysis of complex properties of drought resistance in cereals (phenotyping) shows the limitations of a canonical genocentric approach and the approaches based on molecular genetics to solve the problem of significant hereditary improvement of drought resistance. The priority epigenetic approach that we propose is based on the theory of eco-genetic organization of quantitative traits (TEGOQT). In TEGOQT seven genetic-physiological systems (GPS) involved in harvest increasing, but not particular traits of productivity, are to be operated with. These GPS are attractions; micro-distribution of attractive plastic substances between grains and chaff in ear; adaptability to drought, cold, frost, heat, salt, etc; horizontal immunity; «payment» by dry biomass for a limitative factor of soil nutrition — N, P, K, etc; tolerance to plant density in phytocenosis; hereditary variability in duration of the phases of ontogenesis). In this paper we discuss drought adaptability as a part of GPS complex. It is shown that phenotyping evaluation is necessary to analyze drought tolerance, the complex property to which no less than 22 component characters contribute. This allows to construct a set of eco-genetic portraits (EGP) of parent plants at different types of drought simulated in an artificial climate control chamber. The EGP are histograms which reflect the values of all components of drought resistance for each parent thus allowing to select pairs complementary in the elements of the EGP. Based on a predictive EGP, it is possible to define, through mathematical models that reflect the contribution of each component to the resulting drought tolerance, the optimum combination of components for ensuring maximum positive additive effect, and thus to manage selection of the parents for hybridization in view to create new drought resistant varieties.

Keywords: crop cereals, drought resistance, phenotyping, eco-genetic portraits, management by selection of parental pairs

Evolution did not produce any plant that would prefer drought
(V.V. Kyznetsov, G.A. Dmitreieva)
There are so many hungry people that God cannot appear to them
except in the form of bread
(Korita Kent)

Every year, the Russian grain industry loses 5-7 billion rubles due to drought. In 2015, the damage amounted to 7.9 billion rubles. In Australia, in 2003, the wheat production reduced from 24 to 9 million tons, or by 62.5 %, because of drought [1]. For any plant, the direct effects of moisture deficiency (drought) are reduced cell turgor, stomata closing, suppressed growth, and a decreased yielding [2, p. 218]. There are various agrotechnical methods for combating drought: snow accumulation in fields by forest belts, fallows and watering

(from irrigation to sprinkling and drip irrigation), etc. However, the number of genetic breeding techniques is much greater, and they are cheaper than agricultural technologies, especially those used for irrigation or applied annually.

Is it possible to substantially increase hereditary drought resistance of cultivated cereals? For the answer, it is necessary, according to the Vavilov's law of homological series in hereditary variability, to seek for an increased drought resistance in wild relatives of traditional crops [3]. It is known that some cereals (*Gramineae*) overcome cultivated cereals in drought resistanse. *Phleum phleoides* Wib., *Agrostis vulgaris* With., *Stipa capillata* L., *Lasiagrostis splendens* Kunth., *Poa bulbosa* L., *Festuca ovina* L., *F. sulcata* L., *Bromus inermis* Leyss., *B. sterilis* L., *Agropyrum cristatum* L., *Hordeum spontaneum* C. Koch. grow on dry soils where no cultivated cereals can exist [4]. Consequently, we can talk about the principle possibility of significantly increasing the drought resistance of traditional cereal crops. Wild varieties have increased this property over tens of thousands years by natural selection. Modern geneticists, physiologists and breeders should create new drought resistant varieties in the next decade.

As to the traditional genocentric paradigm, according to which genes determine productivity, adaptability and the genotypic variability of these characteristics, drought resistance is the trait to be studied by the Mendelian genetics. In case of a not clear segregation histogram, the analysis of diallel crosses according to Hayman [5] can be used, based on the same Mendelian genetics postulates. Hence, there is an aspiration to find a gene (polygenes) of drought resistance. The multi-component nature of drought resistance formed during phases of ontogenesis due both to gene differential activity and epigenetic effects (e.g. in hardening and induction by drought of the sleeping genes expression) is not usually considered. These change the hypothetically unambiguous path from the gene to the trait.

There are various types of drought [6], and each depends on many physiological and morphological features of plants. The main ones are the orthotropicity and depth of the root system [7-10]; a labile functionally active part of the root system (for example, a small number of deep roots, reaching the moist soil horizon, increases the moisture absorption by several times, whereas the bulk of the roots in the upper dry horizon practically do not work) [7]; the depth of tillering node, determined by the balance of hormones [11]; osmotic pressure in root hairs [12-14]; energy transport of soil solutions [15, 16]; energy consumed for enzymatic reactions [17]; the temperature limiting catalytic activity of nodal enzymes [17]; ATP synthesis [18]; membrane efficiency [19]; total surface area of the leaves as related to their volume and weight [20]; the thickness and density of the cuticle [11]; the number of stomata per leaf area unit and their size [21]; the osmotic mode of stomata opening and closing [21]; leaf pubescence (i.e. hair density, length, stiffness and light reflection) [11]; leaf ability to twist when drought [14]; vertically oriented leaves [14]; short and narrow leaves [11]; shift of the critical phase of ontogenesis under a typical stressor at a certain time [11]; the intensity of metabolic water production [14]; the structure of chloroplasts, optimal for droughts [14]; CO_2 assimilation per moisture loss [22]; energy expenditures for attraction [23]. The papers on the phenotyping (dissection) of the drought resistance trait into elementary components begin to appear [24].

Each of the components of drought resistance as a complex property cannot be determined by one or two Mendel genes. For example, let us consider the genetic determinism of the component "the osmotic pressure in root hairs". It is known [25] that the total number of genes expressed in human cells is about 24,000, of which about 11,000 are present in cells of any type. If this principle is valid for plants, it can be assumed that there is a pool of products of more than

10,000 genes in each cell of a root hair and each product contributes to the resultant osmotic pressure of hair cells, which allows extracting soil solution from the semi-dry substrate. By the traditional breeding methods (pair crosses and phenotypic selections), it is hardly possible to assemble 10,000 genes for the maximum osmotic activity. A smaller number of genes most likely determine the remaining components of drought resistance; however, geneticists have not found discrete histograms of Mendelian segregation for any of these 22 components. N.L. Udolskaya in 1936 showed [25] that drought tolerance of the variety varies in ontogenesis and depends on the combination of factors that cause drought. Drought resistant varieties can become drought sensitive when precipitation regime changes.

Productivity and yields are determined by genotype × environment interaction (GEI) effects rather than by quantitative trait genes. GEIs are emergent properties of high organization levels (ontogenetic, population, phytocenotic) which are absent at the molecular level [26]. N.I. Vavilov wrote: "We will not be surprised if a thorough study of the heredity of quantitative traits leads to a fundamental revision of simplified Mendel's ideas" [27, p. 275]. The specific genes for productivity, yielding and yield homeostasis (plasticity of the variety), horizontal immunity, species immunity, drought, winter, heat and cold resistance, and so on have not been found, isolated, localized, cloned or sequenced [28]. Similarly, the processes responsible, for example, for consciousness, long before the transition to molecular level will disintegrate to ordinary occurring reactions [29].

Now, it is proposed to radically increase drought resistance with the help of transgenesis, by introducing Mendel genes into non-drought resistant plants. Sometimes, under a certain type of drought, such a gene can slightly increase the yield. Unfortunately, Mendel genes in plants are described very little (only 1-3 %). The products of the remaining 97 % of the genes are, firstly, almost unknown, and, secondly, they are in the most complex interactions with each other and with the constantly changing (even within 24 ours) external limiting factors. As a result, when the LIM factors change, these products alternately determine the components of drought resistance and the resulting manifestation of this property as a whole.

In transgenesis, which currently allows transferring only Mendel genes, there are many weaknesses. The existing methods of plant transformation are effective enough, species- and variety-specific, lead to the random integration of foreign DNA into the recipient genome, impose limitations on the amount of information transferred, etc. The transfer of transgenes from one variety to another requires multiple return crosses and cannot be considered a genetically pure procedure, because in a random recombination, with the transfer of foreign DNA, different DNA fragments of the donor variety are transferred. Transgenes in commercial varieties are expressed constantly and, as a rule, in all plant organs and tissues (similar to cancer behavior when cells escape from the control of the whole organism and divide arbitrarily in any tissues). Effective integration of transgenes into a predefined region of the genome still remains problematic. Manipulating even a few independent traits and coordinating them into hundreds of varieties is an extremely difficult task for breeders [30].

Unfortunately, some geneticists consider such a complex property as drought resistance, which is due to different activity of the genes in ontogenesis, as a feature that is genetically controlled like the elementary Mendel's ones. No attention is paid to the variety specific characters, drought types and duration, the periods of plant sensitivity to drought and the ontogeny phase, in which various productivity elements are pre-formed, the number of which is reduced by the

effect of drought in the corresponding phase of the ontogeny [31]. D.A. Sabinin, the founder of the Russian scientific school of plant physiology, urged to study how various organs and yield components are initiated in ontogenesis.

Breeders and agronomists traditionally estimate drought resistance as a yield value, although there are varieties with the same dry plant weight (that is, in fact, with the same drought resistance), but with different GPS (genetic physiological systems) of attraction, which unequal activity of which results in different grain yield. That is, the grain yield cannot be a correct and strict estimate of drought resistance. Thus, firstly, it is necessary in every particular case to take into account the type of drought in connection with the phase of ontogenesis, in which it occurred, and characterize drought by the force of the action and duration. Secondly, "drought resistance", in our opinion, is biologically very indefinite (this term is used in physics), and it is more appropriate to use the notation "drought tolerance", "drought endurance" or "drought productivity" (the last concept seems to be the most adequate). The final strict quantitative measure of drought productivity should be a reduction in dry plant biomass as compared to that of analogues of the same variety grown in comfortable conditions. In this article, by drought resistance we will understand drought productivity.

Note that the drought productivity, as plant biomass per area of agrophytocenosis, is determined not only by elements of drought resistance, but also by the polygenes of competitiveness and tolerance to sowing density that is, this criterion is much less stringent than the dry biomass production of a single plant at drought.

Breeders test many varieties at different droughts, cross the most drought resistant ones, and in segregating generations, starting with F_2, visually select the most productive individuals by the phenotype. In this case, each productivity trait has a phenotypic variability in the population, including ecological (modification), genotypic, genetic (additive) and epigenetic components of the variability that are not eye-visible. The genotypic variability for yield elements usually amounted to 10-20 %, while ecological one amounted to 80-90 % (if the plants are located at a sufficient distance from each other and do not interact). At the usual density for the nursery, additional noise appears, masking the unique genotypes necessary for the breeder such as genotypic, ecological (competitive) and ontogenetic ones [32]. P.P. Litun [33] on the model wheat populations tested the reliability of selection for phenotypes under drought and found out that of the 10,000 visually isolated plants, only one was genetically drought resistant.

Such a low efficiency of individual genotype identification by phenotype creates a significant obstacle to the inherent increase in drought resistance. The application of the principle of background traits [34] and a new method of genetic analysis of quantitative traits [35] radically solve the problems.

Unfortunately, drought-induced expression of the so-called sleeping genes, like the inducible genes of heat and cold shock proteins [1], has not been adequately studied. The plant response to water deficiency through the synthesis of abscisic acid (ABA), which closes the stomata to reduce water loss, is known [20]. In case of water deficiency, the synthesis of low molecular weight osmotically active substances (mono- and oligosaccharides, as well as amino acid, primarily proline), betaine, polyhydric alcohols and various stress proteins (osmotin, dehydrin) is activated. Proline is of particular importance; its content increases sharply in drought [36, 37]. The *RD29* (responsive to dehydration) genes, for example, are activated in osmotic shock, cooling, water deficiency and by ABA treatment; that is, the genes that are expressed in response to different stressors are known [24].

For a simplified analysis, of all 12 ontogenetic phases in cereals, we sin-

gle out tillering as one of those determining final productivity. In this phase, the drought has a significant effect on the grain number per ear, so it is important to create tolerant varieties for the climatic zones where drought occurred during the onset of this phase (i.e. the most of the grain zones of Siberia). A quantitative measure of drought resistance in tillering (by analogy with the overall resultant drought resistance) is the reduction in the grain number in the ear in a drought-affected plant, compared to the same index for a plant of the same variety under comfortable conditions. The second phase, which we will consider, is grain filling. Saratov type drought, having the greatest impact on this phase, could be estimated by wrinkled grain ratio.

Thus, there are two serious obstacles to the inherent increase in drought resistance: the complexity of the combinatorics of the best polygenes in one class and the very low efficiency of reliable visual identification of genotypes by phenotypes. The second problem is practically removed by the innovative technology of selection, which unlike all existing ones is built on the principles of background traits [34] and "orthogonal" (multidirectional) identification [23]. According to this technology, the productivity traits [35] are not used to visually identify plants that possess the breeder's desired property. They serve as coordinates in which the contributions of genetic and physiological systems are multidirectional. It allows to eliminate interferences that reduce effectiveness of visual identification of genotypes in the field, and help to unerringly identify individual positive hereditary deviations in any of the seven GPS, including the adaptive system, which manifestation at drought leads to drought resistance.

In this paper, we present the development of the formalized approach to selection according to the GPS data, with the example of a hereditary increase in drought resistance in cultivated cereals. We believe that the management of this process should not be carried out at the molecular level (DNA and Mendel genes), but at the level of setting and forming each of the 22 components of the resulting drought resistance and estimating for each component its additive fraction of variance in a specific varieties set. After detection of the genotypes bearing additive polygenes with the maximum contribution to each component of drought resistance, one should try to combine these polygenes by diallelic crosses of 22 varieties with the additive genes, of which the maximum contribution to drought resistance of at least one component is characteristic. To solve this problem, it is proposed to use the model of a quantitative productivity indicator, replacing the traditional model of Ronald Aylmer Fisher [32] for plants. The model proposed by us is formalized in the corresponding algorithms and programs [38].

A success in the selection of wheat indicates that this culture has a very significant additive (genetic) variance [11]. That is why the optimal approach to increasing hereditary drought resistance is the use of ecological genetics methods of quantitative characteristics (one of the branches of epigenetics of quantitative properties) [39]. With its help, it is possible to estimate the additive variance for each of the 22 components of drought resistance and combine the most valuable additive shifts.

Developing our model [38, 40, 41], we proceed from the fact that the plants have the following seven GPS, controlling which breeders achieve an ecogenetic increase in yield. The first is the system of photosynthetic product attraction from stems and leaves to the ear (cereals), capitulum (sunflower), cob (corn), fruit and berries. The second is micro distribution of plastic substances between the grain and the chaff in the ear, the kernel and husk in sunflower, etc. The third one is adaptability (determines the general adaptability to the local conditions of the zone, field and year, as well as adaptability to a certain limiting

factor at a provocative conditions, for example, drought, cold, heat, salinity, soil pH, etc.). The fourth system is horizontal immunity; the fifth one is the dry weight gain per a small dose of soil nutrients (nitrogen, phosphorus, potassium, etc.); the sixth one is tolerance to sowing density; the seventh one is genetic variability of the duration of ontogeny phases. For breeders, the most important types of abiotic adaptability are drought resistance, cold resistance, frost resistance, winter hardiness, heat resistance, tolerance to saline and acidic soils, to lodging and seed germination in ear.

Quantitative geneticists, physiologists and breeders can ignore the complexity of combining multiple genes and their products that affect GPS, and consider only seven GPS (instead of 120,000 genes in soft wheat) as sufficiently indivisible units that are able to combine with each other, gathering in an ideal future variety. Of course, these systems are not 100 % additive, and there are certain interactions between them that do not greatly hamper the historical trend of selective yield increase. This is confirmed by winter wheat varieties for Kuban and Moscow regions with more than 100 c/ha yield which were successfully selected without genetic engineering, genomics, proteomics and metabolomics.

Let us define the formalized form of the criteria for drought resistance of grains in accordance with the verbal definitions formulated above.

For total drought resistance (during grain filling)

$$J_\Sigma(T) = \frac{x_{dr}(T) - \Delta_{dr}(T)}{x_{dr}(T)} \cdot 100 \%, \tag{1}$$

and for the partial drought resistance (during tillering)

$$J_k(T_k) = \frac{n_k(T_k) - \delta_k(T_k)}{n_k(T_k)} \cdot 100 \%, \tag{2}$$

where $x_{dr}(T)$ is plant dry weight at filling T phase onset for favorable conditions, Δ_{dr} is the dry weight loss due to drought, $n_k(T_k)$ is the grain number per ear for favorable conditions at tillering, $\delta_k(T_k)$ is the loss of the grain number because of drought.

When predicting drought resistance criteria, we need the following mathematical models: for the structure "moisture—dry matter"

$$\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \end{bmatrix} = \begin{bmatrix} a_{11} & a_{12} \\ a_{21} & a_{22} \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix} + \begin{bmatrix} b \\ 0 \end{bmatrix} u(t) + \begin{bmatrix} c_{11} & c_{12} & c_{13} \\ 0 & c_{22} & c_{23} \end{bmatrix} \begin{bmatrix} f_1(t) \\ f_2(t) \\ f_3(t) \end{bmatrix} + \begin{bmatrix} \xi_1(t) \\ \xi_2(t) \end{bmatrix}, \tag{3}]$$

$t \in (0,T);$

for grain number per ear

$$n_k = b_1 f_1 + b_2 f_2 + b_3 f_3 + \zeta_k, \tag{4}$$

where x_1 is plant dry weight, x_2 is the green plant biomass, u is soil available nitrogen; f_1 is PAR as a factor of biomass productivity, f_2 is air temperature as a factor of biomass productivity, f_3 is soil moisture as a factor of biomass productivity; ξ_1, ξ_2 are random perturbations reflecting uncertainty of the model; a_{11}_a_{22} are parameters of the dynamic model (3); n_k is the grain number per ear at tillering; b_1, b_2, b_3 are parameters of the model (4); ζ_k is simulation error.

The models (3) and (4) characterize one of the initial varieties used as a parent to produce a new drought resistant generation. The other parents described in the same way will have other parameters. It is necessary to find due combination of drought resistance elements in final variety. In the second (seg-

regating) generation, pairwise combinations of the best plus additive shifts begin to occur. The information-algorithmic basis of these problems is the concept of "ecogenetic portrai" (EGP) [42]. It is a combination of positive shifts in each of the seven GPS (or components within a single GPS, for example, drought resistance components) which are desirable to combine.

Model (3) describes plant state under favorable conditions. Their violation, like GPS effects, leads to disturbances and the appearance of environmental and genetic variances in plants. However, the above 22 physiological and morphological components indicate the possibility of further deepening the proposed EGP-based approach. After all, each of the seven EGP is characterized by its oven number of state components, and most of them can be measured in artificial climate chambers. Let us describe the 22 components that determine drought resistance, as related to all GPS. To do this, we denote these states by the vector $Z = (22 \times 1)$ for all seven EGP, according to their numbering:

$$Z = (z_1\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0),$$

$$Z_2^{\mathrm{T}} = (0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0),$$

$$Z_3^{\mathrm{T}} = (z_1\,z_2\,z_3\,z_4\,z_5\,z_6\,z_7\,z_8\,z_9\,z_{10}\,z_{11}\,z_{12}\,z_{13}\,z_{14}\,z_{15}\,z_{16}\,z_{17}\,z_{18}\,z_{19}\,z_{20}\,z_{21}\,z_{22}),$$

$$Z_4^{\mathrm{T}} = (0\,0\,0\,z_4\,z_5\,z_6\,z_7\,z_8\,z_9\,z_{10}\,0\,0\,z_{13}0\,0\,0\,0\,0\,0\,0\,z_{21}\,0),$$

$$Z_5^{\mathrm{T}} = (0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0),$$

$$Z_6^{\mathrm{T}} = (0\,z_2\,0\,0\,0\,0\,0\,0\,z_{10}\,0\,0\,z_{13}\,0\,0\,0\,0\,0\,0\,0\,z_{21}\,z_{22}),$$

$$Z_7^{\mathrm{T}} = (z_1\,z_2\,z_3\,z_4\,z_5\,z_6\,z_7\,z_8\,z_9\,z_{10}\,0\,0\,z_{13}\,0\,0\,0\,0\,z_{18}\,0\,0\,z_{21}\,z_{22}),$$

where T is the transposition index of the vector and matrix.

As it can be seen from the state vectors structure characterizing drought resistance, the contributions of the EGP to this selection feature are very different. In fact, it is advisable to take into account the contributions of the following EGP, while leaving the most significant states:

$$Z_3^{\mathrm{T}} = (0\,0\,0\,z_4\,0\,0\,z_8\,0\,0\,z_{11}\,0\,0\,0\,0\,0\,0\,0\,0\,z_{21}\,0),$$

$$Z_6^{\mathrm{T}} = (0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,z_{21}\,0),$$

$$Z_7^{\mathrm{T}} = (0\,0\,0\,z_4\,0\,0\,z_8\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,z_{21}\,0),$$

(provides drought resistance at tillering when grain number per ear is formed);

$$Z_3^{\mathrm{T}} = (z_1\,z_2\,0\,z_4\,0\,0\,0\,z_8\,0\,0\,0\,0\,0\,0\,0\,0\,z_{18}\,0\,0\,0\,0),$$

$$Z_6^{\mathrm{T}} = (z_1\,z_2\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0\,0),$$

$$Z_7^{\mathrm{T}} = (z_1\,z_2\,0\,z_4\,z_5\,0\,0\,z_8\,0\,0\,0\,0\,0\,0\,0\,0\,z_{18}\,0\,0\,0\,0),$$

(provides drought resistance at grain filling when 1000 grain weight to one grain weight ratio is formed)

These GPS affect the structure and parameters of the models (3), (4). Strictly speaking, the location and extent of such influence could be identified in the artificial climate chambers. However, when considering general principles of breeding formalization, we confine to the following hypothesis of influence (for mathematical expectation in time scale:

$$\begin{bmatrix} \dot{x}_1 \\ \dot{x}_2 \end{bmatrix} = \begin{bmatrix} a_{11}(Z_3) & a_{12} \\ a_{21} & a_{22}(Z_3) \end{bmatrix} \begin{bmatrix} x_1 \\ x_2 \end{bmatrix} + \begin{bmatrix} b(Z_6) \\ 0 \end{bmatrix} u(t) + \begin{bmatrix} c_{11}(Z_6) & c_{12}(Z_3) & c_{13}(Z_3) \\ 0 & c_{22}(Z_3) & c_{23}(Z_6) \end{bmatrix} \begin{bmatrix} f_1(t) + \delta f_1(t) \\ f_2(t) + \delta f_2(t) \\ f_3(t) + \delta f_3(t) \end{bmatrix}, \quad (5)$$

$$t \in (0, T(Z_7));$$

$$n_k = b_1(Z_6)(f_1 + \delta f_1) + b_2(Z_3)(f_2 + \delta f_2) + b_3(Z_3)(f_3 + \delta f_3) \cdot \tag{6}$$

The considered GPS models influence the overall dynamics of plant biomass accumulation, plant response to fertilizers, light, thermal and water regimes, and also determine the growing season duration.

We convert the models (5), (6) in a more compact and convenient form, where all the variables and parameters are grouped into corresponding vectors and matrices:

$$\hat{X} = A(Z_3)X(t) + b(Z_6)u(t) + C(Z_3, Z_6)(F(t) + DF(t)),$$
$$t \in [t_0; T(Z_7)]; \tag{7}$$

$$n_k = B^T(Z_3, Z_6)(F_k + \Delta F_k), \tag{8}$$

The hypothesis of such GPS effect will be incomplete without the parametric approximations of the parametric perturbations introduced by GPS:

$$A(Z_3) = A_0 + P^T \Delta Z_3, \tag{9}$$

$$b(Z_6) = b_0 + D^T \Delta Z_6, \tag{10}$$

$$C(Z_3, Z_6) = C_0 + G \Delta Z_3 + Q \Delta Z_6, \tag{11}$$

$$B(Z_3, Z_6) = B_0 + V^T \Delta Z_3 + W^T \Delta Z_6, \tag{12}$$

$$T(Z_7) = T \pm dT. \tag{13}$$

Thus, we can model the state of plants for favorable conditions (3), (4), taking into account environmental disturbances and genetic and physiological effects (7)-(12). Based on the simulation results, it is possible to predict the criteria of drought resistance (1), (2). However, such a simulation is possible only when specifying EGP taking into account the state vectors that characterize drought resistance and are the result of GPS effects. Now EGP is a normalized response of the parameters characterizing drought resistance to a disturbed environment, and is formed by three main GPS, the adaptability, tolerance to thickening and genetic variability on the ontogenetic periods.

Now let us formalize this. At first, we combine the criteria of drought resistance (1), (2) in the general criterion, which will facilitate classification and selection of parents:

$$J = J_\Sigma + g J_k, \tag{14}$$

where g is a weighting factor serving as an additional independent variable (argument) in optimization of general selection problem. Crossing plants, we do not know in advance which genotypes and in what quantity we will receive in a new generation. We can only indicate their difference Ω_g, for which the region of the general criterion of drought resistance [14] is divided into N intervals:

$$\Omega_n = \frac{J}{N} * n, \ n = 1, N-1 \cdot \tag{15}$$

Then the EGP will be a combination of the components of GPS vectors $\Delta\varphi_g$ [ΔZ_3, ΔZ_6, ΔZ_7 | Ω_g], which ensures that the criterion of drought resistance falls in the region Ω_g.

Since all these components can be estimated quantitatively in the artificial climate chambers, the EGP formation becomes one of the tasks of preliminary identification of the initial varieties of parental pairs. Let us consider the algorithm of the optimum selection of parental pairs for a hereditary increase in drought resistance. A criterion for the breeding result when crossing (an increased drought resistance), and the typical dynamics of all limiting factors for the place of cultivation of the future variety are at breeder's disposal. In addition, the breeder has an initial base variety or a hybrid which drought resistance must be improved, as well as a data bank for the potential parents in accordance with the above mathemati-

cal models through which the result of increased drought resistance can be predicted for the given dynamics of environmental conditions and the technology used. The base variety itself was previously created by the selection methods, and besides the mathematical model, the breeder has its EGP, obtained in preliminary tests in artificial climate chambers. Using the rapid assessment principle of the additives of GPS [43], which should be confirmed in experiments in artificial climate chambers, we select parents' EGP reflecting all the indicators taken into account, and obtain the predicted EGP of the best transgressions in the F_2 generation, i.e. $\Delta\varphi_g$ [ΔZ_3, ΔZ_6, ΔZ_7 | Ω_g], after input of which into the mathematical model of "dry—wet biomass", it is possible to predict the expected increase in drought resistance J. Comparing it with the required value of J^*, we can decide to stop the process or move to the next possible variant of crossing. By sequentially sorting out the available for cross-breeding options, one is identified that provides the greatest approximation to the required drought resistance J^*.

We understand that we are at the initial stage of the selection process formalization. The simple model of drought resistance components presented in this paper is the initial approach to constructing a complex mathematical model of evolution from the 22 parameters that have been considered, which determine the complex drought resistance property. In particular, the central problem is the identification of drought resistance models in artificial climate chambers, since these models are intended to reveal the possibilities of our proposed theory of ecological and genetic organization of the quantitative trait in the population, an important element of which is the idea of seven GPS and the availability of components within each GPS. The proposed theory replaced the general model of R. Fisher and was adapted for plants. If a breeder knows the contributions of each of the seven GPS to the crop at a typical dynamics of LIM factors, this gives him a tool to control the formation of EGP in real time during the growing season, which makes it possible to transform the selection process from the passive expectation of a probable result to a controlled selection technology.

To implement the proposed simplified scheme, it is necessary, firstly, to perform identification of the mathematical model of plant state dynamics during vegetation (based on this model, the forecast for increasing drought resistance and determining the criteria for selecting parental pairs are determined) at the initial experimental stage in the artificial climate chambers; secondly, to determine the action of all seven GPS and to compare their parameters with the dynamic model based on the results of vegetation; thirdly, to build EGP for initial varieties used as potential parents for subsequent crosses and obtaining the desired variety by the results of vegetation; fourthly, after receiving the F_2 generation, to determine the model of interaction of the parents' EGP, which should be used to form the predicted (required) EGP for a future variety.

Thus, the analysis of the problem of a significant increase in cereals drought resistant both regionally and globally shows that its solution is hardly possible on the basis of classical (Mendelian) and molecular genetics, or modern transgenic methods, in view of the extreme complexity of organizing the multi-component drought resistance, which includes, as a minimum, 22 components. Each of them is formed according to the ontogenesis phases (against the background of the differential activity of genes) and is affected during the vegetation by different types of drought of different duration and intensity. The optimal solution to the problem lies in the application of the recently emerged new direction of genetics, the ecological genetics of quantitative traits (one of the branches of epigenetic). In artificial climate chambers for drought types of a particular breeding zone, at phases critical for the formation of each productivity component, it is necessary to estimate its genotypic and genetic (additive) variability in the set of

varieties of the initial collection created for breeding in the relevant zone, or in world collections, for example, in the collection of VIR (N. I. Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg). Next, we must select varieties that carry at least one maximum additive positive shift for at least one component of drought resistance. Then, the selected 22 varieties (there may be less of them if there are varieties bearing two additive positive shifts or more) should be crossed diallelically. Then, in F_2, the individuals with several positive shifts should be identified and selected. Then they will be crossed again, and finally, the genotype will be selected, which will include 22 (this is the desired maximum) positive additive shifts. It will be a new variety with increased drought resistance, close to the maximum possible.

The structural analysis of the problem of increasing drought resistance in plants has become for us the basis for the further development of the methodological foundations of selection for increasing productivity and yield using the theory of eco-genetic organization of quantitative traits (TEGOQT) [44]. The introduction of measured contributions of the components of the resulting drought resistance into the model allows the construction of EGP of parental pairs when studying drought resistance structure in artificial climate chambers. In addition, it is possible to select parental pairs with complementary contributions from all seven GPS, creating the EGP of each potential parent, and, based on mathematical models reflecting the contributions of each of the seven GPS, to predict yield increase through controlled selection of parental pairs.

Note that in recent years' works on the physiological mechanisms of plant drought resistance [45-47], promising scenarios of phenotyping [48, 49], the study of the inheritance of drought resistance and the relationship of the phenotype to the genotype [50-52], genomics [53] and phenomics [54], the researchers do not yet attempt to approach the phenotyping of this complex adaptive property from the positions of the ecogenetic organization of the seven GPS and EGP for the selection of parental pairs and the management of the selection process on the basis of innovative phytotron technologies.

So, the original phenotyping proposed dissects the complex characteristics of "productivity" and "yield" not into the component traits of the crop structure as in traditional breeding (ear length, ear weight, grain weight per spikelet and per ear, the 1000 grain weight, etc.), but into the contributions of each of the seven genetic and physiological systems (GPS) by which breeders increase yields, most often not having information on the contributions to the yield of each GPS from the used parent variety. Because of this, in traditional breeding, a new variety in the field requires a huge effort and time (P.P. Luk'yanenko and B.I. Sandukhadze created the best domestic varieties for about 30 years). One of the seven GPS (adaptability) is divided into drought, cold, winter, salt resistance, resistance to acidic soils, etc. This paper presents the first attempt of phenotyping such a complex property as drought resistance, which is determined by at least 22 elements, and gives recommendations on the development of innovative phytotron technologies that can quickly and effectively improve the hereditary drought resistance in new varieties of cereals.

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MODERN OPPORTUNITIES FOR IMPROVING QUALITY OF BAKERY PRODUCTS VIA REALIZING THE BREAD WHEAT GENETIC POTENTIAL-BY-ENVIRONMENT INTERACTIONS
(review)

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A b s t r a c t

The purpose of this interdisciplinary research is to analyze the available data on the domestic market of bakery products, assess the factors resulting in increase of the bread consumption, and opportunities of improvement of technological properties of flour and dough through the realization of the genetic potential of bread wheat varieties, taking into account environmental factors. In modern conditions, in the bakery products market of such negative tendencies are observed as decrease in volumes of bread production and deterioration of quality of the products. Among the various factors influencing the formation of these trends, one can point out the poor quality of flour, accompanied by deterioration in the rheological properties of the dough. In the practice, the correction of flour of inadequate quality is increasingly being made through the introduction of chemical improvers, which contributes to improving the technological process. At the same time there is a loss of traditional taste and a change in the consumer characteristics of bread, which leads to the refusal or reduction of consumption of bread by a part of the population of our country. This review summarizes data on the dynamics of average per capita consumption of bread and bakery products and the change in the ratio of these indicators to the consumption of meat products. An alternative approach to solving the problems of flour quality can be attributed to the possibilities of natural improvement of its initial characteristics through the realization of the genetic potential of bread wheat varieties, taking into account environmental factors that ultimately influence the formation of technological properties of flour and dough. In recent years, data have been accumulated that have made significant progress in understanding the complex interaction of various genetic systems and biochemical processes underlying the formation of grain properties that affect the quantity and quality of the flour. Integral components in this complex interaction are the environmental factors, under the influence of which the physiological and biochemical processes are modulated, and the mode of realization of genetic information is changing. The article summarizes the data on the influence of various environmental factors on the technological properties of flour and dough and describes the possibilities of modern IT-support of the selection process, facilitating the evaluation of quantitative characteristics and taking into account the relationship between genotype, phenotype and environmental conditions. Advances in the identification of genetic factors affecting the technological properties of flour and dough are discussed and sources of useful variants of these genes are considered. The importance of the use of winter bread wheat for increasing the share of production of high-quality nutritive grains is emphasized, as well as the results of the search for donors of useful genes among the old varieties of spring bread wheat. Among the latter, varieties with a high content of raw gluten and high elasticity of the dough have been identified. In conclusion, the ways of applying data on the influence of genetic and environmental factors on the formation of technological properties of flour and dough in a selection experiment are dis-

cussed, and the importance of obtaining varieties with genetically determined high strength of flour as a source of natural improver of weak flour is replaced in place of widely used chemical additives.

Domestication of wheat, made it the main foodstuff for millions of people, is a milestone in civilization development. The high nutritional value of bread is due to important nutrients (polysaccharides, proteins, macro and micro-elements, vitamins B1, B2, B5, B9, PP, and also A and E vitamins in the rye bread) which are well metabolized. However, bread popularity is decreasing now [1]. The purpose of this interdisciplinary research is to analyze domestic market of bread and bakery products, factors which affect this market, and ways to improve the nutritional value of bread products basing on knowledges of genetic potential of wheat varieties, biochemical, physiological and ecological mechanism for its realization, bioinformatics and breeding technologies.

Trend in bread production and consumption. Quality problem. In 1925, average per capita wheat and rye bread consumption in proportion to the beef, pork, lamb and fat, was 1.80, and in whole exceeded 4.86 times meat products consumption. According to Federal State Statistics Service (http://www.gks.ru/), in conversion to wheat flour and meat, in 2009 and 2013, the ratio of bread and meat consumption was 1,36 and 1,13, respectively, according to the statistics of budgets inspections, 1,78 and 1,57 according to food balances, and 1,01 (in 2013) in selective surveys of the ration. So the tendency of a growing share of meat products and decreasing in bakery products persists, despite the differences depending on the way of calculation, or indicators are approximately equal (with a survey, the annual consumption of meat and meat products was 88.2 kg, of bread and bread products — 89.4 kg). The "Bread products" group includes flour, cereals, legumes in addition to bread, so the bread and bread products consumption itself is much lower (Table). In analytic publications aggregated figures are often presented, which misinterpret the real data and leads to non-objective conclusions.

Dynamics of average per capita consumption of basic foodstuffs in Russia (kg per year, Federal State Statistics Service data, http:gks.ru/)

Name	Year					2013 год	
	2008	2009	2010	2011	2012	total	to 2008, %
Bread and bakery products (in conversion to wheat) including:	59.9	57.6	57.4	55.2	53.6	51.3	85.6
wheat bread	38.9	36.6	36.5	34.7	33.3	31.7	81.5
Rye bread	17.9	17.8	17.4	16.8	16.5	15.8	88.8
Bakery products	3.1	3.2	3.5	3.7	3.8	3.8	122.5
Meat products (in conversion to meat)	75	73	79	81	83	85	113
Ratio of bread: meat products	0.79	0.78	0.73	0.68	0.65	0.60	75.9

N o t e. The indicators for finished products are overstated due to Federal State Statistics Service uses special coefficients for conversion of bread to flour.

So, from 2008 to 2013 just consumption of bread products had grown while wheat bread had decreased to 81.5% as compared to 2008. Share of bread in the structure of consumption is the highest (65 % in 2008 and 62 % in 2013) that determines the general tendency, and the ratio of bread and meat products had decreased from 0.79 to 0.60 that was due to reduction of per capita bread consumption and its share vs. meat products. New trend consumption behavior of Russian population modifies the structure of nutrition [2].

The linearity between the development of competition and improvement of quality production have recently been challenging [3]. In Krasnoyarsk this year's expertise of the main range showed that 40 % of samples were rejected

from degustation among which only for the first sort wheat bread category these were 5 samples out of 10 ones [4]. The change of crumb taste and structure, bread holes and thick crust, high friability and growing moldy lead to refusing or decreasing of bread consumption by a part of the population, causing the decrease of the production from 9,005 mln tons to 6,539 mln tons in 2014 [5, 6]. A decline in the quality is mainly determined by the flour properties and, as a consequence, by deterioration of the rheological dough indictors. The improving agents are increasingly applied that enhance ease of manufacturing but cause loses in the traditional taste and some consumer qualities. An alternative approach is the natural improvement of the flour characteristics using genetic potential of varieties and environmental factors affecting formation of baking flour properties.

Bread wheat breeding for grain and flour quality. According to data of Ministry of Agriculture of the Russian Federation (spring 2015), in Russia, 17 %, 25 % and 41 % sowings account for winter and spring wheat, the remaining cereals and leguminous, and other crops, respectively. Wheat almost by one third provides the daily human need in the energy and almost a quarter of proteins. From June 2015 to June 2016, Russia supplied 24.5 mln tons of wheat grain on the foreign market, became the leading exporter.

A combination of high yields, grain quality, and resistance to abiotic and biotic factors with a high varietal plasticity and adaptability is under the constant control in the breeding programs to provide the most complete realization of genetic potential. Among current trends the adaptive improving is dominating, that is capability of varieties to maximize productivity in certain environmental conditions. To date, 219 varieties of winter wheat and 175 ones of spring soft wheat have been added to the State Register of Protected Selection Achievements of the Russian Federation [8]. Depending on weather conditions, in some regions, spring wheat and in others regions winter wheat may have an advantage in quality [9]. Winter wheat has a higher biological productivity potential: in Russia its average yield is nearly twice as high as spring wheat has [10]. One of the most urgent tasks is short stature, resistant to lodging winter wheat varieties with high yields. The problem is that almost all short stem varieties have small 1000 grains weight, a poor quality of flour. Moreover, short stems negatively correlate with winter resistance. Largely, the problem was solved by interrupting saturating crossings [11]. As a result, intensive short stem varieties were created which surpass tall-growing standards for yields up by 15 centner per ha and not assented in 1000 grains weight and the flour quality.

Over recent decades, the wheat grain quality is tending to diminish. [2]. Quality formation proceed in the field and is determined both by hereditary characteristics and a complex of soil-climatic and agrotechnical conditions. Wheat strength largely depends on the genotype, so considerable attention is paid to breeding varieties that match good grain quality, high yields and other economically valuable traits. One of the approaches is the creation of varieties which are capable of grain forming in different climatic conditions and suitable for the manufacture of baked goods and grain products.

An example of modern investigations is the WHEALBI programs of the European Union countries (http://www.whealbi.eu/) and BREEDWHEAT in France (http://www.breedwheat.fr/) implemented through public-private partnerships. For example, BREEDWHEAT involves 15 scientific laboratories, 10 private companies, which specialize in plant breeding, seed production and grain production, an innovation cluster and technology transfer company. It is planned to optimize the usage of the genetic and ecophysiological adaptation in wheat based on soil-climatic factors and agrotechnologies, and to implement breeding strategies

based on DNA markers and genomic technologies. A part of this great project is a large-scale phenotyping of more than 4500 varieties and wheat lines on all economically valuable characteristic in 48,000 trial plots from 15 ecological and geographical areas during 9 years. Such large-scale projects include massive data sets. In addition, evaluation of a large number of parameters in large samples necessitates high-performance phenotyping.

Automated estimates of phenotype and relationships between plant genotype, phenotype and environment. Automatic determination of plant phenotypic characteristics [12] by computer image analysis is helpful in rapid collection of accurate information about valuable traits, such as yield and drought resistance. In wheat breeding, the most important traits associated with productivity are 1000 grain weight and the grain number per ear. Their definition manually is arduous. The proposed software and devices allow estimating grain shape and size [12, 13] accurately, and quantitate them. Mainly, this is the recognition of objects on a digital image (as a rule against a light background). Most frequently the grain shape is described by the length and the width [14]. Some methods allow estimation of tens of characteristics (shapes feature, size, and color of grains), and on this basis classification not only the cultivars, but also by the place where the plants grew [15].

Additionally, grain quality and the absence of disease are. For this, technologies based on spectroscopy at near-IR range (λ = 401-1000 nm) and hyperspectral images serve. Such methods allow analyzing of chemical composition, particularly grain protein content, starch [16], fibers [17] and injury by insects [18] and molding [19]. To estimate large grain numbers based on image processing, round-the-clock analyze is developed. Such method is proposed for rice grains [20]. It allows to thrash grains up and to determine their amount, size (length, width), 1000 grain weight; diurnal system capacity is over 1400 plants with an error within 1-3 %.

In phenotyping signs which are linked to drought resistant physiological characteristics (water use efficiency, stomatal conductance, etc.) are essentially added by thermal image data processing [21] with a quantitative estimation of the leaf surface temperature. So, it was shown, that in drought transgenic wheat plants with the alfalfa aldose reductase gene have leaves temperature lower [22]. Infrared spectrum or hyperspectral images from drones or small airplanes [23] are field-used, which allow to control water content in plants over large areas through monitoring of response to arid environment, watering or agrotechnologies.

Therefore, modern methods of mass plant phenotyping allow to consider interrelations among the genotype, phenotype and environmental conditions for the effective selection of stable wheat genotypes in which the grain and flour quality is least affected by adverse environmental factors [24]. These data are also used for automated control of variety cultivating which allows applying a set of measures to ensure the highest quality of grain on time.

The influence of environmental conditions on the technological properties of flour and dough. Main factors are water availability, temperature regime, the composition of chemical elements in the soil (including applied fertilizers), atmospheric air changes, on which depends expression of genetic determinants responsible for technological properties of flour and dough.

Water availability and temperature regime. After pollination, high temperature stress limits starch availability for the reserve accumulation. This results in small grains with low flour outputs [25]. The temperature stress during grain filling increases the protein content and the flour strength; however, at a temperature of more than 30 °C, the composition of proteins and starch changes which significantly affects physical dough properties. Starch biosynthesis is reduced sig-

nificantly while the protein influenced is insignificantly [26]. Simultaneously, the functional properties of starch and protein are modified [27]. High temperatures affect the ratio of amylose to amylopectin which leads to a reducing dough elasticity. Also, there is a different allocation of starch grains in the endosperm [28]. High air temperature (even for several days) during grain filling reduces both the ratio of glutenin/gliadin, and the level of high-molecular polymers in gluten, the content of which correlates to the strength of flour. This is due to reduced activity of the enzymes involved in formation of disulfide bonds and activation of heat shock protein biosynthesis which prevailed in the grain and weaken the elasticity of gluten by disruption of its polymerization [29, 30].

Macro- and microelement content in soil. Nitrogen fertilizers are an inalienable element of agro-technologies. They are necessary for the growth of photosynthetically active leaf biomass, from which about 80 % of the nitrogen is remobilized into the maturing grains [31]. Elevation in nitrogen dose increases the protein content in the grain. This increases grain size and flour yield. Growth in protein and gluten contents are linked, which correlates positively with the physical properties of dough. Meanwhile, the biosynthesis of high-polymeric gluten fractions enhances which make dough more elastic [32-34]. Late N-fertilization and foliar feeding are especially beneficial. The rheological properties of the dough and the bread appearance are improved, and its volume increases. It is known that the water deficit in the seed-filling period deteriorate all qualitative properties. High nitrogen doses under these conditions improve grain quality [35]. Modern Italian varieties when compared to the traditional ones can use soil nitrogen much more effectively that results in increased yield and high grain and flour quality both at low and high doses of nitrogen [36].

High nitrogen content along with deficit in sulfur-containing fertilizers can lead to available sulphur deficiency for the normal grain development [37]. The action of this macro element is linked with the significant function of disulfide bonds in the formation of polymeric forms of gluten which ensure its rheological properties. Sulfur is a part of sulfur-containing amino acids, which form disulfide bonds. Sulfur deficiency changes the gluten composition, increasing the S-poor proteins share (ω-gliadins, high-molecular glutenins) through S-rich (low-molecular glutenins) [38]. The sulfur-containing fertilization has positive effects on crop yield and some gluten quality parameters in the Mediterranean [39]. In Pakistan, this agronomic method reduced the N:S ratio and increased the protein content in the grain with a significant positive effect on baking index [40]. In Estonia, application of sulfur-containing fertilizer increased sulfur-containing amino acid content, crude gluten, improved its quality and all main bread-making properties of winter wheat grain [41].

Foliar feeding with microelements along with mineral fertilizers increased the content of monomeric gliadin and polymeric glutenin. It leads to the formation of more disulfide bonds and higher gluten complex polymerization. Addition of Mn increased grain protein content, gluten, sedimentation index, and grain hardness, Zn was beneficial for high molecular weight glutenins. In gluten, the high- and low-molecular-weight fraction significantly increased as influenced by Zn and Cu [42]. The described effects are related to the fact that microelements serve as cofactors of many cellular enzymes which provide metabolic ways of the proteins biosynthesis in vital processes, including grain formation.

Atmospheric composition. Effects of global climate changes in the Earth's include an increase of the concentration of carbon dioxide in the atmosphere. In a number of countries, its influence on the formation of technological properties of flour and dough is studied [25, 43-45]. In some studies, besides properties, the changes in the grain proteome under the action of elevated CO_2

concentration have been assessed [45, 46]. An increasing CO_2 in the atmosphere affects the gluten content and leads to a deterioration of baking properties, i.e. the bread volume of and flour strength decreases, while dough kneading takes more time. However, dough extensibility and its stability are not vulnerable to this effect. These data are consistent with the proteomic analysis results, which showed that the main changes affect the expression of genes for high-molecular-weight glutenin subunit (HMW-GS). It is well known that, physical and mixing properties of flour and dough depend on the content of high molecular weight glutenin subunits [47-49]. Under elevated CO_2 [50], changes had been observed in the ratio of the main protein fractions of gluten, the glutenins and gliadins. By contrast, elevated CO_2 concentrations do not reduce the expression of low molecular weight glutenins [45] or reduce their amount not as much as high molecular weight ones [46]. With low-molecular-weight glutenin such rheological properties are connected as dough extensibility and stability [51]. Elevated CO_2 decreases the grain protein content, while reducing the S, Ca, Fe, and Zn levels [52]. At high induced CO_2 concentration, the time and rate of nitrogen remobilization from leaves to wheat grain during wilting vary. Therefore, changing dynamics of nitrogen accumulation in leaves can reduce grain protein level [53].

Effects of environment on protein and carbohydrate complexes of the wheat grain. Changing environmental conditions can affect the formation of viscoelastic properties of the gluten protein complex through labile isomerase and oxidoreductase system. They are able to modify folding the storage proteins, their post-translation modification, the polymerization of glutenin subunits, protein deposition, and probably involved in the grain texture formation. Polymerization of glutenin subunits is the most important stage in the gluten protein network forming. The viscoelastic properties of gluten are molecularly based on the distribution of molecular weights of protein polymers [54].

In 11 studied varieties and 5 hybrids of soft wheat grown in eight different geographical locations, 60-84 % of the stability of baking parameters was due to stabile distribution of molecular weight of glutenin polymers. The environment significantly affected the molecular weight of the polymer fraction [55]. Prolamines can interact with foldases, for example with protein disulfide isomerases (PDI), catalyzing the isomerization of disulfide bonds [26, 56]. Significantly reduced level of insoluble glutenin macro-polymers after the addition of bacitracin, a PDI inhibitor, implies that endogenous proteins of PDI family in the flour suppress the depolymerization of glutenin polymers [57].

If the wheat pant grew at high temperatures (37/28 °C, day/night), the heat shock protein expression increased [28]. One of these proteins was localized on the surface of protein bodies [58], accelerating the coagulation and aggregation of prolamins inside them.

Thiol:protein disulfide oxidoreductase (PDO) isolated from maturing wheat grains and classified as glutaredoxin-like protein, catalyzes a disruption of disulfide bonds in gluten proteins and reduces their aggregation capacity [59]. Adding this enzyme to flour of different quality wheat varieties has led to a significant increase in extensibility (from 17 to 49 %). This indicates the essential role of the PDO in the destruction of disulfide bonds in high-polymeric glutenin [60].

Mapping of quantitative trait loci (QTL) for PDO activity and physical dough properties (flour strength and elasticity, water absorbing ability) with use of ITMI mapping population revealed their colocalization [61]. This, probably results from the linkage between the genetic control of PDO activity and the quality of gluten. There is evidence of a close linkage between the content of reduced glutathione, which is maintained by glutathione reductase, and the SS/SH status of storage proteins [62]. Reduced glutathione bound to SH-groups

of glutenin thereby blocks the formation of disulfide bonds, thus preventing polymerization. The increasing in protein-bound glutathione negatively correlates with molecular weights of polymers, and poor quality gluten always has more such proteins [63].

In wheat grains, in addition to the enzymes which catalyze the destruction of S-S bonds, a significant quantities of lipoxygenase (LOX), the key enzyme of lipoxygenase signaling cascade, is present. Its products (fatty acid hydroperoxides) can oxidize SH-groups of storage proteins with the formation of S-S bonds, thereby promoting their polymerization. A correlation analysis performed for three sets of substituted and recombinant wheat lines [64] revealed a close linkage between the activity of endogenous LOX in grains and 15 technological parameters, like flour strength, dough elasticity and mixing properties. At the same time, the high activity of LOX negatively correlated with the main quality parameters, while low activity correlated positively, that is, the nature of correlation depended on the enzyme activity. It was concluded that the positive LOX influence on gluten is due to blocking of excess rheologically active thiol groups in storage proteins. It strengthened gluten and reduce the dough extensibility [64]. However, high activity leads to the formation of a large number of S-S bonds and the loss of the optimal dough viscosity, which reduces the quality. The LOX effect on the grain quality seems to be related to its participation in lipid-protein interactions at the interface between the surface of starch granules and protein bodies which largely determine the texture of the grain. Using the recombinant introgression lines of *Triticum aestivum*/*Aegilops tauschii*, QTL for LOX activity was detected on chromosome 5D [65], coinciding with the position of the main hardness gene *Ha*, which determines the endosperm texture [66, 67]. Successful use of the LOX gene as a target for genomic editing in wheat [68] opens the prospect for the refinement of the effects of mutations in this gene on the properties of grain and flour when using the models obtained. In turn, the LOX expression is changed under the influence of environmental factors [69, 70], therefore one of the mechanisms mediating the influence of the environment on the technological properties of flour and dough can be associated with LOX.

Action of environmental factor through the photosynthetic systems is another mechanism. The influence of structural organization of photosynthesis on the gluten quality and quantity was studied in tall and short-stemmed winter wheat varieties [71]. A positive correlation was found between the active photosynthetic potential, proportional to the biomass (through assimilating surface), and the gluten content in the grain. However, a strong negative correlation was found between the energy efficiency coefficient and the gluten quality (by the measurement of gluten deformation). It appears that short-stemmed varieties accumulate more gluten, but of worse quality.

Thus, environmental impact on the technological properties of grain and flour is mediated by various molecular mechanisms and cellular processes which are involved in the formation of protein and carbohydrate complexes during grain maturation. Despite general trends in the response of different genotypes to the same factors, there are genetically determined differences that can result in the maximum realized quality potential of the variety in certain conditions.

Genes and gene donors for improving technological properties of flour and dough. Bakery quality is an integral parameter which depends both on the genotype and the environment conditions. To date, more than 40 genes and QTL affecting technological properties of flour and dough have been mapped, and closely linked DNA markers have been described [2, 9]. More than half of them are responsible for rheological properties (flour strength,

elasticity, extensibility, water absorption capacity, time of dough formation, stability, and liquefaction). The remaining mapped loci are responsible for milling properties (8 loci), protein and crude gluten content (5 loci), starch properties (3 loci), flour color (3 loci) [2, 9]. The integral characteristic of baking properties (volume of baked bread) differently correlates with the mentioned technological indexes. In particular, this parameter is determined by the composition of gluten proteins, which is controlled by the genes for gliadin and glutenin synthesis localized on chromosomes of homeological groups 1 and 6. However, the bread volume is also determined by individual genetic factors, in particular, *Lvl1* gene located in the region of *Xgwm480* marker on the long arm of chromosome 3A [72]. Recently, *wbm* gene with enhanced expression in the developing grain has been detected and sequenced [73]. In the 54 spring varieties from the CIMMYT collection (International Maize and Wheat Improvement Center, Mexico), different alleles of this gene are associated with low or high baking properties [74].

Among donors of the desired gene alleles affecting technological properties, both modern commercial varieties of soft wheat and lines with fragments of genomes introgressed from wheat relatives are distinguished [2, 75]. Special attention should be paid to the comparative evaluation of the technological properties of flour and dough in modern varieties as compared with wheat cultivated till 1960-1970. As a turning point, it was the 1960s as the period of the "green" revolution and rapid post-war development of science and economy, and the reindustrialization of agriculture. Study of 14 Italian soft wheat varieties cultivated from 1916 to 1994, as well as two populations cultivated in the 1900s, showed that the varieties used after 1970 are higher in flour strength and the ratio of dough elasticity to tensile [36]. The authors concluded that modern varieties that are better in nitrogen absorption and have higher productivity and quality under N deficit are of interest even for organic farming. A low quality of German varieties of the pre-war and early post-war period is also noted [76].

Russian old-fashioned varieties have a lot of potential. Thus, 45 Siberian varieties of different selection periods were analyzed for the following features: 1000 grains weight which affects the flour output; the grain hardness and the flour particle size which determine the flour use; crude gluten content in grain which determines use and nutritive value of flour; flour strength; dough extensibility and resilience which determine baking properties; resilience to extensibility ratio affecting dough resilience [77]. Note, weight of 1000 grains is more variable in modern varieties and as a whole better than in the old ones. Altai 50 (32.6 g), Fora (31.9 g) and Irtyshanka 10 (29.4 g) are especially distinguished on this trait. Old-fashioned varieties are more variable in grain vitreousity, however, higher vitreousity have the modern varieties with the highest index for Novosibirskaya 89 (96.4 %), Novosibirsk 67 (93.9 %) and Altai 60 (92.3 %), and the old-fashioned varieties Cesium 94 (93.8 %) and Krokhinskaya 10 (92.2 %). The average content of raw gluten in grain is remarkably higher in the old varieties for which a great variability on this feature is also characteristic. The varieties with the largest content of raw gluten were identified, i.e. Tarskaya 2 (43.8 %) and Alenka (40.9 %). For flour strength, dough extensibility and resilience, the modern varieties exceeded the old ones. DNA markers are found which admittedly are linked to the genetic factors determining high expression of studied traits [77]. The largest number of such markers was revealed in old varieties for milling properties and gluten content in grain. These markers can be used in selection after further verification.

Among the traditional Siberian varieties of soft wheat, putative gene donors have been detected that can be involved in breeding varieties with specified technological properties. After the primary structure sequencing of the identified

genetic factors, they can undergo target modification by CRISPR/Cas genomic editing technology. This promising method which allows for non-transgenic modified plant varieties is already being used to improve yielding and resistance in wheat [78].

So, the molecular, biochemical and physiological data on mechanisms that mediate influence of the environment and genetic trait control provide a basis for optimization of genotype × environment interaction, for example in selection of a variety in combination with agrotechnology to maximize its biological potential on yield and quality. Study of the role of genetic factors in expression of flour and dough technological properties will allow to develop diagnostic DNA markers application of which speeds up breeding and increases its accuracy when creating varieties with genetically determined high strength of flour. Instead of chemical additives, such flour can be a natural improver of weak flour for better quality of bread products. Besides, high technological properties will contribute to an increase Russian grain export.

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A REVIEW OF MOLECULAR MARKERS TO GENES INVOLVED IN MINERAL NUTRITION EFFICIENCY CONTROL IN RICE (*Oryza sativa* L.)

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A b s t r a c t

To date, the doses of mineral fertilizers per hectare in Asia and Europe are the highest in the world. This leads to serious problems for environment contamination, including water pollution, an increase in emission of hotbed gases and a decrease of pH in soils and water. Nitrogen utilization in rice, wheat and corn is 26-30 %, and in vegetables less than 20 % (K. Vinod et al., 2012). During last fifty years high yielding varieties are being bred at high mineral nutrition. An increase in doses of fertilizers has resulted in smaller efficiency of their application and an increasing adverse impact on the environment. As a rule, the varieties which are high productive due to introduced high doses of fertilizers are less effective in their utilization. Besides, productivity of such varieties is very unstable as being considerably influenced by doses of the introduced fertilizers, terms of their introduction, ambient temperature, etc. In the paper we briefly reviewed the mechanisms of plants adaptation to low nitrogen and phosphorus nutrition, and the genotypic distinctions in the efficiency of using these elements. The efficiency is noted to be influenced no only by various responses of genotypes to the applied doses, but also by a source of element and the interactions between a genotype and the environment. Comparison of genotypes of rice has shown 20-fold distinction in efficiency of phosphorus use between extreme types (M. Wissuwa et al., 2001). All highly effective genotypes are cultivars with age-old longevity or endemics. Most P-containing organic compounds that plants have to extract from soil should be turned into the accessible form by phosphatases. So a genotypic diversity in the efficiency of phosphorus use is related to different activity of phosphatases. Phytin acid produced by roots and the rhizosphere microorganisms is one of the agents promoting phosphorus accessibility (A.E. Richardson et al., 2001). Therefore, the plant ability to support favorable microbial communities in the rhizosphere serves as the additional adaptive mechanism. Plant adaptation to low nitrogen and phosphorus levels can be due to root system development, intensification of absorption and utilization, and also to biosynthesis and excretion of the organic acids to increase availability of the mineral elements in the rhizosphere (H. Lambers et al., 2006). Redistribution of the absorbed elements between generative and vegetative organs, between leaves on one or different stems stands for an internal efficiency of nutrient utilization. Variability on the efficiency of nutrition utilization among 30 rice genotypes resulted mainly from distinctions in the growth of root system which increased the absorbing area. Variability of genotypes on the tolerance to a lack of phosphorus was mainly due to different ability to P absorption, while the changes in the efficiency of P utilization were insignificant. OTL related to N and P utilization and specific molecular markers flanking the loci, RM 53, RM 25, RM 600, RM 242, RM 235, RM 247, RM 322, RM 13, RM 261, RM 19 (D. Wei et al., 2012; Y. Cho et al., 2007), are found in foreign and domestic rice varieties. In the Russian rice varieties all studied markers to QTL involved in the expression of effective absorption of mineral elements are polymorphic (Yu.K. Goncharova et al., 2015) that allows to use these markers in marker-assisted selection and screening population to reveal donors of desirable traits.

Keywords: rice, mineral nutrition, efficiency of use nitrogen and phosphorus, QTL, microsatellite (SSR) markers

Nitrogen and phosphorus availability is one of the most important factors which determines crop productivity. Nutrient in soil are not replenished when there is shortage of fertilizer, and it leads to soil degradation and low yields [1-3]. Whiles, over-fertilization at the EU, had led to soil nitrogen accretion, which menaces to 70 % of agrocoenosis. Till now, attention has not been attracted to

increased inorganic nutrition efficiency in creating varieties of rice and other crops, however, these works are indispensable. It is important to study both mechanism of plant resistance to the soil deficient in mineral nutrients, and genes encoding the traits. In this review the main achievements and challenges in genetics of rice (*Oryza sativa* L.) inorganic nutrition are discussed as well as outlook for molecular-genetic technologies and increasing its efficiency.

In the last 40 years mineral fertilizers provide 40 % gain in food production. From 1961 to 2002 its using in the world had been increased by almost 350 % (from 33 to 146 million tons) and often causes damage to the environment [4, 5]. To date, Asia and Europe have the highest rates of fertilizer consumption per hectare. As a consequence, acidification of soil and water is observed, as well as pollution of surface and underground water bodies, increase of hotbed blowout gases. Environmental impact of mineral fertilizers is determined by their quantity, methods and time of application. The problem is to abandon improper methods and move to agriculture, which will provide reliable basis for sustainable intensification of plant growing.

Nitrogen as the main component of many organic molecule, nucleic acids, proteins, is necessary for development of plants much more than another mineral elements. Though N is the most accessible element in atmosphere (78 %), it becomes a limiting factor in agricultural ecosystems. Its soil reserves are equal to 2-20 t/ha, but plants receive just small amount of it [3]. Nitrogen use efficiency is 26-30 % for rice, wheat, maize, and makes less than 20 % for vegetable crops [6-8].

Some quantity of ammonium and ammonium fertilizers which accumulates during mineralization of the organic compounds is fixed by soil minerals (illite, montmorillonite, vermiculite and others). Soil nitrogen, as a part of humic compounds, is difficult to decompose by microorganisms. Small amount of nitrogen is a part of another organic compounds such as amino acids, amino sugars, nucleic acids, etc., and also minerals compounds (preferentially ammonium and nitric acid). Ammonium nitrogen pool formed due to ammonification and fertilizers is unstable. Under the influence of nitrifying bacteria, it is oxidized to nitric acid. Nitrates are not absorbed by soil colloid and can be washed out of soil. Also, nitrogen get into the atmosphere because of nitrate nitrogen reduction to free molecular nitrogen (N_2) or to gaseous oxide or nitrous oxide (NO and N_2O). Just small amount of nitrogen remains in soil and is available to the next generations of plants. In most cases, on well aerated soils they use nitrate nitrogen (NO_3), on bad aerated and flooded soils they use ammonium nitrogen (NH_4^+) [4]. Although absorption of NH_4^+ needs less energy, only some of plants (including rice) are capable to grow using just these ions [9].

Phosphorus is the second most important mineral in plant nutrition after nitrogen and one of the least available. Mainly, it is in the bound state in the soil. Approximately, 5.7 billion hectares of soils all over the world contain insufficient quantity of plant available phosphorus. Almost 50 % of rice occupied soils are phosphorus poor. Deficit of phosphorus can significantly affect rice growth and development. Plants cultivated on soils with phosphorus deficit are scraggy with dark green leaves, inhibited root system and lowered tillering [4, 5]. Their maturation is delayed, and grains are incompletely filled that leads to loss of grain quality. This deficit could be overcome by fertilizer application which farmers do not apply because of high price. As well as, natural phosphates of earth's formations which easy to accesses for plants are resources which can be exhausted by the end of XXI century [10]. Creating varieties of cultivated plants which are capable of more efficient absorption of bound phosphorus from soil with high yielding at low doses of phosphorus fertilizers and more efficient use of

absorbed phosphorus has crucial importance [11].

From 2008 to 2012, a steady growth of fertilizer consumption up to 1.7 % (or 15 million tons annually) had been reported, and in 2012 nitrogen consumption raised by 1.4 %, or 73 mln. t. At the same time there is loss of consumption efficiency per 1 kilo of getting products, and the rate of rice yield gain in 2010 was less than 1 % compared to 1.6 % in 1990 [5]. Other factors which forced to change strategy of fertilizer consumption have become their price growth, limited reserves, and also environment pollution because of uncontrolled use.

Last 50 years efforts is mainly focused on breeding plant varieties with high yield under high mineral nutrition [13]. Application of ever large doses of fertilizers has led to a decrease of their efficiency and an increase of environmental effects on productivity [14]. Moreover, crop yield of these varieties is unstable.

Genotypic differences in the absorption efficiency of elements of mineral nutrition. Not only on nitrogen doses, but on its source genotypes react differently. Genotype × environment interaction is an important factor as well [15]. There are several parameters of nitrogen consumption among which are nitrogen deficit tolerance (NDT) and nitrogen use efficiency (NUE) [16]. It is detected that samples of rice plants differ genotypically on their ability to take up phosphorus when it is in deficit [17-19]. Comparison of 30 genotypes showed a twenty-fold difference in this parameter between extreme types [20]. All high-efficient genotypes are age-old samples or endemics. Modern high-yielding varieties are not enough adapted to deficit of mineral nutrition.

Plant adaptation to low content of nitrogen and phosphorus can be due to an increase of root surface, intensification of elements absorption and internal efficiency of their use. The internal efficiency is a redistribution of incoming phosphorus between generative and vegetative organs, between leaves of one or different shoots, etc. M. Wissuwa [21] who studied the efficiency of mineral nutrition in 30 rice genotypes, including high-yielding varieties and endemic samples, attributed the variability on this trait to distinctions in the growth of root system and thereafter increasing the plant nutrition area. In rice, resistance to phosphorus shortage is related to ability of genotypes to absorb this element, while internal use efficiency exerts slight influence [21]. With phosphorus shortage plants re-distribute more assimilates to the roots slowing the growth of shoots. A high deficit also impedes the root growth [22, 23]. Effective way to increase root surface is to increase root length and number, and also quantity of root hairs. In *Arabidopsis thaliana* some mutations affect root development. Homologues of some genes responsible for root hair development are identified in rice genome [24]. However, it has not yet been established whether they differentiate rice genotypes on stable and unstable.

Architectonic modifications of the root system in soil connected with stability to the phosphorus shortage were marked in white lupine and other legumes and rice. Absorbing root capacity is the main factor of adaptation to phosphorus shortage in legumes [26]. Meaning of this mechanism in an increase of rice plant stability to P shortage has not been determined. Differences in the root length do not always provide distinction in efficiency of absorption of mineral elements [22]. So it is necessary to consider all interconnected factors that control adaptation. Factors involved in effective remobilization and relocation of phosphorus and nitrogen in plants, and regulation of this processes at the genome level are studied a little [27, 28].

The most part of phosphorus can be found in soils in organic complexes which are converted by phosphatases to form available for plants. In wheat, the

genotypic differences in phosphorus use efficiency are expressed as not identical phosphatase activity [29, 30]. Such differences have not been found in rice [23]. Soils often contain phytic acid (substance which conduces conversion of fixed phosphorus to the available), but plant roots excrete it in very small amounts, while microorganisms can produce much more acid, so ability of roots to support useful microbial community serves as an additional adaptive mechanism [31]. The majority of plants, as rice, are able to symbiotic associations with mycorrhizal fungi. These interactions are genetically determined [14]. But for Nipponbare variety, soil sterilization did not reduce efficiency of phosphorus absorption [21]. Organic acid biosynthesis and secretion by rhizosphere, which increase availability of elements, positively affected the tolerance of wheat, barley, and rice plants to nitrogen, zinc and phosphorus deficit [32, 33].

Prospects of improving rice varieties one links with identification of the genetic determinants of tolerance to mineral element deficit and their introduction to high yielding varieties using MAS methods.

Quantitative trait loci (QTL) which determine tolerance to nitrogen deficit. In marking different rice populations, from 7 to 8 QTL linked to nitrogen-deficiency tolerance (NDT) was identified on the chromosomes 1, 2, 3, 4, 7, 9, 10 and 11. NDT is the ratio of the trait value at low nitrogen (LN, 0 kg/ha; mostly without additional fertilizers) to its value at optimal mineral nutrition (NN, 130 kg/ha).

Indicators of varietal adaptation to nitrogen deficiency are plant productivity, the yield per plot, biomass, plant height, root length, the content of pigments, etc. [34-36]. But analysis of these traits showed that high relative biomass value was characteristic of the genotypes with low total biomass [35]. The most informative indicators were the relative grain yield (grain yield LN/grain yield NN) × 100, relative biomass, relative nitrogen content in grain and in biomass, nitrogen response (NR kg/ha, as TNf – TNo, the difference between nitrogen consumption by the plant at optimum and low mineral nutrition).

Nitrogen use efficiency is determined as the difference between the values at optimal and low mineral nutrition [36], and physiological NUE (PE, kg grain/kg nitrogen) is the ratio of the grain yield response (GYf – GYc) to the total response to nitrogen (TNf – TNo).

QTL for NUE were mapped on chromosomes 1, 2, 3, 4, 6, 7, 9, 10 and 11 in rice variety of subspecies *indica* (Zhenshan 97 × Minghui 63) (Table 1) [33-40]. Four genomic regions in chromosomes 1, 3, 4 and 7 involved in expression of both studied traits, NDT and NUE, are flanked by markers G393-C922, RM232-C63, G235-G102 and RG678-R1440, receptively. However, phenotypic manifestation of many of detected loci was influenced by year conditions (weather or others unaccounted factors) [34]. Three chromosomal segments mapped in chromosomes 4, 6 and 10 were linked to maximum nitrogen response determining 11.1; 16.6 and 7.5 % of the trait phenotypic manifestation, respectively. In this work, dry biomass was measured by cutting plants to the root and dried at 75 °C for 4 days, and nitrogen was assessed by Kjeldahl method. There was a negative correlation between indicated traits [36]. In another research [37], the loci were allocated which determine much higher phenotypic manifestation of the trait in the rice varieties of subspecies *japonica*. So, 32.1 % of grain nitrogen level was controlled by QTL *ncg 8.2*, mapped on chromosome 8 and flanked by markers S8055-RM72. The crop yield index in stress was 23.1 % influenced by locus *hi7b* on chromosome 7 flanked by SSR markers RM420-RM248 (Table 2). Plant biomass was determined by locus *sy8.1* (23.8 %) of chromosome 8 with flanking markers S8055-RM72 [38].

1. QTL for nitrogen use efficiency detected in varieties of rice (*Oryza sativa* L.) subspecies *indica* (Zhenshan 97 × Minghui 63) [37]

Trait	1	2	3	4	5	6
Relative grain yield, % (RGY)	qRGY3	3 (2006)	RM232-C63	180.2	171.2-190.6	10.8
Relative biomass, % (RBM)	qRBM9-2	9 (2006)	RM242-RM257	35.5	32.0-41.6	13.1
	qRBM1	1 (2007)	RG101-C922	70.8	66.9-78.2	11.5
	qRBM2	2 (2007)	C777-R1843	28.4	23.7-32.1	9.3
	qRBM4	4 (2007)	G235-G102	42.3	33.1-51.6	14.5
Relative grain nitrogen, % (RGN)	qRGN9	9 (2006)	RG667-RG570	20.5	16.3-27.8	8.6
	qRGN1	1 (2006-2007)	G393-RG101	67.9	66.0-74.3	13.2
Relative biomass nitrogen, % (RBN)	qRBN2	2 (2006-2007)	RZ599-RM53	54.5	48.8-59.4	8.9
	qRBN9	9 (2006)	RG667-RG570	18.2	10.8-24.5	9.4
	qRBN4-2	4 (2007)	G235-G102	42.3	41.6-48.0	14.6
Nitrogen response, kg/ha (NR)	qNR6	6 (2006)	RZ398-C764	167.3	165.3-170.6	16.6
	qNR10	10 (2006)	R2625-RG561	8.6	4.0-11.4	7.5
	qNR4	4 (2007)	G235-G102	42.3	36.6-57.5	11.01
Grain yield response, kg/ha (GR)	qGR3	3 (2006)	RM232-C63	180.2	170.5-185.3	16.2
	qGR9	9 (2006)	C472-RM201	77.4	57.4-95.7	12.5
	qGR1-1	1 (2007)	RM212-R2201	49.0	46.3-57.8	12.9
	qGR1-2	1 (2006)	G393-RG101	66.9	63.2-75.0	9.9
Physiological nitrogen-use efficiency, kg grain/kg nitrogen (PE)	qPE3	3 (2006-2007)	RM232-C63	180.2	166.2-185.3	12.8
	qPE7	7 (2007)	RG678-R1440	68.9	50.6-76.2	8.8

N o t i c e. 1 — locus; 2 — chromosome (year); 3 — flanking markers; 4 — the most probable gene position, cM; 5 — position on the chromosome, cM; 6 — phenotypic manifestation of traits, %; $RGY = (GY_0/GY_F) \times 100$; $RBM = (BM_0/BM_F) \times 100$; $RGN = (GN_0/GN_F) \times 100$; $RBN = (BN_0/BN_F) \times 100$; $NR = TN_F - TN_0$; $GR = GY_F - GY_0$; $PE = (GY_F - GY_0)/(TN_F - TN_0)$; GN_0 and GN_F; BM_0 and BM_F; BN_0 and BN_F; TN_0 and TN_F; GY_0 and GY_F — nitrogen content in grain; biomass; nitrogen content in biomass; nitrogen content per plant; grain yield (no fertilizer — 0, fertilizer application — F).

2. QTL for nitrogen use efficiency detected in varieties of rice (*Oryza sativa* L.) subspecies *japonica* [38]

Trait	Locus	Chromosome	Flanking markers	Trait manifestation, %
Nitrogen content in grain (NCG)	ncg8.1	8	RM25-S8090	10.2
	ncg9	9	S9058-S9062	10.2
	ncg10	10	S10023-S1001B	7.5
	ncg8.2	8	S8055-RM72	32.1
Nitrogen content in straw (NCS)	ncs9	9	S9093-S9073	11.1
	ncs10	10	S10019-S1001B	13.3
Nitrogen content in shoots (TNCS)	tncs1b	1	RM600-RM312	19.7
	tncs10	10	S1003-S10013	14.1
Harvest index (HI)	hi5b	5	RM87-RM31	8.7
	hi7b	7	RM420-RM248	23.1
Grain yield (GY)	gy2b	2	S2052-S2054	6.9
	gy9	9	RM242-RM215	12.9
Straw biomass (SY)	sy1b	1	RM600-RM312	12.1
	sy8.1	8	S8055-RM72	23.8
	sy8.2	8	RM25-S8090	8.4
	sy5a	5	S5014B-S5029	9.6
	sy5b	5	RM87-RM31	9.1
	sy7b	7	RM420-RM248	8.4
	sy12b	12	S12038-RM277	9.9
Physiological N-use efficiency (PNUE)	pnue9	9	S9093-S9073	15.3
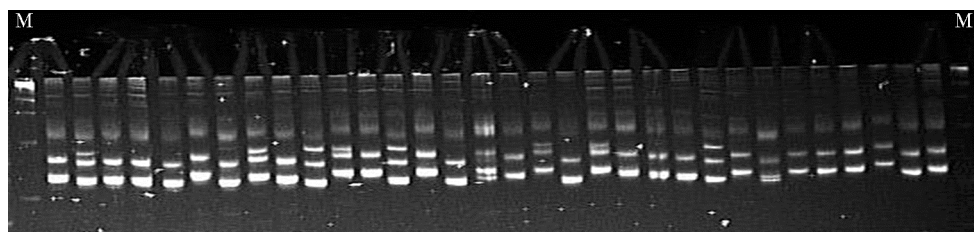
Fig. 1. Polymorphism of Russian rice (*Oryza sativa* L.) varieties on marker RM 600 to genes for nitrogen use efficiency: from left to right — Hankai, Sadko, Primorskii, Liman, Garant, Pavlovskii, Rapan, Novator, Serpantin, Boyarin, Regul, Yantar, Zhemchug, Lider, Khazar, Amethyst, Narciss, Druzhnyi, Sprint, Viola, Dal'nevostochnyi, Fontan, Kasun, Yupiter, Atlant, Kurchanka, Fakel, Snezhinka, Sharm, Anait, Flagman, Izumrud; M is a molecular weight marker [12].

Study of Russian varieties using molecular markers to genes for nitrogen

use efficiency showed polymorphism in some of them [12]. The maximum number of polymorphic alleles was found using the marker RM 600 located on chromosome 1 (Table 3, Fig. 1).

3. Polymorphism of Russian rice (*Oryza sativa* L.) varieties on SSR markers to genes for nitrogen use efficiency [12]

Marker	Trait	Fragment size, bp	Chromosome	Polymorphism
RM 53	Relative biomass nitrogen (RBN)	182	2	Polymorphic, 2 alleles
RM 25	Straw yield (SY), nitrogen content in grain (NCG), drought adaptiveness, photosynthetic efficiency	146	8	Polymorphic, 2 alleles
RM 600	Nitrogen content in shoots (TNCS), straw yield (SY)	220	1	Polymorphic, 5 alleles
RM 242	Grain yield (GY), relative biomass nitrogen (RBM), adaptiveness to law temperatures	225	9	Polymorphic, 2 alleles

Markers RM 242, RM 53, RM 25 showed 2 alleles. This polymorphism allows marker-assisted selection (MAS) for nitrogen use efficiency, and identification of donors of appropriated genes among Russian varieties. Interestingly, these markers were also associated to adaptiveness to other stresses (drought, low temperature).

QTL associated to tolerance to phosphorus deficiency. Although the wide genetic variability on this trait, the progress in breeding rice varieties, which are high-yielding and effectively use phosphorus, is small. It is found that inheritance of traits, associated to phosphorus shortage tolerance is largely specified by two independent QTL [41]. The basic locus named *Pup1* and flanked by the RM235 and RM247 markers separated by a distance of 0.2 cM is located on chromosome 12. Using the flanking markers, the *Pup1* gene was identified in the verities Dular, IAC 47, IAC 25, AUS 257, and Vary Lava 701. Clusterization of 80 varieties carrying different allelic variant of gene *Pup1* identified the allele associated to tolerance in varieties adopted to drought. Practically, this allele was not find in the varieties cultivated under irrigation. Donor varieties have longer and more branchy root system in comparison to others genotypes, that confirms necessity of selection for this trait when creating forms tolerant to phosphorus deficiency. Molecular markers were successfully used in introgression of *Pup1* gene to stress sensitive varieties IR64, IR74, Dodokah, Batur, and Sity Bagendit [42, 43]. The *Pup1* gene determines almost 80 % of phenotypic differences in the population [21].

A locus with less significant effect was detected in chromosome 6. QTL on chromosome 6 determined 25-34 % phenotypic differences in this trait, but field trials showed its less effect [23]. Few minor loci were mapped on other chromosomes [43-45]. Some of these QTL were located in the same chromosomal regions as the gene-candidates for phosphorus shortage tolerance which were identified earlier.

In another marked population (Kasalath × Gimbozu) QTL, causing roots elongation with shortage of phosphorus, is also mapped on chromosome 6 [45]. Later, several loci involved in phosphorus metabolism were detected on chromosomes 1, 2, 5, and 9 [43]. As a rule, the genes determining adaptiveness is situated in clusters, however, another genes associated to the traits, except *Pup1* on chromosome 12, were not detected. The positive effect of *Pup1* gene was more clear when phosphorus shortage was coincide with water shortage.

The distance between other markers, which flank the genes encoding tolerance to phosphorous shortage does not exceed 12.9 cM. It allows us to involve them in genetic and selection researches, because use of two markers to control allele introgression significantly reduces the probability of dual crossing and

increases the reliability of estimates. For two flanking markers with a distance of 10 cM the probability of false gene identification is not more than 1.2 %, which is lower than admitted in biological research [35]. In more recent papers, the effective use of selected markers in control of introgression was shown in different population.

Polymorphism of Russian and foreign varieties has been shown with all investigated markers to genes encoding effective use of phosphorus, which makes it possible to involve them in breeding [46-48]. The maximum number of alleles is noted for RM 247 (chromosome 12) (Fig. 2). All Russian varieties, except Yantar, Dal'nevostochnyi, Anait, are monomorphic in their RM 322 marker. There are three alleles for RM13 (in varieties Pavlovskii, Boyarin, Regul, Yantar, Druzhnyi, Kasun the size of amplified DNA fragments is bigger than in most others), and two alleles for RM 261 (molecular weight of the fragments is bigger in the varieties Sadko, Novator, and Regul).
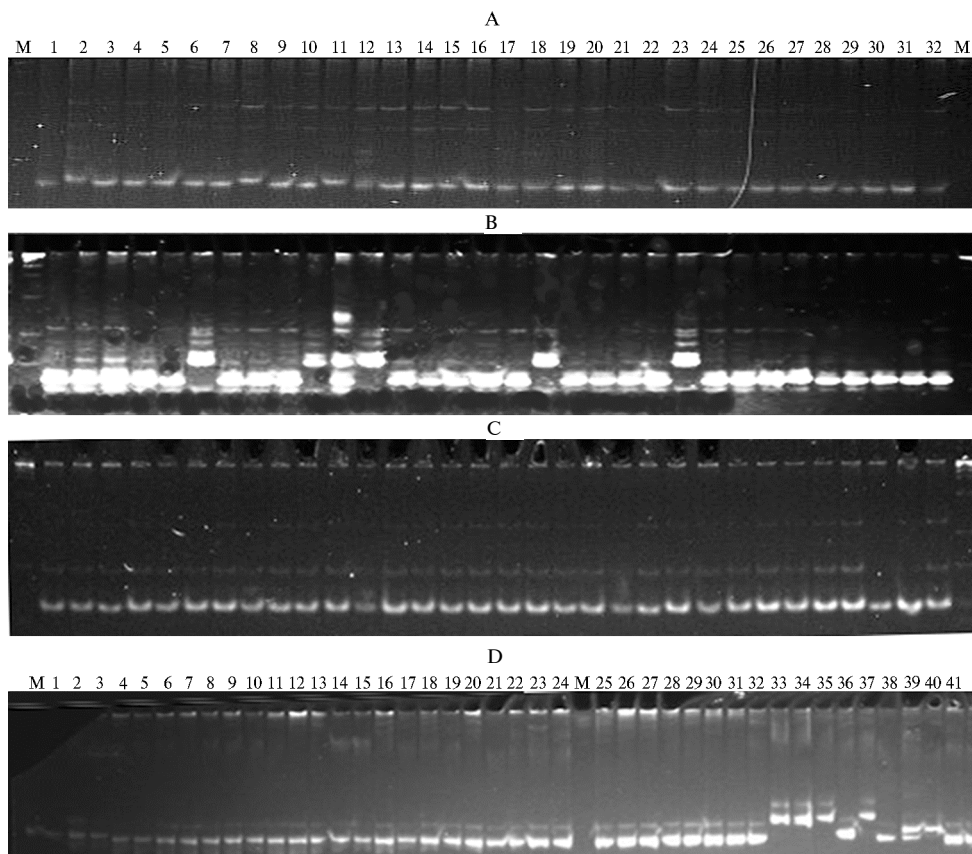
Fig. 2. Polymorphism of Russian and foreign rice varieties (*Oryza sativa* L.) on markers RM 261 (A), RM 13 (B), RM 322 (C) and RM 19 (D) to genes for effective use of phosphorus: 1 — Khankai, 2 — Sadko, 3 — Primorsky, 4 — Liman, 5 — Garant, 6 — Pavlovskii, 7 — Rapan, 8 — Novator, 9 — Serpentine, 10 — Boyarin, 11 — Regul, 12 — Yantar, 13 — Zhemchug, 14 — Leader, 15 — Khazar, 16 — Amethyst, 17 — Narciss, 18 — Druzhnyi 19 — Sprint, 20 — Viola, 21 — Dal'nevostochnyi, 22 — Fotan, 23 — Kasun, 24 — Yupiter, 25 — Atlant, 26 — Kurchanka, 27 — Fakel, 28 — Snezhinka, 29 — Sharm, 30 — Anait, 31 — Flagman, 32 — Izumrud; 33 — IR 66, 34 — NSIC RC 158, 35 — IR68897B, 36 — IR 73328B (donors of genes *WC*); 37 — Moroberican, 38 — N 22, 39 — Azusena, 40 — Dular, — - Taipei 309; M is a molecular weight marker [48].

All studied markers associated to phosphorus absorption capability of plants are suitable for breeding and selection of donor genotypes among Russian varieties because it is shown that the donor and recipient varieties carry different target alleles, so the plants receiving the donor allele can be detected by electro-

phoretic separation of PCR products.

Thus, mechanisms for plants adaptation to low nitrogen and phosphorus levels in soil are associated to growth and development of root system, intensification of absorption and internal efficiency of the elements use, and also to their availability due to synthesis and excretion of organic acids to the rhizosphere. Crops differ genotypically in the efficiency of nitrogen and phosphorus utilization. The variability in this trait among rice genotype is mainly due to differences in root system growth, which increases its absorbing surface, and to genotypic differences in capability to absorb mineral nutrients, while the change of internal efficiency of their use affects slightly. There is a set of molecular markers associated to studied traits. In Russian rice verities all studied markers associated to efficiency of mineral element intake are polymorphic. All this allows more effective seeking for donors of the target genes and facilitates breeding by using molecular markers.

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VARIABILITY OF AGRONOMICALLY IMPORTANT TRAITS IN SPRING WHEAT HYBRIDS OBTAINED BY MARKER-ASSISTED SELECTION FROM CROSSES OF WINTER WHEAT WITH SPRING WHEAT DONORS OF RESISTANCE GENES

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A b s t r a c t

Spring wheat is the main cereal crop in the West Siberian region of Russia and occupies more than 40 % of the acreage. The widening of the genetic diversity of this culture and the creation of new varieties with high productivity and resistance to environmental factors have always been a key problem of breeding. Winter wheat varieties which are characterized by a wide diversity of useful characters such as tolerance to abiotic stressors, productive tillers and yield can be used as a promising source of agronomically important traits. Despite the fact that the methodological approach to the introgression of the gene pool of winter wheat into the genome of spring wheat has been in use for a long time to increase the yield of spring varieties, at the moment there is little available information concerning the manifestation of valuable traits in offspring obtained from the winter and spring wheat hybridization. However, such results are important for assessing the combining ability of winter and spring wheat varieties and for the selection of potential parents by a complex of characters. For the identification of the winter and spring genotypes in the offspring resulting from the hybridization of winter and spring parents with the help of convenient breeding methods, a large sample size populations and a long period of cultivation of plant material are required. The application of MAS technologies and markers linked to the target loci can significantly decrease the time of development of new breeding lines. In creation of breeding lines resistant to leaf rust, the winter wheat varieties Filatovka and Biyskaya ozymaya, adapted for cultivation in the West Siberian region, were involved in a scheme of hybridization. Spring wheat introgression line 5366-180 (*Triticum aestivum*/*T. timopheevii*) и spring wheat cultivar Tulaikovskaya 10, containing genetic material of *Thinopyrum intermedium* were the donors of effective leaf rust resistance genes *LrTt2* and *Lr6Ai#2*. The aim of this work was to evaluate the diversity of spring wheat forms obtained from these crosses on economically important traits and analysis of the genotype influence on their manifestation. The plants differing in winter and spring habits were selected in F_2 progeny by means of allele-specific markers to the *VRN-1* genes. To identify genotypes containing leaf rust resistance genes, a microsatellite marker *Xbarc232* and primers MF2/MR1r2 and MF2/MR4 specific for *T. timopheevii* and *Th. intermedium* translocations, respectively, were used. As a result of hybridization of winter varieties with spring donors, 122 F_2 plants were obtained, of which 30 homozygous spring plants with different allelic composition of *VRN-1* genes were selected. Of these, 12 plants according to molecular data with *Lr*-specific primers contained alien translocations. For the comparative analysis of agronomic traits, six F_2 plants were used which possessed the same allelic composition of *VRN-1* genes as in the initial spring donors but differed in the presence or absence of *Lr* genes. F_{3-4} families obtained by selfing of the corresponding F_2 plants were studied during two field seasons on the following traits: heading date, productive tillers, grain number per spike, grain weight per spike, and 1000-grain weight. The results of heading date evaluation showed that, despite the same allelic composition of the *VRN-1* genes, there was a significant difference in the heading date between F_{3-4} hybrids and spring donors. Depending on the year of the field evaluation, the differences were three to eight days, and it was found that the presence of alien genetic material does not affect this trait. Analysis of the productivity traits of F_{3-4} hybrids indicated that the main impact to the manifestation of the traits was made by environmental factors. A significant contribution of the genotype to the phenotypic manifestation of the traits grain number per spike and 1000-grain weight was detected in the hybrid 70-12

containing the translocation from *Th. intermedium*. The obtained data demonstrated the effectiveness of MAS for the identification of homozygous genotypes with desired traits in the early breeding stages using small sample population. Agronomic trait variations observed in the F_{3-4} progenies allows to select breeding lines with the optimal expression of valuable traits for further breeding.

In Russia, spring soft wheat is one of the most common cereal crops. When searching for promising sources of valuable traits for spring wheat and their use in diverse hybridization schemes, the gene pool of winter wheat is interesting as a source material for the enrichment of spring forms. It is known that winter soft wheat is characterized by a wide variability in tolerance to abiotic factors, productive tillers, ear fertility and yield [1, 2]. Winter wheat has been used to expand the range of spring varieties in the world breeding practice since ancient times [3, 4]. Soviet scientists applied winter and spring wheat crossings to study and select seeds by earing time [5, 6]. It was shown that in F_2 and F_3 there were plants with an earlier earing as compared to the spring parent, but they had no tillering ability and had only one stalk.

Productive and drought-resistant spring wheat varieties were obtained by the hybridization of the winter varieties Bezostaya 1, Aurora, and the Caucasus with spring ones [7, 8]. At the International Maize and Wheat Improvement Center (CIMMYT), breeding lines and varieties of spring soft wheat with high yields, resistance to fungal diseases and a short stem have been created by the hybridization of winter and spring forms [9]. The spring wheat variety Extrakolben was obtained in Sweden by crossing a spring wheat variety with a winter one. Its yields are 10 % higher than in the best initial varieties, indicating the possibility of significantly increasing productivity of spring varieties when crossing with winter forms [10]. By using the winter varieties Bezostaya 1, Myronivska 808, Early 12 as a maternal form and the spring variety Vardenik 9 as a pollinator, spring hybrid forms were received, exceeding the spring parent by 1000-grain weight [11]. In addition, constant lines with high productivity and good grain quality were identified among the hybrid generations.

The combinative ability [12, 13] and heterosis in hybrid wheat forms obtained by crossing varieties which differ in their mode of life have been reported [14-17]. However, we have little available information about economically important traits in the offspring of such crosses.

In recent years, marker-assisted selection (MAS) has been used in addition to traditional methods. Its main advantages include the identification of genotypes homozygous for the target trait by chromosome-specific markers and the ability to detect target genotypes on small samples [18].

To create a new selection material and transfer effective leaf rust diseases resistance genes into the genome of winter and spring soft wheat, we used a hybridization scheme where winter wheat varieties adapted for growth in the Western Siberian region were used as recipients. Spring forms containing alien genetic material with resistance genes inherited from *Triticum timopheevii* and *Thinopyrum intermedium* were donors [19, 20]. Obtaining winter genotypes with molecular markers for identification of the genes controlling winter or spring mode of life and resistance to leaf rust diseases, and evaluating their winter hardiness and susceptibility to the pathogen have been described earlier [21]. This report presents the first results of study on spring offspring from crossing the winter and spring parents based on the sprouting period and harvesting components. Here, the effect of genetic material inherited from *Triticum timopheevii* and *Thinopyrum intermedium* on the manifestation of the traits is analyzed.

The aim of this work is to compare the productive properties of spring

wheat hybrids with introgressed leaf rust resistance.

Technique. The winter soft wheat varieties Filatovka and Biyskaya ozy-maya were used as maternal forms in hybridization. The spring introgressive line 5366-180 (*T. aestivum*/*T. timopheevii*), and the soft wheat variety Tulai-kovskaya 10 (*T. aestivum*/*Th. Intermedium*) with leaf rust resistance genes *LrTt2* and *Lr6Ai#2*, respectively, were the donors [19, 20]. Before the hybridization, the winter varieties were vernalized for 60 days at 3 °C. Crossing with spring donors of resistance genes and growing of F_1 and F_2 hybrids were carried out in a hydroponic greenhouse (Center for Collective Use of Artificial Plants Growing Laboratory, FRC Institute of Cytology and Genetics).

The F_2 plants obtained by hybridization were genotyped using allele-specific markers to *VRN-1* genes and an alien genetic material containing *Lr* genes. Genomic DNA was isolated from the leaves of young plants using sodium bisulfite $Na_2S_2O_5$ as described [22]. Spring and winter plants were selected using markers to dominant and recessive alleles *Vrn-A1*, *Vrn-B1* and *Vrn-D1*. To select plants carrying the *LrTt2* gene from *Triticum timopheevii*, we used the SSR marker *Xbarc232* [23]. To identify the *Lr6Ai#2* locus from *Thinopyrum intermedium*, we used the two pairs of primers, *MF2/MR1r2* and *MF2/MR4*, developed by us [24]. The structure of the primer to *VRN-1* genes, PCR protocol for the primers to the *VRN-1* genes, *MF2/MR1r2* and *MF2/MR4* [25], as well as the PCR protocol for the *Xbarc232* marker [22] were described previously. PCR was performed on a Bio-Rad T-100 Thermal Cycler; the PCR product was separated in a 1.5 % agarose gel with ethidium bromide and visualized using the gel documentation system Gel Doc XR+ (Bio-Rad, USA).

F_{3-4} from self-pollination of the spring plants F_2, selected by molecular markers, were evaluated under field conditions in 2014 and 2016 (experimental field, Novosibirsk region, Krasnoobsk settlement) by the traits of the sprouting-earing time, productive tillers, the grain number per spike, the grain weight per ear and the 1000-grain weight. Sowing (on May 14, 2014 and May 17, 2016) was carried out manually in 2 replicates (initial spring forms were the control). Plants were grown in 1 m plots in width, with 40-60 seedlings in a row with a distance between rows of 20 cm. The sprouting-earing time was considered to be the time from the appearance of sprouts to the exit of the ear from the tube. The number of productive shoots was determined as the number of all lacustrine shoots of the plant. The 1000-grain weight was counted in 2-3 ears for 20 plants of each sample. For the statistical processing of remaining traits data, 25 plants were randomly selected.

In determining the reliability of the differences between the mean values of the two sample collections, Student's *t*-test was used. Samples were compared by variance factor analysis; reliability was assessed according to the Fisher *F*-criterion; the contribution of factors was calculated according to L.A. Vasilyeva [26]. The statistical processing was carried out using the Statistica 10.0 software package (StatSoft, Inc., USA). The tables and figures show the arithmetic mean (*x*) and the mean error ($\pm s_x$).

Results. The weather conditions during the vegetation period differed in the amount of precipitation and the temperature regime from year to year. In 2014, they were close to the average long-term, while in May there was a heat deficit and excessive moisture. In 2016, there was a decrease in moisture supply throughout the entire field season and higher temperatures as compared to 2014.

The winter soft wheat varieties Filatovka and Biyskaya ozymaya, adapted to the soil and climatic conditions of Western Siberia, have high winter hardiness, are highly productive, resistant to abiotic factors, but sensitive to most varieties of leaf rust [2]. In hybridization of the Filatovka and Biyskaya ozymaya va-

rieties with the introgression line 5366-180 and Tulaikovskaya 10, 122 descendants were obtained, of which 30 homozygous spring plants with different allelic composition of *VRN-1* genes were selected by molecular marking in F_2. Of these, 12 plants contained alien translocations with *Lr* genes. As an example, an electrophoregram of PCR amplification products of the genomic DNA of parents and F_2 progeny with SSR marker *Xbarc232* is shown (Fig. 1). In the winter varieties Biyskaya ozymaya and Tulaikovskaya 10 with *Xbarc232*, a 200 bp fragment specific for the long arm of 5B chromosome was synthesized; and in the introgressive line 5366-180 and plant No. 6 from the F_2 population this fragment was absent (zero allele) that indicates the presence of alien translocation with the gene *LrTt2*.
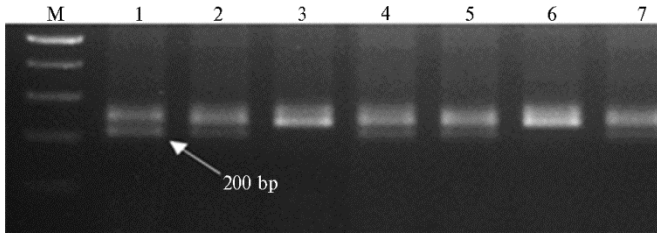
Fig. 1. Identification of genotypes with leaf rust resistance gene *LrTt2* using the *Xbarc232* DNA marker: 1 and 2 — the varieties Biyskaya ozymaya and Tulaikovskaya 10, respectively; 3 — introgressive line 5366-180; 4-7 — the F_2 plants; M — the fragment length marker (100 bp ladder, Biosan, Novosibirsk). The arrow indicates a diagnostic 200 bp fragment.

We compared economically valuable traits in six F_2 offspring plants that had the same allelic composition of *VRN-1* genes as in the spring parent, but differed from it by the presence or absence of *Lr* genes (Table 1). Hybrids containing *Lr* genes according to the results of molecular analysis have low susceptibility to the West Siberian population of leaf rust (*Puccinia triticina* Erikss.) (data not shown).
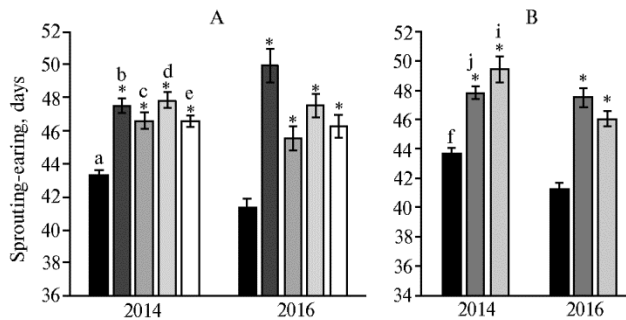
Fig. 2. Sprouting-earing in spring plants F_{3-4} from crossing Filatovka × 5366-180 (donor of gene *LrTt2*) (A) and Biyskaya ozymaya × Tulaikovskaya 10 (donor of *Lr6Ai#2* gene) (B) in different years, as compared to the parental forms: a — 5366-180 (control), b, c, d, e — lines 90-7, 88-11, 88-3, 89-12, f — Tulaikovskaya 10 (control), g, i — lines 70-12, 71-12 (experimental field, Novosibirsk region).

* Differences between hybrid lines and parental forms are statistically significant at $p < 0.001$.

It is known that the combination of dominant and recessive alleles of *VRN-1* not only determines the type of development, but also affects the rate of development and the time of earing [27, 28]. There is also the assumption of an earlier earing and higher yielding in soft wheat varieties with two dominant genes *Vrn-A1* and *Vrn-B1* [29]. The evaluation of the earing time in the spring offspring F_{3-4}, obtained by crossing winter varieties and spring resistant donors, showed that hybrid plants were eared much later than the spring parent (Fig. 2). Thus, for plants derived from crossing Filatovka × 5366-180, the difference in earing onset compared to the spring parent was on average 3.3-4.5 days in 2014 and 4.2-8.6 days in 2016 (Fig. 2, A). It should be noted that there are no differences in the earing time in samples containing and not containing alien translocations with *Lr* genes. In the offspring from crossing varieties Biyskaya ozymaya and Tulaikovskaya 10 (see Fig. 2, B), the lines 70-12 and 71-12 were identical to

the parent Tulaikovskaya 10 plants in *VRN-1* alleles, but the line 70-12 possessed introgression. The evaluation of the earing time showed a delay in earing onset in both lines compared to parent variety regardless of the year of field tests.

Grain number per spike in all studied Filatovka × 5366-180 hybrids significantly increased in 2014, but in 2016, no differences were observed. In crossing Biyskaya ozymaya × Tulaikovskaya 10, the sample 70-12 with translocation from *Th. intermedium* significantly exceeded the original variety in both years, whereas the line 71-12 without translocation did not differ from the spring parent (Table 2). Grain weight per spike in the first combination significantly exceeded that in the control lines 90-7, 88-11 and 88-3 in 2014, and in the second combination the line 70-12 1.2 times exceeded the control in 2016. The 1000-grain weight for the samples 88-11 and 88-3 in the progeny Filatovka × 5366-180 was 35.7 and 35.1 g, respectively, which is significantly lower than that for the line 5366-180 (38.2 g), and the samples 90-7 and 89-12 did not differ from the control. In 2016, no samples from the first combination showed significant differences from the control 1000-grain weight, and in the second combination, it was significantly lower in the line 70-12 in both years (Table 2).

2. Economically valuable characteristics of F$_{3-4}$ progeny and parental forms of spring soft wheat in different years for two hybrid combinations ($x \pm s_x$, experimental field, Novosibirsk region)

Sample, variety, line	Traits	2014	2016
F i l a t o v k a × 5366-180			
5366-180 (control)	Productive tillers, pcs.	2.50±0.14	3.13±0.21
	Grain number per spike, pcs.	28.79±0.71	23.45±1.21
	Grain weight per spike, g	1.15±0.04	0.64±0.04
	1000-grain weight, g	38.15±0.60	25.79±0.79
90-7	Productive tillers, pcs.	3.23±0.21**	2.31±0.26*
	Grain number per spike, pcs.	32.74±1.23*	25.58±2.22
	Grain weight per spike, g	1.32±0.06*	0.65±0.07
	1000-grain weight, g	36.64±0.57	24.17±1.58
88-11	Productive tillers, pcs.	2.46±0.19	2.60±0.23
	Grain number per spike, pcs.	36.40±1.52***	26.50±1.45
	Grain weight per spike, g	1.38±0.07**	0.70±0.04
	1000-grain weight g	35.65±0.70**	25.63±0.72
88-3	Productive tillers, pcs.	2.64±0.17	2.17±0.25**
	Grain number per spike, pcs.	36.03±1.34***	20.27±1.69
	Grain weight per spike, g	1.37±0.06**	0.55±0.06
	1000-grain weight, g	35.09±0.72**	24.34±1.14
89-12	Productive tillers, pcs.	2.41±0.15	2.68±0.36
	Grain number per spike, pcs.	31.59±1.16*	22.06±1.83
	Grain weight per spike g	1.26±0.06	0.61±0.06
	1000-grain weight, g	36.93±0.93	26.15±1.25
B i y s k a y a o z y m a y a × T u l a i k o v s k a y a 10			
Tulaikovskaya 10 (control)	Productive tillers, pcs.	2.28±0.13	2.71±0.17
	Grain number per spike, pcs.	33.52±1.14	28.98±1.30
	Grain weight per spike, g	1.18±0.05	0.87±0.04
	1000-grain weight, g	33.93±0.57	28.96±0.58
70-12	Productive tillers, pcs.	2.03±0.22	1.64±0.16***
	Number of grains in the ear, pcs.	41.19±2.39**	38.94±1.93***
	Grain weight per spike, g	1.33±0.12	1.04±0.08*
	1000-grain weight, g	29.22±1.30***	26.48±1.08*
71-12	Productive tillers, pcs.	2.27±0.19	2.19±0.20*
	Grain number per spike, pcs.	35.22±2.11	32.30±1.62
	Grain weight per spike, g	1.21±0.09	0.79±0.06
	1000-grain weight, g	32.68±1.02	23.27±0.99***

*, **, *** Differences between hybrid lines and parental forms are statistically significant at p < 0.05; p < 0.01; p < 0.001.

It should be noted that almost all of the samples, including parents, had quantitative indicators declining in 2016, which may be due to weather conditions. The significant influence of environmental factors on quantitative indicators was also supported by the factor analysis (Table 3).

The factor variance analysis, based on the evaluation of economically important traits, showed that the genotype of hybrid lines contributed significantly to

sprouting-earing time while productive tillering depended only on environmental conditions (see Table 3).

3. Factor analysis of variance based on economically valuable characteristics of F$_{3-4}$ progeny and parental forms of spring soft wheat for two hybrids combinations (experimental field, Novosibirsk Region, 2014 and 2016)

| Traits | Filatovka × 5366-180 | | | | Biyskaya ozymaya × Tulaikovskaya 10 | | | |
| | introgression | | no introgression | | introgression | | no introgression | |
	r_{wg}	r_{we}	r_{wg}	r_{we}	r_{wg}	r_{we}	r_{wg}	r_{we}
Sprouting—earing period	0.94*****	0.05	97.1*****	2.70	0.91*****	0.08	0.99*****	0.01
Productive tillers	0.01	0.98*****	0.05	0.92*****	0.12	0.86****	0.01	0.98*****
Grain number per spike	0.22**	0.75****	0.09	0.78****	0.83****	0.16*	0.23	0.74**
Grain weight per spike	0.05	0.92*****	0.04	0.93*****	0.20	0.80***	0.03	0.95**
1000-grain weight	0.02	0.96***	0.02	0.98***	0.55***	0.41*	0.26	0.68**

N o t e . r_{wg} and r_{we} — correlation coefficients determining the contribution of the genotype and the environment respectively to the trait phenotypic manifestation.
*, **, ***, ****, ***** The influence of the genotype and the external environment is statistically significant at $p < 0.05$, $p < 0.01$, $p < 0.001$, $p < 0.0001$ and $p < 0.00001$.

The factor analysis suggests that the translocations do not affect sprouting—earing time and productive tillering in the hybrids. However, in the sample 70-12 from Biyskaya ozymaya × Tulaikovskaya 10 crossing, the grain number per spike and the 1000-grain weight are more influenced by the genotype, whereas in the sample 71-12 these are mainly influences by the environmental conditions. It is not excluded that the trait variability in these hybrids is due to the influence of the alien genetic material, since the line 70-12 contains a translocation from *Th. intermedium*.

In all the studied spring lines selected from crossing with winter varieties the earing delayed as compared to the spring parent, regardless of their haplotype by the *VRN-1* genes and the presence of alien translocations. It should be noted that at the beginning of the 20th century the attempts to obtain earlier spring samples from the crossing of spring and winter wheat varieties were unsuccessful [3-6]. First, spring character is ensured by dominant *VRN-1* gene, but the earing depends on the interaction of many regulatory and structural genes, which can be introduced from the winter genotype [30, 31]. In addition, variations were observed within the lines both for earing time and other economically important traits (see Fig. 2, Table 2), which makes it possible to select spring forms of plants that are superior to the parent forms in productivity and close to them in earing onset. We cannot exclude that when using winter varieties from the European region in crossings with spring wheat, the delay in earing of the spring progeny can be much less.

Thus, our results testify to the effectiveness of allele-specific markers in the selection of genotypes by target loci. The genotyping of samples simultaneously by several genetic loci can significantly reduce the sample size and allows to identify the homozygous offspring in F$_2$ population. Considerable delay in earing in the spring offsprings, obtained by hybridization of winter and spring soft wheat, is apparently not related to the effect of *VRN-1* genes which determine the timing of earing. The evaluation of the F$_{3-4}$ spring families by quantitative characteristics did not reveal reliable differences from the parental forms. Field study of the samples during two seasons showed that the environmental factors mainly contribute to the manifestation of these characteristics. The sample 70-12, in which the significant influence of the genotype on the manifestation of quantitative traits can be explained by the presence of alien genetic material, is an exception.

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PLUG MARKERS TO DETECT ALIEN GENETIC MATERIAL IN BREAD WHEAT (*Triticum aestivum* L.) HYBRIDS DURING BREEDING

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A b s t r a c t

Molecular markers make it possible to monitor the directional introgression of alien genetic material to the genome of wheat. Among the diverse molecular markers, the PLUG (PCR-based landmark unique gene) markers have the greatest advantage for analyzing distant hybrids due to the ability to amplify polymorphism fragments on closely related species and to detect simultaneously three genomes within one homoeological group in one PCR. A collection of 41 entries of different breeding stages developed in Russian State Agrarian University as a result of crossing of bread wheat (*Triticum aestivum* L.) and wheat *T. timopheevii* Zhuk. was studied. All hybrids were resistant to leaf rust and powedery mildew natural infection. Using this collection of distant hybrids, we demonstrated for the first time the possibilities of PLUG markers for identifying alien introgressions in the genome of bread wheat from *T. timopheevii*. In order to increase the degree of polymorphism between the entries, we additionally processed the products of amplification of PLUG markers with high-frequency restriction endonucleases TaqI and HaeIII. The entries were found to carry both single and multiple introgressions. The latter entries with introgressions in more than one homeological group are of particular interest. The heterogeneity of not only the late generations of entries, but also the entries from the nursery of the preliminary cultivar estimation, which may be associated with secondary chasmogamy, is shown. As a result of the studies, 14 PLUG markers for the long and short chromosome arm of each homeological group have been selected, which can be recommended for the detection of the genetic material of *T. timopheevii* in distant hybrids of *T. timopheevii* × *T. aestivum*. The advantages of PLUG markers as a tool for monitoring the transfer of alien hereditary material into the genome of bread wheat are discussed.

Keywords: common wheat, *Triticum aestivum*, *Triticum timopheevii*, wide hybridization, molecular markers, PLUG, PCR

The predominance of bread wheat (*Triticum aestivum* L., A^{u}BD) over other wheat species is determined by ecological plasticity, resistance to low and high temperatures, excess and shortage of moisture, diseases and pests. Currently, this is the main food crop for about one third of the world's population [1, 2]. However, in connection with impoverishment of genetic resources of bread wheat, evolution of pathogens, loss of efficiency of the present disease and pest resistance genes, a problem of search for new resistance genes for transfer into the *T. aestivum* genome at distant hybridization becomes important.

As a source of such genes, relatives of bread wheat are used, in particular,

T. timopheevii Zhuk. (A^{b}G). In this species, a plurality of valuable resistance genes have been found, part of which has been introduced into bread wheat, e.g. two leaf rust resistance genes (*Lr18* and *Lr50*), three stem rust resistance genes (*Sr36*, *Sr37*, *Sr40*), and three mildew resistance genes (*Pm6*, *Pm27*, *Pm37*) [3]. It is also reported that the introduced gene *LrTt2* can be a new allele of *Lr18* [4].

T. timopheevii wheat, which possesses resistance to brown rust, loose and covered kernel smut [5, 6], to Swedish and Hessian fly [7, 8], can be used for further enrichment of the bread wheat genotype. Moreover, *T. timopheevii* has increased cold resistance, high drought resistance, excess moisture resistance, and high bread baking qualities: the protein content in the seed is 19 to 30 %, which is significantly higher than that in the bread wheat [9-11]. Therefore, distant hybrids obtained involving *T. timopheevii* are a promising material for bread wheat selection.

The hybrid combinations of *T. aestivum* × *T. timopheevii* were obtained in 1994 by V.F. Kozlovskaya and M.M. Starostyonkova (Altai Research Institute of Agriculture). Using these hybrids, at P.I. Lisitsyn Selection Station (Russian State Agrarian University— Timiryazev Moscow Agricultural Academy) a sample collection has been provided involving the genetic material of *T. timopheevii*. These samples (populations, families, lines) are the breeding products of different completeness which were selected for yielding and resistance to mildew and brown rust at natural infectious background [12].

Molecular markers are widely used in order to reveal introgressions in the progeny of distant hybrids, which can hardly be identified phenotypically. There are different marker types (expressed sequence tag—simple sequence repeats, EST-SSR; expressed sequence tag—sequence-tagged sites, EST-STS; SSR; random amplification of polymorphic DNA, RAPD; etc.) which allow detecting alien genetic material by polymerase chain reaction (PCR) and direct identifying by marker-assisted selection — MAS. For hexaploid bread wheat, PLUG (PCR-based landmark unique gene)-PCR markers are the most convenient. These markers have been developed by G. Ishikawa et al. [13] basing on conservative orthologous genes. The primers for the PLUG markers have been selected on exon sites that flank the intron. Due to the fact that in cereals the exons are high conservative, the PLUG markers developed for bread wheat, due to high homology, can be amplified using DNA of closely related species as well. At the same time, the inner parts of the amplicon are introns in which high species variability is observed (insertions/deletions, nucleotide replacements). Therefore, the products of amplification in various species would be different in size or nucleotide composition. One can detect such differences by electrophoretic separation of the PCR products (directly or, in the second case, after pre-treatment with restriction endonucleases). The main advantage of these markers is a simultaneous identifying in the distant bread wheat hybrids both all three own sub-genomes and alien introgressions within one homeological group [14].

PLUG markers allowed identifying alien chromosome obtained from other genera, e.g. *Thinopyrum elongatum* [15], *Th. intermedium* [16], *Leymus mollis* [17], *Th. ponticum* [18], in the distant hybrids of bread wheat. However, there is not a single paper on using PLUG markers to study distant hybrids from crossing *Triticum* genus representatives.

For the first time, we have carried out the investigation of hybrids of two wheat species from the working collection of selected samples using molecular PLUG markers and found forms with both single and multiple introgressions (from the latter, of special interest are the samples with introgressions in more than one homeological group). Heterogeneity was shown not only for latter generations of selection lines, but also for the line from the nursery of the prelimi-

nary cultivar estimation, which can be associated with secondary chasmogamy.

The aim of the present paper was to find genetic material of *Triticum timopheevii* in the distant hybrid samples using PLUG markers in the course of breeding.

Technique. The collection of F_7-F_{10} distant hybrids (P.I. Lisitsyn Selection Station at Russian State Agrarian University— Timiryazev Moscow Agricultural Academy, collection is based on the hybrids ontained by V.F. Kozlovskaya and M.M. Starostyonkova in Altai Research Institute of Agriculture) comprised the lines from the combination of L-6 [♀(Zhnitsa × *T. timopheevii* K-47793) × ♂Zhnitsa] , i.e. L-6-1, L-6-2, L-6-3, L-6-4, L-6-5, L-6-6, L-6-7, L-6-8, L-6-9, L-6-10, L-6-11, L-6-12, L-6-13, L-6-14, L-6-15, L-6-16, L-6-17, L-6-18, L-6-19, and also the lines from the combination L-25 [♀(Novosibirskaya 67 × *T. timopheevii* K-47793) × ♂Novosibirskaya 67], i.e. L-25-11, L-25-12, L-25-13, L-25-14, L-25-15, L-25-16, L-25-17, L-25-18, L-25-19, L-25-20, L-25-21, L-25-22, L-25-23, L-25-24, L-25-25, L-25-26, L-25-27, L-25-28, L-25-29, L-25-30. All these lines were first selected for productivity and resistance to mildew and leaf rust under natural infection. The L-6-6, L-6-7 and L-6-10 lines were involved in further selection. The parental forms of the distant hybrids, Zhnitsa and Novosibirskaya 67 bread wheat varieties, and *T. timopheevii* K-47793 served as control.

The genome DNA was extracted by CTAB method from the etiolated seedlings by J.J. Doyle and J.L. Doyle procedure [19].

Basing on the literature data, 52 molecular PLUG markers were selected for PCR analysis [10, 12]. The PCR mixture (25 μl) consists of 0.5 μl genomic DNA (500-1300 ng/μl), 2.5 μl dNTP (Thermo Fisher Scientific, USA), 2.5 μl Taq-buffer with 25 mM $MgCl_2$ (Silex, Moscow), 1 μl forward and reverse primers (in total; Sintol, Moscow), 0.5 μl Taq-polymerase colored (2.5 u/μl, 5000 UA, Silex, Moscow). Amplification protocol was as follows: initial denaturation at 94 °C for 5 min; 35 cycles including 94 °C for 45 s; 55 °C for 45 s; 72 °C for 2 min; final elongation at 72 °C for 10 min (a DNA engine Tetrad 2 amplifier, Bio-Rad, USA). The PCR product was treated with restriction endonucleases, TaqI and HaeIII, for 12 h at 65 and 37 °C, respectively, according to the manufacturer's protocol (SibEnzim-M, Russia). Amplicons and their restricts were separated by electrophoresis in 2 % agarose gel with TBE buffer at 6 V/cm; 100 bp Ladder (Fermentas, Lithuania) was a molecular weight marker.

Results. In the collection, the populations ♀Omskaya 36 × ♂L-6-7, ♀Trizo × ♂L-6-7, ♀Esther × ♂L-6-10 are in the hybridization nursery, the populations ♀Esther × ♂L-6-10, ♀L-6-6 × ♂Trizo, ♀L-6-6 × ♂Ivolga are in the selection nursery, the lines ♀L-6-6 × ♂Trizo, ♀L-6-10 × ♂Trizo, ♀Ivolga × ♂L-6-7, ♀L-6-7 × ♂Trizo are in the nursery of the preliminary cultivar estimation, and the line ♀[(Zhnitsa × *T. timopheevii* K-47793) × Zhnitsa] × ♂Trizo is involved in the variety testing.

Successful use of molecular markers in selection, when dealing with distant hybrids, necessitates polymorphism between the parental forms. The PLUG markers used by us were selected in such a way so for each chromosome arm of each homeological group three fragments would be amplified that are characteristic of each bread wheat sub-genome: TNAC 1009 (1S), TNAC 1010 (1S), TNAC 1026 (1L), TNAC 1102 (2S), TNAC 1178 (2S), TNAC 1233 (2S), TNAC 1021 (2L), TNAC 1118 (2L), TNAC 1204 (2L), TNAC 1248 (3S), TNAC 1300 (3S), TNAC 1263 (3L), TNAC 1383 (3L), TNAC 1408 (4AS 4BDL), TNAC 1421 (4AS 4BDL), TNAC 1428 (4AS 4BDL), TNAC 1457 (4AL 4BDS), TNAC 1464 (4AL 4BDS), TNAC 1510 (4AL 4BDS), TNAC 1663 (4AL 4BDS), TNAC 1485 (5S), TNAC 1497 (5S), TNAC 1588 (5S), TNAC 1496 (5B), TNAC 1528

(5L), TNAC 1535 (5L), TNAC 1540 (5L), TNAC 1541 (5L), TNAC 1545 (5L), TNAC 1554 (5L), TNAC 1559 (5L), TNAC 1562 (5L), TNAC 1567 (5L), TNAC 1577 (5L), TNAC 1605 (5L), TNAC 1610 (5L), TNAC 1613 (5L), TNAC 1614 (5L), TNAC 1616 (5L), TNAC 1618 (5L), TNAC 1864 (5L), TNAC 1674 (6S), TNAC 1683 (6S), TNAC 1685 (6S), TNAC 1702 (6L), TNAC 1752 (6L), TNAC 1763 (6L), TNAC 1805 (7S), TNAC 1806 (7S), TNAC 1926 (7S), TNAC 1867 (7L), TNAC 1903 (7L), TNAC 1957 (7L).

Of these, after PCR with DNA of the parental forms (Zhnitsa and Novosibirskaya 67 bread wheat varieties, and *T. timopheevii* K-47793) and electrophoretic separation only those markers have been selected with which three fragments are amplified from each sub-genome of the *T. aestivum* (note that *T. timopheevii* and *T. aestivum* differ in such fragment profiles) (Fig. 1).

Fig. 1. Target fragment (680 bp) **characteristic of *Triticum timopheevii* which was revealed by TNAC 1867 marker to the long arm of chromosome (bread wheat homeological group-7):** 1 — Novosibirskaya 67, 2 — Zhnitsa, 3 — *T. timopheevii* K-47793; M — molecular weight marker 100 bp Ladder (Fermentas, Lithuania).

The treatment with TaqI and HaeIII, carried out for further detection of polymorphism between *T. timopheevii* and *T. aestivum*, has revealed 14 markers: TNAC 1026 (1L), TNAC 1010 (1S), TNAC 1021 (2L), TNAC 1178 (2S), TNAC 1383 (3L), TNAC 1248 (3S), TNAC 1464 (4AL-4BDS), TNAC 1421 (4AS-4BDL), TNAC 1535 (5L), TNAC 1485 (5S), TNAC 1752 (6L), TNAC 1674 (6S), TNAC 1867 (7L), TNAC 1806 (7S). These markers can be used for studying forms resulted from hybridization of *T. timopheevii* and *T. aestivum*, because they simultaneously detect the absence of fragments characteristic of subgenomes of bread wheat and the presence of fragments specific for *T. timopheevii*, indicating genomic changes in samples.
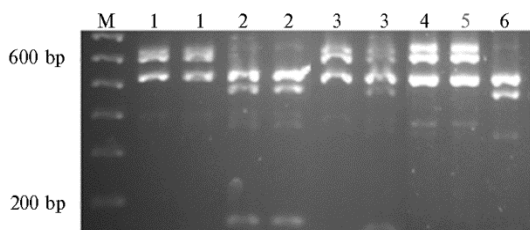
Fig. 2. Target fragment (590 bp) **characteristic of *Triticum timopheevii*, absence of two fragments characteristic of bread wheat/the presence of an extra fragment** (150 bp) **not characteristic of *T. timopheevii* and bread wheat in L-6-2 line, and also a heterogeneity of L-6-10 line on *T. timopheevii* chromatin content which were revealed by TNAC 1867 marker to the long arm of chromosome (bread wheat homeological group-7) and**

TaqI: 1 — L-6-1, 2 — L-6-2, 3 — L-6-10, 4 — Omskaya 36 variety, 5 — Esther variety, 6 — *T. timopheevii* K-47793; M — molecular weight marker 100 bp Ladder (Fermentas, Lithuania).

Such forms are the lines, selected from L-6 and L-25 combinations which were obtained by crossing *T. timopheevii* K-47793 with Zhnitsa and Novosibirskaya 67, respectively. Using the selected 14 PLUG markers, we have analyzed 19 lines from L-6 combination and 11 lines from L-25 combination and found the absence of the fragment characteristic of bread wheat and the presence of extra fragments or heterogeneity in the studied forms. It has been shown that extra fragments specific for *T. timopheevii* are present in the L-6-7, L-6-8, L-6-15, L-6-18, L-6-20 (marker TNAC 1421_TaqI), L-6-10, L-6-16, L-6-17 (TNAC 1867), L-6-2 (TNAC 1806), L-6-9, L-6-11 (TNAC 1806_TaqI) samples, and, moreover, the L-6-2 line not only contains an extra fragment of *T. timopheevii* DNA but also differs from the others by the absence of the fragment

characteristic of wheat (TNAC 1867) (Fig. 2). These results indicate the introgressions from *T. timopheevii* in the genome of the said samples.

It also has been shown that in the samples L-6-6, L-6-7, L-25-26 (TNAC 1026_HaeIII); L-25-20 (TNAC 1178), L-25-21 (TNAC 1178_ TaqI); L-6-5, L-6-6, L-6-11, L-6-14, L-25-28 (TNAC 1248), L-6-3, L-25-20, L-25-21 (TNAC 1248_TaqI, TNAC 1248_HaeIII), L-25-22 (TNAC 1248_TaqI), L-25-27 (TNAC 1248_HaeIII) there is no fragment characteristic of bread wheat. In L-6-16 (TNAC 1752) there was an extra fragment different to those of the bread wheat but not characteristic of *T. timopheevii*. In L-6-2, L-6-11, L-6-12, L-6-13, L-6-14, L-6-17 (TNAC 1752) there was no fragment characteristic of bread wheat but an extra fragment appeared not characteristic of *T. timopheevii*. The absence of the wheat fragment may indicate a presence in the corresponding locus of an alien introgression (an amplified extra fragment for such an introgression is possibly not visualized because its length corresponds to the sizes of wheat fragments). Extra fragments not characteristic of *T. timopheevii* are possibly due to polymorphism of the *T. timopheevii* samples. Among the studied lines, L-6-2, L-6-6, L-6-7, L-6-8, L-6-10, L-6-11, L-6-12, L-6-14, L-6-16, L-6-17, L-6-18, L-6-20, L-25-20, L-25-21, L-25-22, L-25-27 and L-25-28 exhibited heterogeneity which may be associated with the secondary chasmogamy.

The lines L-6-6, L-6-7 and L-6-10 were involved in producing 11 samples which selection at the moment is differently finalized. We have used 4 PLUG markers for their analysis: TNAC 1026_HaeIII (1L), TNAC 1248_TaqI (3S), TNAC 1421_TaqI (4AS-4BDL), TNAC 1867 (7L). These markers were chosen based on the fact that, according to our data, the L-6-6, L-6-7 and L-6-10 profiles differed from the bread wheat profile either in the absence of bands specific to bread wheat or in the presence of extra bands.
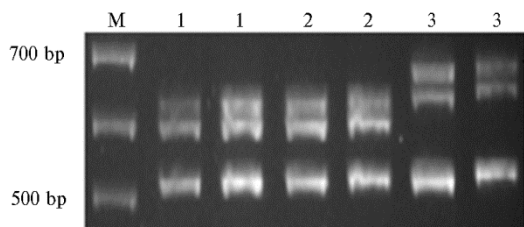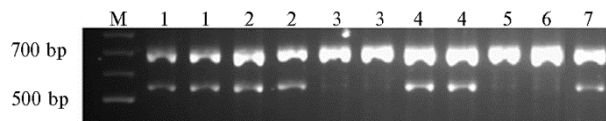

Fig. 3. Target fragment (550 bp) characteristic of *Triticum timopheevii* which was revealed by TNAC 1421_TaqI marker to the long arm of chromosome of homeological group-4 for subgenome A and the long arm of chromosomes of homeological group-4 for subgenomes B and D in bread wheat: 1 — ♀[(Zhnitsa × *T. timopheevii* K-47793) × Zhnitsa] × ♂Trizo, 2 — ♀Omskaya 36 × ♂L-6-7, 3 — ♀Esther × ♂L-6-10, 4 — ♀L-6-6 × ♂Trizo, 5 — Omskaya 36, 6 — Esther, 7 — *T. timopheevii* M-47793; M — molecular weight marker 100 bp Ladder (Fermentas, Lithuania).

Using TNAC 1421_TaqI marker, the extra fragment of *T. timopheevii* type was found in the populations from the hybridization nursery ♀Omskaya 36 × ♂L-6-7 and ♀Trizo × ♂L-6-7, in the population from the selection nursery L-6-6 × ♂Trizo, in the line from the nursery of the preliminary cultivar estimation ♀Ivolga × ♂L-6-7, and also in the line involved in variety testing ♀[(Zhnitsa × *T. timopheevii* K-47793) × Zhnitsa] × ♂Trizo, which indicates an introgression from *T. timopheevii* in these samples (Fig. 3, Table). By TNAC 1248_TaqI marker, the fragment characteristic of bread wheat was not found in the populations from the hybridization nursery ♀Omskaya 36 × ♂L-6-7 and ♀Trizo × ♂L-6-7, and in the lines from the nursery of the preliminary cultivar estimation ♀L-6-6 × ♂Trizo and ♀Ivolga × ♂L-6-7, which also confirms the presence of the *T. timopheevii* genetic material in the listed samples (see Table).

Molecular PLUG marking is the most convenient method in plant selection due to ability of simultaneous identification of the presence or absence of introduced genetic material in several subgenomes within a homeological group in polyploidy species. Using PLUG markers for studying the distant hybrids of bread wheat and *T. timopheevii* at sequential steps of selection, we have obtained

data about the presence of an alien genetic material in a number of studied forms and also about the heterogeneity of some samples.

Characterization of *Triticum aestivum* and *T. timopheevii* distant hybrids' progeny at sequential steps of selection using two PLUG markers

Sample	Selection step	PCR amplification profile
TNAC 1248 (3S)_TaqI		
♀Omskaya 36 × ♂L-6-7	Hybridization nursery	Absence of fragment characteristic of bread wheat
♀Trizo × ♂L-6-7	Hybridization nursery	Absence of fragment characteristic of bread wheat
♀L-6-6 × ♂Trizo	Selection nursery	Absence of fragment characteristic of bread wheat
♀Ivolga × ♂L-6-7	Preliminary cultivar estimation	Absence of fragment characteristic of bread wheat
TNAC 1421 (4AS-4BDL)_TaqI		
♀Omskaya 36 × ♂L-6-7	Hybridization nursery	Extra fragment characteristic of *T. timopheevii*
♀Trizo × ♂L-6-7	Hybridization nursery	Extra fragment characteristic of *T. timopheevii*
♀L-6-6 × ♂Trizo	Selection nursery	Extra fragment characteristic of *T. timopheevii*
♀Ivolga × ♂L-6-7	Preliminary cultivar estimation	Extra fragment characteristic of *T. timopheevii*
♀[(Zhnitsa × *T. timopheevii* K-47793) × Zhnitsa] × ♂Trizo	Variety testing	Extra fragment characteristic of *T. timopheevii*

Specific PLUG markers we used allowed three types of PCR product profiles. The first type matched that of bread wheat, which indicated the absence of introgressions. The second type was characterized by the absence of wheat-specific product. This may indicate a loss of certain chromosome site of bread wheat as a result of substitution by an alien fragment, which was not identified visually (possibly due to size matching with the wheat fragment). For the third type, the absence of the wheat fragment and the presence of the extra fragment of amplified alien introgression were characteristic.

In the earlier studies, the PLUG markers were used in wheat for identification chromatin of distant genetic species *Thinopyrum elongatum* [15], *Th. intermedium* [16], *Leymus mollis* [17], *Th. ponticum* [18]. We have shown for the first time a possibility to obtain distinctive profiles in such genetically close species as bread wheat and *T. timopheevii*.

Additional amplified fragments found in the studied samples and not matching to those present in the profiles of bread wheat could be associated with stability to brown rust and mildew. The PLUG markers selected by us and allowing for amplifying the specific fragments can be used for assessment of segregating populations in order to find an alien introgression associated with plant resistance. Earlier, we demonstrated the efficiency of such an approach using PLUG markers in studying 6Ai#2(6D) substitution which determines resistance to leaf rust in the Tulaykovskaya 10 bread wheat variety [16].

We have found forms both with single and multiple introgressions. The samples with multiple introgressions in several homeological groups are of special interest. Thus, L-6-6 has introgressions in homeological groups 1 and 3, L-6-7 — in homeological groups 1 and 4, L-25-20 and L-25-21 — in homeological groups 2 and 3, L-6-11 — in homeological groups 3, 6 and 7, L-6-14 — in homeological groups 3 and 6, L-6-2, L-6-16 and L-6-17 — in homeological groups 6 and 7. The markers we chose, when using the collection of nulli-tetrasomics as control, allow to locate single and multiple introgressions more precisely. Taking into account that PLUG markers are high conservative, one can thus expect that extra DNA fragments in the profiles testify to alien genetic material introgressed exactly from the homeological group of *T. timopheevii* to which the corresponding PLUG marker was developed.

Moreover, by PLUG markers, the heterogeneity could be studied, and the most stable lines not segregating by markers could be revealed. In particular, we showed the heterogeneity not only in the latter generations of lines selected from the L-6 and L-25 crossing combinations, but even in the line from the nursery of preliminary cultivar estimation, ♀L-6-6 × ♂Trizo that can be associated with secondary chasmogamy [20].

Thus, PLUG marking facilitates monitoring and a directed introgression of alien genetic material into the bread wheat genotypes. This is especially helpful for *Triticum timopheevii*, involving of which in wheat breeding is as valuable as complicated. As a whole, PLUG markers are helpful when working with distant hybrids as they are capable of identifying alien introgressions even in the absence of information about donor species, because these markers allow to conclude about the introgressions by comparing PCR product profiles in the hybrid form and in bread wheat.

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ESTIMATION AND SELECTION OF PARENTAL FORMS FOR BREEDING KAZAKHSTAN SALT TOLERANT RICE VARIETIES

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A b s t r a c t

About 25 % of the entire land surface is represented by saline soils, and up to 90 % of the total irrigated area — in some regions of Central Asia and the Caucasus, Ukraine and Kazakhstan. Rice-growing region of the Republic of Kazakhstan is also located in the area with high salinity. A challenge for rice growing in Kazakhstan is deteriorating humus and reclamation of soil, rise in soil secondary salinity and degradation. In Kyzylorda region, pollution of surface (up to 3-5 g/l) and ground water (up to 6-7 g/l) by salt residues reaches a critical point. The dominating sulfate-chloride-sodium type of salinity is especially toxic for crops. Akdalinski and Karatal zones of irrigation in the Almaty region are also located within the provinces of sulfate-soda and boric biogeo-chemical soil salinity. According the data of Kazakh Rice Research Institute, in recent years the rice yields and yield quality have sharply decreased — from 50 to 35-48 centners per hectare, and from 65 to 45-50 % of the groats output, respectively. In this regard, the rice breeding for salt tolerance is the most important to ensure food security in Kazakhstan. The objective of our study was the evaluation and selection a promising starting material of rice resistant to different types of salinity. To this end, we studied the rice (*Oryza sativa* L.) varieties, collection samples and their hybrid combinations of different generations derived from Russia, Kazakhstan and Philippine (34 genotypes in total). For the initial assessment at the seedling stage we used the laboratory screening for tolerance to various types of salinity, i.e. chloride, sulphate and carbonate. Saline stress negatively affected seedling growth and total weight in the studied samples. It was revealed that the carbonate type of salinity is the most toxic for rice plants; the chloride and sulfate types were less adverse. The hybrid collection sample F_2 Khankai 429 × 4-09 and as well as varieties Marzhan and Madina accumulated the highest percentage of biomass compared to the control at the salinity of all three types. Therefore, these genotypes are valuable in selection for salt tolerance. Molecular screening of chromosomal DNA regions linked to quantitative trait loci of salt tolerance (*Saltol* QTL) by PCR with microsatellite markers closely linked to the target chromosomal regions, RM 493 and AP 3206, showed RM 493 to produce polymorphism which allowed to distinguish the studied genotypes contrasting in salt tolerance. Thus RM 493 is informative to rank the rice genetic plasma by salt tolerance.

Keywords: *Oryza sativa* L., rice, chloride salinity, sulfate salinization, carbonate salinity, molecular screening for salt tolerance, SSR markers, selection

About 950 million hectares of agricultural lands in the world are saline,

and 77 million hectares are irrigated by saline water. According to forecasts, salinity will affect over 50 % of cultivated areas by 2050 [1]. In Russia, saline soils occupy 15 million hectares, including 196.4 thous. ha in Krasnodar Krai, of which 76.4 thous. ha are irrigated areas, and over 85 % of them are in rice irrigation systems.

Soil salinity of more than 0.35 % kills rice plants. One of serious challenges for rice growing in the Republic of Kazakhstan is deteriorating humus and reclamation conditions of soil, rise in soil secondary salinity and degradation. Currently, the area of saline and alkaline soils is 111.6 million hectares, or 41.0 % of the entire territory of the republic. In Kyzylorda region, pollution of surface (up to 3-5 g/l) and ground water (up to 6-7 g/l) by salt residues reaches a critical point. In the most (30 %) irrigated rice-planting areas, groundwater salinity reaches 5-10 g/l. As a consequence, the crop yields there decrease by 20-50 % [2]. By chemical composition, sulfate-chloride-sodium type of salinity dominates there, which is especially toxic for agricultural plants. Akdala and Karatal zones of irrigation in Almaty region (another important rice-planting region of Kazakhstan) are also located within the provinces of sulfate-soda and boric biogeochemical soil salinity. Excess amounts of water-soluble salts in soil cause thinning of seedlings, retards plant growth and development. According to the data of Kazakh Rice Research Institute, in recent years it has resulted in sharp decrease in rice yields (from 50 to 35-48 c/ha) and rice quality (groats output has reduced from 65 to 45-50 %) [3].

Nowadays, the leading rice varieties cultivated in Kazakhstan cannot meet the increased needs of the processing and food industry because of expansion of re-saline soil areas. The yield decrease in saline soils may be overcome by soil reclamation and increasing rice tolerance to salinity. Rice selection for the leading rice-planting regions of the Republic of Kazakhstan (Kyzylorda and Almaty regions) shall be aimed at creation of varieties tolerant to salinity, resistant to diseases and pests, having high groats productivity and quality. Introducing the salt tolerant varieties into agricultural practices is a cost-effective method and a primary objective for the modern rice growing in Kazakhstan.

The ability of plants to survive and complete a reproduction cycle in conditions where there are salt concentrations unfavorable for the most of other species is commonly called salt tolerance. According to B.P. Strogonov, there are biological and agronomical types of salt tolerance [4, 5]. Salt tolerance is a polygenic trait manifested in a number of various physiological responses affecting plant metabolism and occurring at different levels [6]. There are direct and indirect methods for estimation of plant salt tolerance which involve the assessment of yields and productivity, seed germination rate, cell plasmolysis intensity, chlorophyll bleaching in leaves of seedlings placed into saline solutions [7].

It is expedient to estimate salt tolerance by the agricultural resistance, i.e. by plant ability to keep yields typical for nonsaline conditions when grown in saline soil. Resistance to soil salinity varies in vegetation stages; there are two critical phases in the ontogenesis of rice plants: during germination—formation of seedlings and blooming [8-11].

In this work, we for the first time have conducted screening of the Kazakhstan rice lines for salt tolerance at the early stage of vegetation and their molecular analysis using microsatellite markers.

The objective of our study was to evaluate and select a promising starting material for breeding rice for tolerance to different types of salinity.

Technique. The following rice (*Oryza sativa* L.) varieties and collection samples (including their hybrid combinations of different generations) were the subjects of the study: Liman, Regul, Dariy 23, Sonata, VNIIR10173, Slavyanets,

Khankai 429, Fisht, Yantar, Rapan, Kuban 3, Atlant, Serpantin, Kurchanka varieties and collection samples 4-09 and 49-09 derived from Russia; Madina, Analog II, Bakanassky, Marzhan varieties, collection sample 34-09, and F_2 Sonata × Liman, F_2 Regul × Kurchanka, F_2 Dariy 23 × collection sample 49-09, F_2 Khankai 429 × collection sample 4-09, F_2 Marzhan × Kurchanka, F_2 Bakanassky × Analog II, F_2 Dariy 23 × Analog II, F_2 Kuban 3 × collection sample 34-09, F_2 Khankai 429 × Kurchanka hybrid lines derived from Kazakhstan; FL 478 HB 9093, BINA dhan 8 HB 9106, BRRI dhan 47 HB 9114 varieties derived from Philippines. The varieties of Marzhan (Kazakh salt tolerant standard), Kurchanka (Russian salt tolerant standard), FL 478 HB9093, BINA dhan 8 HB9106, BRRI dhan 47 HB9114 (salt tolerant samples obtained from the International Rice Research Institute — IRRI, the Philippines) were used as a positive control in all experiments.

Phenological observations were made by P.S. Yerygin [12].

The varieties, collection samples and hybrid lines were screened for salt tolerance at the germination stage under laboratory conditions according to the procedure of All-Russian Rice Research Institute [13]. For that, the seeds were sprouted in a thermostat at 29 °C for 4 days under salinity stress (0.75 % NaCl, 0.75 % Na_2SO_4, 0.075 % Na_2CO_3) (in 3 replicates). On day 5, 20 typical germs of each sample were taken and weighted to 0.01 g.

Molecular screening of chromosomal DNA regions linked to QTL (quantitative trait loci) of salt tolerance (*Saltol* QTL) was carried out by PCR analysis in F_3 and F_4 hybrid combinations. DNA was extracted by the CTAB method using 7-day old non-chlorophyllic seedlings [14]. Microsatellite markers closely linked to the target chromosomal regions, RM 493 and AP 3206, were applied [15]. The PCR was conducted using 40-50 ng of DNA in a final volume of 25 μl. The reaction mixture was composed of 0.05 mM of dNTPs, 0.3 mM of each primer, 25 mM of KCl, 60 mM of Tris-HCl (pH 8.5), 0.1 % Triton X-100, 10 mM of 2-mercapto-ethanol, 1.5 mM of $MgCl_2$, 1 unit of Taq-polymerase (New England Biolabs, Inc., USA). DNA amplification was made as follows: initial denaturation for 4 min at 94 °C; denaturation for 1 min at 94 °C, annealing of primers for 1 min at 55 °C, elongation for 1 min at 72 °C (30 cycles); last cycle of synthesis for 5 min at 72 °C. Primer pairs for the markers concerned were synthesized by Sintol (Moscow). Nucleotide sequences of primer pairs for RM493 were TAGCTCCAACAGGATCGACC (5′→3′), GTACGTAAACGCG-GAAGGTG (5′→3′); and for AP3206 were GGAGGAGGAGAGGAAGAAG (5′→3′), GCAAGAATTAATCCATGTGAAAGA (5′→3′). The PCR was carried out in a Tertsik DNA amplifier (NPO DNA Technology, Russia). Polyacrylamide gel (8 %) based on 1× TBE (Tris-HCl — 0.09 M, boric acid — 0.09 M, EDTA — 2 mM, pH 8.2) was used for electrophoretic separation of the PCR products. Visualisation was performed under the UV-light after gels had been colored by ethydium bromide.

The data obtained were processed in Microsoft Excel. The table presents arithmetical mean values (*M*) and errors of mean (±*m*).

Results. The salinity stress negatively affected the gain in total biomass of seedlings in the samples studied (see the Table). The maximum (as a percentage compared to the control) gain in biomass in the conditions of chloride salinity was observed for the varieties derived from Kazakhstan, Bakanassky and Marzhan; medium vales — for the varieties derived from Russia, Rapan, Yantar, Fisht, Sonata and Khankai 429. The minimum gain in the total biomass was noted in IRRI varieties: BINA dhan 8HB9106, FL 478 HB9093, BRRI dhan 47 HB9114, although they were considered to be standards for salt tolerance. Probably, salt tolerance in these samples is expressed at later stages of ontogenesis.

Screening of rice (*Oryza sativa* L.) samples by the total biomass of seedlings compared to the control (%) **at different types of salinity** (laboratory experiment, $M\pm m$)

Variety, sample, hybrid	Salinity		
	chloride (0.75 %)	sulfate (0.75 %)	carbonate (0.075 %)
BINA dhan 8 HB9106	45.45±2.73	87.61±16.15	61.50±10.45
BRRI dhan 47 HB9114	46.19±4.89	87.01±14.19	74.43±8.15
FL 478 HB9093	47.75±4.86	79.47±5.77	61.97±6.66
Akdala	74.98±8.25	66.31±6.22	59.65±6.46
Analog II	73.37±6.01	97.02±19.46	61.78±8.39
Atlant	75.26±4.91	75.36±11.62	66.90±10.71
Bakanassky	81.97±16.42	65.39±8.75	75.30±15.28
VNIIR10173	73.24±9.69	69.71±8.85	63.00±32.50
Dariy 23	71.72±12.26	82.33±13.51	62.10±5.61
Collection sample 34-09	75.86±6.33	67.25±7.94	73.68±5.76
Collection sample 4-09	61.93±4.31	87.47±22.14	54.80±9.58
Collection sample 49-09	70.50±3.09	71.11±5.56	74.31±6.07
Kuban 3	79.29±6.50	82.67±14.44	68.86±9.06
Kurchanka	69.79±4.59	74.02±12.02	67.16±4.46
Liman	65.03±8.19	70.50±7.49	45.11±5.37
Madina	85.64±5.02	78.41±10.95	74.29±7.11
Marzhan	80.90±15.55	78.90±7.37	76.04±6.65
Rapan	71.15±14.91	69.10±20.49	73.03±17.24
Regul	70.36±12.61	98.36±10.36	75.15±13.77
Serpantin	81.42±5.14	79.67±8.15	60.15±7.11
Slavyanets	80.77±11.44	65.10±3.52	58.44±8.88
Sonata	70.23±6.56	79.50±7.02	55.62±3.15
Fisht	77.40±8.23	83.97±8.76	72.83±3.84
Khankai 429	66.13±6.17	75.39±4.12	58.25±6.59
Yantar	72.97±12.66	78.07±16.28	57.29±4.06
F_2 Bakanassky × Analog II	78.01±8.05	71.90±6.78	70.23±10.10
F_2 Dariy × Analog II	73.08±9.68	74.00±6.78	62.84±3.97
F_2 Dariy 23 × collection sample 49-09	67.34±6.05	81.03±8.43	77.06±7.07
F_2 Kuban 3 × collection sample 34-09	89.48±7.44	71.73±7.04	72.26±4.63
F_2 Marzhan × Kurchanka	68.37±11.37	71.37±6.41	67.76±10.91
F_2 Regul × Kurchanka	70.63±7.46	58.79±5.36	69.03±4.33
F_2 Sonata × Liman	69.78±7.75	77.56±6.15	69.64±8.21
F_2 Khankai 429 × Kurchanka	80.56±10.36	72.60±6.25	67.88±7.63
F_2 Khankai 429 × collection sample 4-09	70.71±6.69	96.74±31.91	80.04±4.73

By their tolerance to sulfate salinity, the tested genotypes were divided into three groups: high tolerance genotypes (76-100 %), medium tolerance genotypes (70-75%), and low tolerance genotypes (0-69 %). The high tolerance was observed in the varieties derived from Kazakhstan, Marzhan (78 %) and Madina (77%); in varieties obtained from the IRRI, FL 478 HB 9093 (79 %), BRRI dhan 47 HB 9114 (85 %), and BINA dhan 8 HB 9106 (87 %); and also in Russian varieties, Kuban 3 (82 %), Fisht (82 %), Sonata (80 %), Atlant (93 %), Regul (97 %), Dariy 23 × collection sample 49-09 (80 %), Khankai 429 × collection sample 4-09 (91 %). The low tolerance to this selective factor was noted for seedlings of varieties and hybrids Bakanassky, Rapan, Slavyanets, Akdala, VNIIR10173, F_2 Regul × Kurchanka, and collection sample 34-09.

For sodium carbonate salinity, more damaging impact on the absorption capacity of rice roots is characteristic as compared to the chloride and sulfate ones [16]. In our experiments, the varieties derived from All-Russian Rice Research Institute, i.e. Liman, Sonata, Yantar, Slavyanets, Khankai 429, VNIIR10173, and Serpantin, were the most sensitive to carbonate salinity. The maximum gain in biomass was characteristic of Marzhan, Regul, Madina, F_2 Dariy 23 × collection sample 49-09, and F_2 Khankai 429 × collection sample 4-09.

There are various theories which explain the inhibition of plants in saline conditions. According to one of theories, the phenomenon is defined by osmotic influence of saline solutions, while the other one says that the plant growth and development are inhibited as a result of toxic effects of absorbed ions on physio-

logical and biochemical processes [17]. In our experiments, carbonate salinity was the most toxic, while chloride and sulfate types of salinity were less adverse.
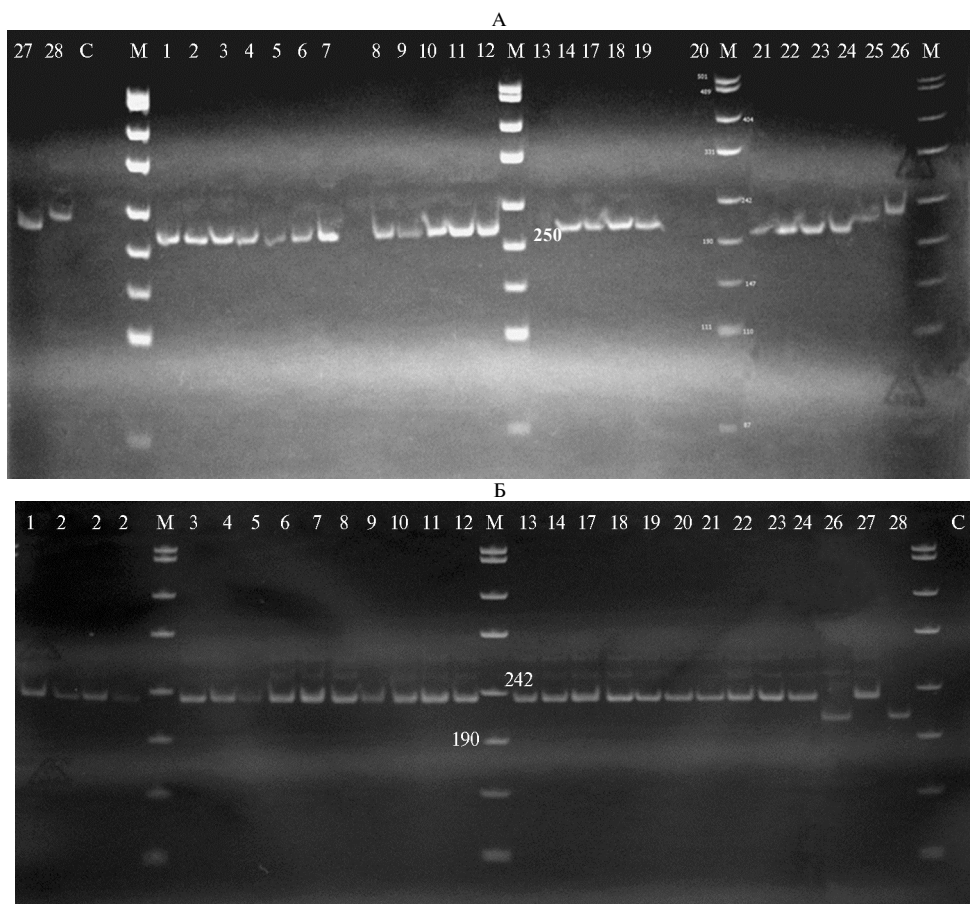
Microsatellite profiles in rice (*Oryza sativa* L.) samples derived from Russia, Kazakhstan and global selection for RM 493 (A) **and AP3206 loci** (B)**:** 1 — Marzhan, 2 — Kurchanka, 3 — F_4 Marzhan × Kurchanka var. *dichroa*; 4 — F_4 Marzhan × Kurchanka var. *zeravschanica*, 5 — F_4 Marzhan × Kurchanka var. *italica*, 6 — F_4 Marzhan × Kurchanka var. *vulgaris*, 7 — Regul, 8 — F_3 Regul × Kurchanka var. *italica*, 9 — F_3 Regul × Kurchanka var. *zeravschanica*, 10 — F_3 Regul × Kurchanka var. *dichroa*, 11 — Sonata, 12 — Liman, 13 — F_4 Sonata × Liman var. *italica*, 14 — F_4 Sonata × Liman var. *nigroapiculata*, 17 — F_3 Kuban 3 × collection sample 34-09 var. *zeravschanica*, 18 — Khankai 429, 19 — F_4 Khankai 429 × Kurchanka var. *dichroa*, 20 — Bakanassky, 21 — Analog II, 22 — F_4 Bakanassky × Analog II var. *nigroapiculata*, 23 — Dariy 23, 24 — F_3 Dariy 23 × Analog II var. *italica*, 25 — FL 478 HB9093, 26 — BINA dhan8 HB9106, 27 — BRRI dhan47 HB9114, 28 — Madina, C — control (H_2O); M — molecular mass marker (bp) 100 bp (Sintol, Moscow).

For the PCR analysis of DNA (see the Figure), we used microsatellite markers RM 493 and AP 3206 defined inside of *Saltol* region in chromosome 1. Comparison of microsatellite profiles in the samples tested allows for the conclusion about the presence of three alleles in RM 493 locus: the first one was found in BINA dhan8 HB9106 genotypes (salt tolerant standard, IRRI) and Madina variety derived from Kazakhstan; the second one — in FL 478 HB9093 and BRRI dhan 47 HB9114 genotypes (salt tolerant standards, IRRI); and the third one — in all other samples (see the Figure). It is notable that the latter also includes salt tolerant standards, Marzhan variety derived from Kazakhstan and Kurchanka variety derived from Russia. Salt tolerance of these rice genotypes of Russian and Kazakhstan origin was more likely defined by other QTL.

The use of AP 3206 made it possible to reveal polymorphism, but this

microsatellite marker appeared to be less informative as compared to RM 493 (see the Figure). Profiles of PCR products were similar almost in all samples studied, except for two ones — BINA dhan8 HB9106 (path 26) and Madina (path 28), that provided evidence of existence of two salt tolerance alleles for AP 3206 locus.

Marker-assisted selection (MAS) widely used now [18], including for increase of salt tolerance in plants, has enabled to make great progress as compared to traditional methods [19-22]. It helps significantly cut time required for creation of new genotypes, assess the purity and identity of varieties, study the genetic diversity of modern varieties, define genes and quantitative trait loci (QTL), reveal markers closely linked to genes and QTL of target traits, monitor types of crossings and introgression of genes and QTL in different MAS patterns, and implement gene stacking [23, 24].

Salt tolerance in rice is controlled by several independent loci responsible for the trait formation at different stages of ontogenesis. The key loci, among others, are *SKC1* and *Saltol. Saltol* belongs to QTL and has been defined in chromosome 1 of salt tolerant Pokkali rice [25, 26]. A technology of molecular genetic screening for salt tolerance enables to immediately select forms of plants having target genes and create varieties with predefined properties and without using challenges [27, 28]. According to literature data, introduction of *Saltol* QTL into highly productive varieties stabilizes the rice yields in saline soils [29]. Currently, we have already carried out hybridization among Bakanassky, Marzhan, Madina, Kuban 3 varieties and BINA dhan 8HB 9106, FL 478 HB 9093, BRRI dhan 47 HB 9114 salt tolerance donors for the purpose of *Saltol* QTL introgression into rice varieties grown in Kazakhstan (no data provided). The next stages in the selection pattern will be a series of backcrossings with Kazakh varieties as recipient forms and further testing of the obtained lines under field conditions, DNA identification of *Saltol*-loci in the lines and creating salt tolerant Kazakh rice varieties based on them.

It has been reported that polymorphic SSR markers linked to QTL of salt tolerance, such as RM 490, RM 1287, RM 10694, AP 3206, AP 3206f, RM 8094, RM 3412b, RM 10748, RM 493, RM 10793, SalT 1, RM 562, RM 7075, are the most promising in the molecular genetic analysis of target DNA regions [30]. In our studies, microsatellite marker RM 493 has appeared to be informative to rank the rice genetic plasma for the presence of *Saltol* locus of salt tolerance.

Thus, the studied rice genotypes of different origin have shown heterogeneity in tolerance to carbonate, chloride and sulfate types of salinity during screening at the seedling stage (the carbonate salinity appeared to be the most toxic). In the molecular genetic analysis of samples contrasting in salt tolerance, polymorphism has been revealed in one of the two microsatellite markers used, i.e. RM 493 defined inside of *Saltol* region. Three alleles of this microsatellite locus have been found in the genotypes under study; one of them is characteristic of the most genotypes studied, the second one has been identified in two varieties derived from the Philippines, and the last one has been found in one Philippine and one Kazakh varieties.

So, the screening of 34 genotypes of rice (varieties, collection samples derived from Russia, Kazakhstan and the Philippines, and their different hybrid combinations) has revealed the most promising salt tolerant varieties and hybrids which may be grown in saline soils as well as used as a starting material for breeding higher salt tolerant forms. Hybrid F_2 Khankai 429 × collection sample 4-09 as well as Marzhan and Madina varieties gained the maximum percentage biomass in three types of salinity (chloride, sulfate and carbonate). Hence, these genotypes are of value as a starting material for breeding programs aimed at improving salt

tolerance. To monitor the introgression of *Saltol* locus, polymorphic microsatellite marker RM 493 may be used, which appeared to be the most informative during the analysis of the genotypes contrasting in the studied trait.

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BIOLOGICAL AND AGRONOMICAL ASSESSMENT OF WHEAT LANDRACES CULTIVATED IN MOUNTAIN AREAS OF UZBEKISTAN

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A b s t r a c t

Wheat cultivation for thousands of years under extreme environment of the Central Asia resulted in local wheat cultivars adapted to local soil and climatic conditions. In the past, main wheat production in Uzbekistan was concentrated on dry-farming land of piedmont and mountain regions where precipitation and warm were sufficient as minimal conditions for stable wheat cultivation. Expansion of area under cultivation of intensive high productive commercial wheat cultivars brought decreasing of areas on which wheat landraces had been cultivated. However, wheat landraces were being kept by local farmers in remote districts where no intensive cultivars had been spread widely. Employment of valuable genetic resources of locally originated cultivars is important in plant breeding programs. The aim of current study is to characterize landraces of winter wheat of Uzbekistan by morphological and quality traits (e.g. plant heights, yielding, etc.) with geo-information data on the points of landraces origin, and also to analyze elements of crop productivity. Wheat landraces such as Kzyl-bugday, Ak-bugday, Tyuya-Tish, unnamed landrace and also Surhak (created from local landrace) were used in this study. Landraces differed from commercial cultivars by morphological traits such as plant height, length and density of spike. Selection in the region was carried out by morphological traits. Using GPS-navigation during expedition to survey wheat landraces, a spreading of the studied landraces was mapped. Collected samples of wheat landraces were cultivated during four sessions. Commercial Krasnodarskay 99 served as a control cultivar for comparison. Result of statistical analysis of fifteen lines, i.e. Kzyl-bugday 2-6T, Kzyl-bugday 3-7T, Kzyl-bugday 9-4T, Kzyl-bugday 5-4T, Kzyl-bugday 11-4T, Kzyl-bugday 1-4T, Ak-bugday 8-2T, Ak-bugday 7-3T, Ak-bugday 16-5T, Surhak 12-3T, Surhak 15-6T, Tyuya-Tish 10-4T, 13-8T (unnamed), 14-3T (unnamed), showed significant difference in plant height compared to the control cultivar. Although the average productivity of studied landraces did not differ from that in the control variety, two landraces, Tyuya-Tish 10-4T and Kzyl-bugday 1-4T, differed from the control cultivar in productivity. The weight of 1000 kernel was higher in nine landraces, Kzyl-bugday 2-6T, Kzyl-bugday 3-7T, Kzyl-bugday 9-4T, Kzyl-bugday 5-4T, Surhak 12-3T, Kzyl-bugday 1-4T, 14-3T (unnamed), Ak-bugday 16-5T, Surhak 15-6T, compared to the control cultivar. Gluten content was significantly higher in Kzyl-bugday 9-4T, Ak-bugday 8-2T, Ak-bugday 16-5T and 13-8T (unnamed) landraces as compared to Krasnodarskaya 99 cultivar, while medium gluten content was characteristic of other landraces.

Keywords: wheat landraces, morphological analysis, quantitative traits, gluten content

The selection of the majority of agricultural crops faces a serious problem of their extremely sparse genetic diversity. The problem solution is associated with attracting the genetic material of wild relatives and related species [1, 2], i.e. overcoming the primary gene pool erosion at the expense of the secondary one included in it. However, the drastic reduction in native habitats of such potential donor species and their polymorphism restriction as a result of keeping insufficiently representative by their composition and small by their size popula-

tions in gene banks lead to the secondary gene pool loss that limits inevitably the biological diversity expandability for the species cultivated [3-6].

A great number of modern cultivars of wheat (like other crops) are often genetically similar and have a rather limited genetic base [7, 8]. The landraces originated from the natural selection in conjunction with the performance selection carried out by farmers [9-11] have generally wider genetic base that can provide characteristics important for the selection [12]. Such landraces are characterized by their tolerance to local stress factors [13, 14], stable yielding, wider intravariety genetic diversity by protein content and some other grain qualities as compared to existing commercial cultivars [15]. Therefore, they represent a valuable portion of the gene pool [13, 15] and shall be involved in breeding new commercial cultivars. Furthermore, traditional varieties-populations not subjected to scientific breeding are of interest for studying plant genetics, physiology and ecology as scientific selection bases [16-18]. Commercial cultivars have completely replaced the traditional landraces in the present-day grain production, therefore, the populations of the latter are of particular value in areas where local landraces are still cultivated, i.e. kept and evolve in natural conditions until the present. In particular, such unique areas include mountain regions of Uzbekistan.

We are the first who has analyzed the distribution of and traits significant for selection in 30 main landraces of wheat gathered in traditionally cultivated areas of mountainous Uzbekistan, and identified samples possessing high baking and taste qualities which are genetically determined and can be introduced to the modern commercial cultivars.

The aim of the study is to describe soft wheat landraces of Uzbekistan by their quantitative and qualitative traits, carry out an analysis of yield components, as well as to show the distribution and crop areas of the landraces, including geoinformation data on cultivated locations.

Technique. The subjects of the study were soft spring wheat landraces such as Kzyl-bugday, Ak-bugday (Graecum), Tyuya-Tish, Pashmak, Khivit, Boboki, Muslimka, Kairaktash, Kzyl-Shark, unnamed landrace, and also Surkhak (bred from a local landrace). Samples were taken during an expedition in 2010 and 2013 in villages (kishlaks) of different districts in three regions.

The samples were cultivated during four seasons (2011-2012, 2012-2013, 2013-2014 and 2014-2015), under irrigation, on the experimental base of the Institute of Genetics and Experimental Biology of Plants of the Academy of Science of Uzbekistan (Tashkent region) in accordance with the described winter wheat cultivation technology [19]. In the first, third and fourth seasons the samples were sown in the III decade of October, while in the second season they were sown in spring. For that, the agricultural practice without vegetative irrigation generally accepted in the study area was used. Ammophos (200 kg/ha) was applied to soil before sowing. The samples were sown by hand. Each sample was planted in 3 replicates randomized throughout a 1 m^2 plot (with row spacing of 15 cm, row length of 1 m, plot spacing of 30 cm). Commercial Krasnodarskaya 99 wheat cultivar was used as a control.

Agronomical estimate and recording of major yield structure components were conducted by conventional methods [20]. Gluten content was determined according to State Standard GOST 13586.1-68 (Grain. Methods for determination of gluten quantity and quality in wheat).

Statistical data analysis was carried out using the Student's *t*-test (Stat-View 5.0, SAS Institute, Inc., USA) (the analysis did not include data of 2012-2013 season). Tables contain mean values (*M*) and significance levels of differences (p).

Results. Locations of primary cultivation, area planted, and spreading of the studied landraces were identified during the field research using a GPS-navigator (Table 1).

1. Geographical location and description of traditional wheat landraces sampled

Latitude, longitude; elevation, m	Cultivar	Sowing time	Pure line or impurity (%) and impurity type in a mixture	Grain color	Plant height, cm
Y i e l d s o f 2 0 1 0					
S u r k h a n d a r y i n s k a y a r e g i o n					
Baisunsky District					
Duoba Kishlak					
38°32′121″N, 67°38′181″E; 1391	Kzyl-bugday	March	Mixture, 2 %, various	Red	120
38°32′480″N, 67°36′566″E; 1544	Boboki	March	Pure line	Red	120
38°32′025″N, 67°36′594″E; 1431	Kzyl-bugday	March	Pure line	Red	130
Kurgancha Kishlak					
38°37′921″N, 67°41′462″E; 1633	Kzyl-bugday	March	Mixture, 5 %, having red ears	Red	120
Gumatak Kishlak					
38°35′699″N, 67°37′737″E; 2136	Kzyl-bugday	March	Pure line	Red	130
38°35′986″N, 67°07′070″E; 2174	Kzyl-bugday	March	Mixture, 5 %, barley	Red	130
38°35′057″N, 67°42′538″E; 2143	Kzyl-bugday	March	Pure line	Red	100
Pulkhokim Kishlak					
38°16′484″N, 67°38′905″E; 1050	Boboki	October	Pure line	Red	140
K a s h k a d a r y i n s k a y a r e g i o n					
Yakkabagsky District					
Guldara Kishlak					
38°78′582″N, 66°81′014″E; 1159	Ak-bugday	March	Pure line	White	120
38°77′369″N, 66°82′451″E; 1270	Graecum	March	Mixture, 20 %, Surkhak landrace	White	120
Terakli Kishlak					
38°75′540″N, 66°81′783″E; 1634	Surkhak	November	Pure line	Red	130
38°75′934″N, 66°82′558″E; 1500	Ak-bugday	November	Pure line	White	120
Kamashinsky District					
Kuga Kishlak					
38°66′376″N, 66°92′626″E; 2249	Ak-bugday	March	Pure line	White	120
38°63′243″E, 66°94′461″E; 1988	Ak-bugday	March	Pure line	White	90
38°64′701″N, 66°93′114″E; 1731	Kzyl-bugday	March	Pure line	Red	120
Kzyltom Kishlak					
38°61′663″N, 66°93′731″E; 1753	Tyuya-Tish	March	Mixture, 15 %, having red ears	Red	120
38°66′376″N, 66°92′626″E; 2249	Unnamed	March	Mixture, 20 %, having red ears	Red	100
38°65′243″N, 66°90′205″E; 2147	Unnamed	March	Mixture, 20 %, various	Red	100
38°59′266″N, 66°91′480″E; 1317	Ak-bugday	March	Pure line	White	130
D z h i z a k s k a y a r e g i o n					
Bakhmalsky District					
Muzbulak Kishlak					
39°71′376″N, 68°12′882″E; 1520	Ak-bugday	October	Pure line	White	115
Zartepa Kishlak					
39°70′017″N, 68°19′329″E; 1763	Surkhak	March	Mixture, 10 %, barley	Red	120
Gallyaaralsky District					
Yonbosh Kishlak					
40°12′471″N, 67°41′983″E; 1449	Ak-bugday	November	Pure line	White	130
Lalmikor Kishlak					
39°93′540″N, 67°45′574″E; 740	Ak-bugday	November	Mixture, 5 %, barley	White	110

Table 1 (continued)

Y i e l d s   o f   2 0 1 3

S u r k h a n d a r y i n s k a y a   r e g i o n

Saryasiysky District

38°61′500″N, 67°58′411″E; 2008	Pashmak	October	Pure line	White	90
38°60′202″N, 67°56′589″E; 1650	Khivit	August	Pure line	White	90
38°57′685″N, 67°58′622″E; 1558	Kzyl-bugday	March	Pure line	Red	110

Altynsaisky District

| 38°33′086″N, 67°65′667″E; 1301 | Graecum | October | Pure line | White | 105 |

Uzunsky District

38°31′318″N, 67°04′989″N; 1289	Muslimka	October	Mixture, 20 %, various	Red	110
38°58′531″N, 67°57′554″E; 1615	Kairaktash	March	Mixture, 20 %, various	White	100
38°49′779″N, 67°69′071″E; 957	Kzyl-Shark	October	Mixture, 10 %, various	Red	110
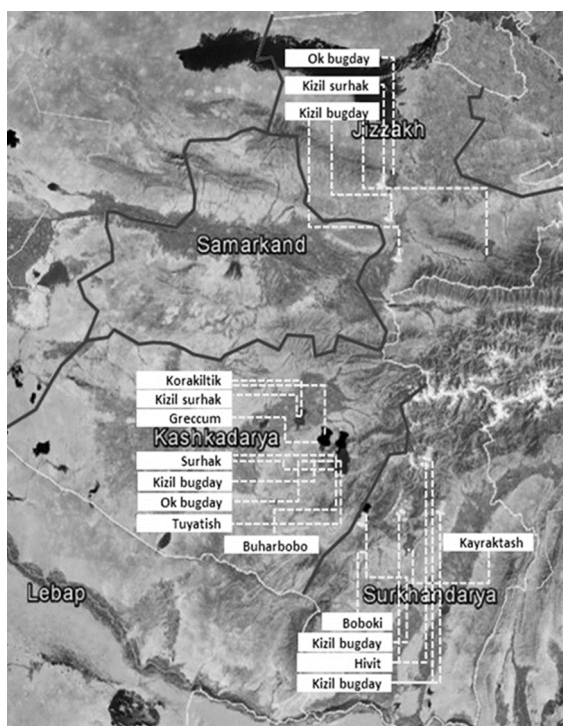
Distribution of traditional spring wheat landraces in Uzbekistan.

Based on this and numerous polls of the local population, a distribution map of traditional landraces in Uzbekistan has been compiled (see the Figure).

Fifteen lines characterizing the landraces by homogeneity and yields have been selected for 3-year trial plot tests.

In some areas of Uzbekistan where spring wheat is spread, the commercial cultivar Surkhak is grown (it was selected from a traditional landrace in 1940s). The cultivar is relatively resistant to drought, high temperature and shattering (hardly threshed), and nonresistant to lodging. The height of plants is 110-140 cm. Its ear with white glabrous glumes and red grains corresponds to *erythrospermum* species. It has large kernels (1000 seeds weight up to 50 g). The cultivar is characterized by poor winter resistance, but in Uzbekistan lives through winters well. Its vegetation period lasts for 210-240 days. Baking qualities are good, crop yields under optimal conditions reach 3 t/ha. Many crops of the cultivar are extremely unhomogeneous and weedy. Generally, it is grown on large cooperative farms and many neighboring small private farms.

The traditional soft wheat landrace Kzyl-bugday is cultivated on small farms in remote areas at the elevation of 1500-2500 m above sea level. The landrace belongs to *erythrospermum* variety as well. Its plants are rather high (120-140 cm) and highly productive (its yield under optimal conditions reaches 3.5 t/ha). It has red, large kernels; the weight of 1000 seeds is 55-60 g. The ears are long, up to 14 cm, with medium awns.

The soft wheat landrace Ak-bugday (known as Graecum in some areas) has white glumes, white awns and relatively large white grain (1000 seeds weight

45-48 g) that corresponds to characteristics of *graecum* variety. Its plants are 95-100 cm high, and do not lodge on bogharic lands. It is grown mainly in two are-as (Kashkadaryinskaya and Dzhizakskaya regions). The landrace keeps well, is relatively homogeneous morphologically, and possesses good baking qualities. Its yield reaches up to 3 t/ha. Its straw is very soft, highly respected as a building material for interior finish in houses, as well as is used as fodder for livestock.

The Boboki landrace that is morphologically similar to Kzyl-bugday and referred to *erythrospermum* variety is grown mainly in mountain areas of southern provinces of Uzbekistan. Its plants are very high (150-160 cm) with filled stems, they do not lodge in boghara conditions. It is distinguished among traditional landraces by its morphological homogeneity, good quality and high crop yields.

The wheat of Kairaktash landrace (*graecum* variety) is relatively rare. Its plants are of medium height, resistant to lodging even in irrigation conditions. It possesses excellent baking qualities.

The traditional wheat of Tyuya-Tish landrace is very rare. According to old residents, this landrace was highly productive, had rough grains, possessed good baking qualities, however, it was sown over small areas, primarily, in private households. Currently, it differs from earlier descriptions and corresponds to *erythrospermum* variety. The landrace plants are high and have large kernels.

Also, there were several unnamed samples (probably, they did not attract attention of peasants or the latter bred the plants they like for their own needs without giving names).

2. Plant stand height and yield in lines of traditional wheat landraces by years of studies (Republic of Uzbekistan)

Line	Plant stand height, cm					Yields, g/m²				
	2012	2014	2015	*M*	p	2012	2014	2015	*M*	p
Krasnodarskaya 99	70	79	77	75.33		450	568	655	558	
Kzyl-bugday 2-6T	115	124	92	110.33	0.0242	432	601	260	431	0.3324
Kzyl-bugday 3-7T	110	139	100	116.33	0.0269	482	673	320	492	0.6059
Kzyl-bugday 9-4T	115	113	111	113.00	0.0002	400	580	360	447	0.2851
Kzyl-bugday 5-4T	110	110	107	109.00	0.0003	454	576	331	454	0.3232
Kzyl-bugday 11-4T	115	113	112	113.33	0.0002	447	462	250	386	0.1313
Ak-bugday 7-3T	90	110	106	102.00	0.0163	287	446	370	368	0.0646
Surkhak 12-3T	105	120	106	110.33	0.0032	390	538	350	426	0.1855
Ak-bugday 8-2T	90	119	105	104.67	0.0291	448	426	313	396	0.0896
Tyuya-Tish 10-4T	115	130	119	121.33	0.0009	388	405	385	393	0.0500
Kzyl-bugday 1-4T	90	126	115	110.33	0.0334	421	325	310	352	0.0404
13-8T (unnamed)	110	116	107	111.00	0.0007	272	492	432	399	0.1470
14-3T (unnamed)	117	129	115	120.33	0.0009	440	417	458	438	0.1202
Ak-bugday 16-5T	105	104	105	104.67	0.0004	417	416	471	435	0.1188
Surkhak 15-6T	110	118	110	112.67	0.0006	391	440	385	405	0.0696

N o t e. *M* — arithmetical mean, p — significance level by the Student's *t*-test.

Results of the statistical analysis (Table 2) showed significant difference of all studied 15 lines of traditional wheat landraces from the control cultivar in the plant height, and all the lines were relatively tall-growing. The average yield analysis has shown that in the traditional landraces it was lesser as compared to the control cultivar (Krasnodarskaya 99 cultivar). However, it should be noted that the differences were insignificant, and two lines only (Tyuya-Tish 10-4T and Kzyl-bugday 1-4T) had statistically significant differences from the control cultivar.

The traditional wheat landraces can show high productivity depending on the conditions observed in the relevant year and cultivated location. So, the Kzyl-bugday 3-7T yield in 2014 reached 673 g/m² that exceeded the maximum yield of Krasnodarskaya 99 cultivar (655 g/m²) for the period of 2012 through 2015. It should be noted that deep spring frosts in 2015 caused considerable decrease in the yield of the most traditional landraces, while the Krasnodarskaya

99 plants were not affected negatively by them. Since the commercial Krasnodarskaya 99 cultivar is characterized by the high yield stability, its average yields have exceeded that of the traditional wheat landraces.

Based on the comparison of the weight of 1000 seeds, it was found out that in nine lines it was higher than in the control cultivar, but the differences were not significant (Table 3). All the lines of traditional landraces had a high content of gluten. It should also be noted that Kzyl-bugday 9-4T, Ak-bugday 8-2T, Ak-bugday 16-5T and 13-8T (unnamed) lines differed significantly from the control cultivar in this trait (see Table 3). All the traditional wheat landraces grown in the mountain and piedmont areas of Uzbekistan were characterized not only by the relatively high content of gluten, but also good baking properties and great taste qualities as compared to the commercial cultivars grown in Uzbekistan. That is one of the reasons why the traditional landraces have been kept and cultivated in the areas concerned for many years.

3. Weight of 1000 seeds and gluten content in lines of the traditional wheat landraces by years of studies (Republic of Uzbekistan)

Line	Weight of 1000 seeds, g					Gluten content, %			
	2012	2014	2015	M	p	2012	2014	M	p
Krasnodarskaya 99	38.0	51.0	37.0	42.00		30.5	29.2	29.85	
Kzyl-bugday 2-6T	52.0	42.7	35.0	43.23	0.8623	33.5	34.2	33.85	0.0324
Kzyl-bugday 3-7T	52.0	43.0	37.5	44.17	0.7436	35.1	29.2	32.15	0.5259
Kzyl-bugday 9-4T	54.0	37.0	42.0	44.33	0.7476	35.0	33.5	34.25	0.0473
Kzyl-bugday 5-4T	54.0	42.5	45.0	47.17	0.4162	22.0	35.6	28.80	0.8919
Kzyl-bugday 11-4T	50.0	37.0	40.5	42.50	0.9371	34.5	29.8	32.15	0.4451
Ak-bugday 7-3T	44.0	30.0	43.0	39.00	0.6625	32.0	28.0	30.00	0.9496
Surkhak 12-3T	48.0	43.0	40.0	43.67	0.7592	28.0	26.0	27.00	0.1394
Ak-bugday 8-2T	48.0	39.0	38.5	41.83	0.9771	37.5	36.4	36.95	0.0141
Tyuya-Tish 10-4T	48.0	37.0	37.5	40.83	0.8494	32.0	31.6	31.80	0.1032
Kzyl-bugday 1-4T	50.0	37.0	42.0	43.00	0.8734	29.0	32.0	30.50	0.7293
13-8T (unnamed)	42.0	40.5	39.0	40.50	0.3267	40.5	39.6	40.05	0.0060
14-3T (unnamed)	52.0	35.0	44.5	43.83	0.7971	35.0	32.4	33.70	0.1179
Ak-bugday 16-5T	48.0	44.5	44.0	45.50	0.4962	35.0	36.0	35.50	0.0204
Surkhak 15-6T	48.0	47.3	43.0	46.10	0.4387	35.5	29.6	32.55	0.4657

N o t e. M — arithmetical mean, p — significance level by the Student's t-test.

The data obtained show that some of the traditional Uzbek wheat landraces have a high potential productivity and elevated gluten content, though they are exceeded by the commercial Krasnodarskaya 99 cultivar in the yield stability. Since the natural productivity is implemented during a critical period from VI to IX organogenesis stages (beginning of stem elongation—end of blooming), in the traditional landraces it may be additionally stimulated by agrochemical exposures, for example, growth regulators (retardants) to be applied during the critical period [21], as well as by genetic selection methods.

Thus, the traditional landraces of Uzbekistan have many traits useful for selection. The collection and study of these landraces are of great importance for preserving the wheat gene pool in the natural conditions. Among them, there are landraces possessing high baking and taste qualities. The traits are genetically determined that makes it possible to use the traditional landraces as donors of high-quality genes in the selection process. The highest weights of 1000 seeds and gluten contents have been found in Kzyl-bugday 2-6T, Kzyl-bugday 3-7T, Kzyl-bugday 9-4T, Kzyl-bugday 11-4T, Ak-bugday 16-5T, Surkhak 15-6T and 14-3T (unnamed) samples.

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WHEAT (*Triticum* L.) CULTIVARS FROM GRIN COLLECTION (USA) SELECTED FOR DURABLE RESISTANCE TO *Septoria tritici* AND *Stagonospora nodorum* BLOTCH

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A b s t r a c t

Septoria tritici blotch (STB) or *Stagonospora nodorum* blotch (SNB) are among the most harmful and economically significant diseases of wheat in the grain growing regions of the world, especially in the countries with a temperate climate. In epiphytotic years the losses from the disease can reach 30-40 %. In Russia the diseases holds a dominant position in a pathogenic complex of fungus diseases of grain crops. In this paper we first determined the parameters of partial resistance in the cultivars of wheat (genus *Triticum*) from the collection of the Germplasm Resources Information Network (GRIN, USA) using the stable strains of *Septoria tritici* and *Stagonospora nodorum* pathogens. The aim of our study was to select wheat varieties with long-term resistance to blotch based on field and laboratory tests. A long-term study (2009-2015) of the disease development on the wheat cultivars from GRIN Collection were conducted at artificial infection in infection nursery (Central region of Russia, Moscow Province). The samples studied belonged to various genetic groups. A total of 20 samples were diploids ($2n = 14$), 409 samples were tetraploids ($2n = 28$), 1688 samples were hexaploids ($2n = 42$), and also 397 lines derived from crossing of *Triticum aestivum* with *Aegilotriticum* were tested. The area under disease progress curve was determined, and the index of resistance (IR) was calculated. The cultivars, that characterized by slow development of the disease in the field, i.e. with high-and middle IR, were selected for laboratory studies. The plants were grown in artificial climate chambers till the 3rd leaf fully unfolded. Pieces of leaves were inoculated by a drop of spore suspension of *S. tritici* (4 isolates) or *St. nodorum* (4 isolates), 10 replications per each variety-to-pathotype combination. The samples were grouped according to the latent period length and size of infectious spots. As a result, 191 samples of *T. aestivum* subsp. *aestivum* and a sample of *T. aestivum* subsp. *spelta* with a high index of resistance to the disease were selected among hexaploid wheat; 16 samples were found in tetraploid wheat, including 8 samples of *T. turgidum* subsp. *durum*, 2 samples of *T. turgidum* subsp. *turgidum*, 3 samples of *T. turgidum* subsp. *dicoccon*, 3 samples of *T. timopheevii* subsp. *timopheevii*; and 4 samples were selected from diploid wheat *T. monococcum* subsp. *aegilopoides*. Eleven lines derived from crossing of *T. aestivum* and *Aegilotriticum* sp. showed the sloweddown in the disease development. The selected hexaploid wheat cultivars were mostly from North American ecology-geographical group of *T. aestivum* subsp. *aestivum*, including 77 cultivars from the USA and 18 — from Canada (34.5 % in total). Selected tetraploid wheat samples of *T. turgidum* subsp. *durum* were from North and Central America, and those of *T. turgidum* subsp. *turgidum*, *T. timopheevii* subsp. *timopheevii* and *T. turgidum* subsp. *dicoccon* from Europe and Asia. The samples from Iraq and Hungary with a high index of blotch resistance were found among diploid wheat *T. monococcum* subsp. *aegilopoides*. The synthetic lines of wheat from the USA and Mexico were also characterized by a sloweddown development of the disease. Thus the wheat cultivars with partial resistance have been revealed, including 10 cultivars with partial resistance to *Septoria tritici* blotch and 40 cultivars — to *Stagonospora nodorum* blotch. The accessions PI 494096 Tadinia, Cltr 17904 Owens, Cltr 15645 II-62-4 (USA), VIR 63915 Flame (England), Cltr 14492 Azteca, PI 520555 Alondora 'S' (Mexico), PI 404115 Timson (Australia), Cltr 11765 Chinese 166 (Germany), PI 422413 CNT 1 (Brazil), PI 168724 Benvenuto, Cltr 15378 Piamontes, PI 344468 Piamontes Inta (Argentina), PI 306551 2944 (Romania), PI 355706 69Z5.715 (Azerbaijan), PI 355560 SK 1B (Switzerland), PI 94743 290 (Russia) are of special interest for breeding as a source of long term resistance.

Keywords: *Septoria tritici* blotch, STB, *Stagonospora nodorum* blotch, SNB, partial re-

sistance, long-term resistance, *Triticum* L., diploid, tetraploid and hexaploid wheat, synthetic lines, the Germplasm Resources Information Network (GRIN) Collection

Septoria blotch is one of the most dangerous and economically significant diseases of grain crops in the majority of regions with a temperate climate [1, 2]. The greatest harmfulness of the disease was noted in France, Great Britain, Germany, Poland, Belgium, Czech Republic, the Netherlands, in Scandinavian countries (Norway, Sweden, and Finland), Lithuania, and Latvia [3-7]. Cases of Septoria blotch were recorded in North Africa (Tunisia, Algeria, Morocco, Ethiopia) and Australia, Northern Caucasia (Georgia), North America (the USA, Canada) and South America (Mexico, Argentina) [8-15]. In Russia, Septoria blotch holds a dominant position in a pathogenic complex of fungus diseases of wheat plantings. The disease poses the highest priority danger in Central, Southern, North Caucasian, North-Western, Privolzhsky, and Siberian Federal regions [16-18]. In epiphytotic years, the crop losses from the disease can reach 20-40 % [19-23]. Septoria blotch reduces the photosynthetic activity of plants and causes the ear malformation. The assimilation surface of leaves decreases, the stems are die-back and folded (as a result of severe node damages), early ripening and shortfall of grains are observed. In case of severe damage of crops, Septoria blotch may cause seedlessness and death of individual plants. If the disease appears later the grain yield diminishing does not generally exceed 5-7 % [24-26]. The Septoria blotch pathogens can affect all the aboveground organs of plants. There are about 16 names of these fungi in the literature, but their taxonomy generates a lot of differences. The best known species now are *Septoria tritici* Rob. Et Desm.; *Stagonospora nodorum* (Berk.) E. Castellani and E.G. Germano and *Stagonospora avenae* Bissett f. sp. *triticea* T. Johnson [25].

There is almost no wheat breeding for resistance to Septoria blotch in our country, although the disease has been starting to spread throughout Russia since early 1970s, and currently it is present in nearly every region where spring and winter wheat is cultivated. The development of resistant cultivars requires donors, the sources of resistance genes, which can be searched for only among varieties of world collections. The most long-lasting protection against Septoria blotch is provided by partially resistant varieties which are characterized by slower development of the disease in field, can reduce the probability of epiphytotics and extend the duration of resistance retention in a cultivar [25, 27].

In this paper, we have determined for the first time the parameters of partial resistance in the cultivars of wheat from the collection of the Germplasm Resources Information Network (GRIN, USA) using the stable strains of *Septoria tritici* and *Stagonospora nodorum* pathogens.

The aim of our study was to select wheat cultivars with the durable resistance to Septoria blotch based on field and laboratory tests.

Technique. Dynamics of Septoria blotch development in wheat samples (genus *Triticum*) from the GRIN collection (Germplasm Resources Information Network, USA) was studied in 2009-2015 in an infection nursery located at the Central Region of Russia (Moscow Province, Odintsovo Region, trial fields of All-Russian Research Institute of Phytopathology, ARRIP). The study was conducted in 2117 wheat samples from various genetic groups, including 20 diploids ($2n = 14$), 409 tetraploids ($2n = 28$), 1688 hexaploids ($2n = 42$), and also 397 selection samples derived from crossing of *Triticum aestivum* with *Aegilotriticum* sp.

Area under disease progress curve (ADPC) was determined by the method of D.A. Johnson et al. [28] based on results of 5 and more measurements of Septoria blotch affection intensity for plants during the growing season:
$$S = \tfrac{1}{2}(x_1 + x_2)(t_2 - t_1) + ... + (x_{n-1} + x_n)(t_n - t_{n-1}),$$
where S is the area under disease progress curve; *n* is the number of measure-

ments; x_1, x_2, x_n are a degree of the disease development at the time of the 1st, 2nd and last measurements, respectively, %; ($t_2 - t_1$) is the time interval between the 2nd and the 1st measurements, days; ($t_n - t_{n-1}$) is the time interval between the last and second to last measurements, days.

Index of resistance (IR) was calculated by the formula proposed by A.A. Makarov et al. [29] as a ratio of the ADPC for the cultivar tested to that for the susceptible control: $IR = ADPC_{cultivar}/ADPC_{control}$. Based on the IR value, the cultivars were conditionally divided into 4 groups: cultivars having high (0.10-0.35), medium (0.36-0.65), and low (0.66-0.80) IR, and highly susceptible cultivars (IR > 0.81). The cultivars characterized by slower development of the disease in the field conditions, i.e. those having the high and medium IR, were selected for laboratory experiments.

Parameters of quantitative resistance to *Septoria tritici* pathogen (latent period and size of infectious spots) were studied in 39 wheat samples, and those to *Stagonospora nodorum* — in 60 wheat samples. The susceptible Priokskaya cultivar was used as a control. The pathogen strains were obtained from the National Collection of phytopathogenic microorganisms and varieties of plants identifying pathogenic strains of microorganisms (ARRIP).

The selected plants were grown in artificial climate chambers till the 3rd leaf fully unfolded. A leaf was cut into 9-10 cm long pieces and placed in trays onto filter paper wetted with aqueous solution of benzimidazole (0.4 g/l). The pieces of leaves were inoculated by a drop of spore suspension of each of *S. tritici* isolates (V-35/ChI1, 5/23, 5/8, 5/9; 10^7 spores/ml) or *St. nodorum* isolates (R-8, V-81, V-28/KG$_3$, NAlt-1; 10^6 spores/ml), 10 replications per each variety-to-pathotype combination.

According to latent period and size of infectious spots, the varieties were grouped as follows: I — varieties with small infectious spots and long latent period; II — varieties with small infectious spots and short latent period; III — varieties with large infectious spots and long latent period; IV — varieties with large infectious spots and short latent period. The varieties with a long latent period included those for which it lasted for or longer than 15 and 5 days for *S. tritici* and *St. nodorum*, respectively, and the varieties with a short latent period included those for which it lasted for less than 15 and 5 days. Spots with the area of < 20 mm^2 were considered small, and those of > 20 mm^2 were referred to large ones.

Statistical processing was carried out by the analysis-of-variance method using the program developed at All-Russian Research Institute of Phytopathology (version 1.02.1995, № 006). Tables include mean values and LSD$_{95}$.

Results. Based on findings of multi-year research, wheat cultivars and hybrid forms derived from crossing of *T. aestivum* with *Aegilotriticum* sp. showing different rates of Septoria blotch development have been identified. The most long-lasting protection was provided by cultivars with the high index of resistance (i.e. partially resistant to the disease) characterized by slower development of Septoria blotch under field conditions.

Wheat cultivars with the high IR were selected from various genetic groups taking into account their origin. As a result, the studied samples were distributed into 10 ecology-geographical groups according to N.I. Vavilov's classification [30, 31].

The maximum number of samples (85.7 %) with the high index of resistance was identified in cultivars of *T. aestivum* subsp. *aestivum* (Table 1).

The majority (34.5 %) of the studied hexaploid wheat cultivars that demonstrated resistance originated from the North American ecogeographical group (species *T. aestivum* subsp. *aestivum*): high IR was characteristic of 77 varieties from the USA and 18 ones from Canada (see Table 1). They were mainly se-

lection samples, as well as cultivars Chaparral, Minnpro, Anderson (the USA) and Agatha (Canada). Anderson and Agatha cultivars which demonstrated the high IR to Septoria blotch for 3 and more years of studies were of particular interest.

1. Wheat cultivars from the collection of the Germplasm Resources Information Network (GRIN, the USA) with the high index of resistance to Septoria blotch (infection nursery, Central Region of Russia, Moscow Province, 2009-2015)

Ecogeographical group	Country	Number of cultivars	Name
Triticum aestivum subsp. *aestivum* (2n = 42)			
North American	USA	77	N No. 1226; N No. 1121; N No. 1265; Rival; N 1827; II-36-67; 1415 A-1-1-1-1; Ns. 3880.227; Ns. 3880.127; II-52-238; ND 364; Chaparral; II-56-10; II-54-89; II-54-102; II-54-1; II-58-51; II-60-115; II-59-11; II-62-51; II-62-48; II-62-15; II-62-69; II-62-65; II-62-76; II-62-77; II-62-79; II-62-75; II-62-82; II-62-81; II-62-83; II-62-84; II-62-25; II-54-79; II-62-49; II-62-32; II-62-31; II-60-222; II-61-1; II-61-15; II-62-71; II-60-220; II-60-218; II-62-72; II-62-35; II-62-20; II-54-46; II-56-32; II-56-29; II-56-33; II-58-15; II-58-57; II-58-14; II-56-12-7; II-60-100; II-60-46; II-60-126; II-60-130; II-52-324; CI 15756; ND 466-2; ND 468; Frohberg 12-107; MN 6796; MN 6864; MN 6955S; MN 6983S; MN 7570; ND 573; ND 599; MN 7444; MN 7533; ND 617; Minnpro; Cltr 15645; Anderson; WA 6101
	Canada	18	RL 988; RL 2520; RL 4137; Agatha; RL 4110; RL 4151; RL 4170; RL 4203; RL 4204; 60 GRR 92; Q 254-43; Q 2331-34; RL 6003; RL 6005; RL 6008; RL 6009; RL 6010, P8917-B4D4
Central American	Mexico	36	CI 2817; II-1776-2C; II-3962-2H-2Y-3C-1Y; VI-136-49-6H-1R; Huamantla Rojo; II-7242-5C-1H-2R-1M; II-7232-17R-1M-1B-2M; II-7527-5M-2R-5M-1R; II-7740-26-2H-1R-4M; II-8192-2R-2M-1R-4M; II-8495-4Y-9C-2Y-2C-1Y-2C-1Y; II-8523-11M-1R-4M; 8738-4R-5M-1R-1M-1R; P-9897-8T-2B-1T-1B; P-9916-9T-1B-2T-1B; P-11380-1B-7T-1B; P-11380-1B-7T-1B; II-11996-4R-5M-1R; JARAL 66; Bajio; Potam 70; II-19008-52M-6Y-3M-3Y-2C; II-19005-23M-3Y-3M-2Y-2C; II-19865-58M-100Y-104C; II-19021-4M-3Y-102M-2C; II-19008-83M-4R-4C-2Y-4C; D 19329-28M-11Y; Alondra 'S'; Cocora-Que F75; Mor 'S'; SWM 4127-1Y-1M-4Y-2M-1Y-OM; Bobwhite 'S'; Veery No. 3; Bobwhite 'S'; Alondra 'S'; II-1462-2C-2C-15C
	Brazil	8	BH 941; CNT 1; Trareano; 16-52-2, BH 2845; Itapeva; Colotano; 266/51
South American	Argentina	18	Americano; IF 1054; Klein H211-t-1422; Benvenuto Inca; Benvenuto 3085; La Prevision 25; 38 M.A.; Klein Lucero; Piamontes; D.I.V. 6656; I-1055; Magnif 142; Piamontes Inta; Klein Impacto; Vivela Mar; Taganrog Buck Balcarse; Mole II; 1352
	Peru	3	Mult 757; Mult 760; Mult 764
West European	Germany	1	Chinese 166
	Belgium	1	ALBA
	Macedonia	1	I/24
	Czechoslovakia	2	Dobrovicka Drogerowa B I 32; Stupicka Bastard
	Bulgaria	2	Bogdan, Experiment station no.85
	Portugal	3	Richelle Blanche Hative; Portugues; WS-9
East Asian	China	13	3867; CI 8328; Chiu mai; 7045; 7049; 848; 7177; 7227; 7329; I13; 357; ST-56; Daqingshan No. 4
Mediterranean	Turkey	1	1403
South-Western Asian	Iran	1	158e
	Afghanistan	2	BlackShanazi; Sirhosha
South Asian	Nepal	1	57-336
Australian	Australia	2	AB 21/10-1-1-2; Timson
African	Ethiopia	1	ELS 6404-26
Total		191	
Triticum aestivum subsp. *spelta* (2n = 42)			
West European	Spain	1	69Z6.886
Triticum turgidum subsp. *durum* (2n = 28)			
North American	USA	1	ND 63-36
	Canada	1	UM 6001

Table 1 (continued)

Central American	Mexico	6	RF 427-19; RF 427-30; Scoter 'S'; Gambridge 010, Pinguno 'S'; Pinguno
			Triticum turgidum subsp. *turgidum* (2n = 28)
South-Western Asian	Armenia	1	37
West European	Portugal	1	Rubiao
			Triticum turgidum subsp. *dicoccon* (2n = 28)
West European	Italy	1	Paganuzzi
	Spain	1	103
	Switzerland	1	Subletshchumicum
			Triticum timopheevii subsp. *timopheevii* (2n = 28)
South-Western Asian	Georgia	1	WIR 38555
West European	Hungary	1	01.01.2004
Mediterranean	Israel	1	Kurazim
			Triticum monococcum subsp. *aegilopoides* (2n = 14)
South-Western Asian	Iraq	3	G2292, G2706, G2885
West European	Hungary	1	1-1-1752
			Triticum aestivum × *Aegilotriticum* sp.
North American	USA	2	SW34
Central American	Mexico	9	BW27722; BW27723; BW27777; BW27778; BW27779; BW27830; BW27985; BW28153; BW28154
Total		223	

The Central American group included cultivars from Mexico and Brazil. Among Mexican samples we have selected 36 cultivars having high IR. Slower development of the disease in the samples originated from Brazil (BH 941, CNT 1, Trareano, 16-52-2, BH 2845) was noted throughout multi-year studies. Eighteen cultivars with the high IR from Argentina and three lines from Peru have been identified among 54 studied samples from South America. Ten similar cultivars have been found out in West European countries, and 13 samples from China have been revealed in the East Asian ecogeographical group (see Table 1).

The Mediterranean, South-Western Asian, South Asian, Australian and African ecogeographical groups appeared to be represented by a small number of *T. aestivum* subsp. *aestivum* cultivars. However, we succeeded in finding samples with high partial resistance to the disease among them, i.e. sample 1403 (Turkey), 158e (Iran), Black Shanazi and Sirhosha (Afghanistan), 57-336 (Nepal), AB 21/10-1-1-2 and Timson (Australia), ELS 6404-26 (Ethiopia). In the genetic group of hexaploid wheat, sample 69Z6.886 (Spain) referred to *T. aestivum* subsp. *spelta* species has been selected (see Table 1).

Among tetraploid wheat cultivars of *T. turgidum* subsp. *durum*, we have revealed 16 ones having the high IR (originating generally from North and Central America). Two European samples were identified in *turgidum* subsp. *turgidum* group, three European samples were found among *T. timopheevii* subsp. *timopheevii* forms, as well as three ones — among *T. turgidum* subsp. *dicoccon* (see Table 1).

Cultivars with high index of resistance to Septoria blotch included 4 diploid wheat samples of *T. monococcum* subsp. *aegilopoides* originated from Iraq and Hungary. Slower development of the disease was also characteristic of synthetic lines of wheat from USA and Mexico (see Table 1).

When assessing the quantitative resistance of wheat cultivars to Septoria blotch pathogens *S. tritici* and *St. nodorum*, the samples were divided into four groups. Group I cultivars, the slower development of the disease in which is defined by a longer latent period and smaller size of infectious spots as compared to those in the susceptible control, attract the most interest for selection. Among the studied samples from the GRIN collection, we have not found any cultivars belonging to group I by their resistance to *S. tritici*. Group I by resistance to *St. nodorum* included 13 cultivars (Table 2).

The infection development in group II cultivars was slowed down due to the reduction in sizes of infectious spots. Group II by resistance to *S. tritici* in-

cluded 8 cultivars, and by resistance to *St. nodorum* there were 8 cultivars as well from the GRIN collection (see Tables 2, 3).

2. Wheat cultivars from the collection of the Germplasm Resources Information Network (GRIN, USA) partially resistant to *Stagonospora nodorum*

№ as per the GRIN catalog	Cultivar	Origin	Spot size, mm^2	Latent period, days
		G r o u p I		
PI 520555	Alondora 'S'	Mexico	18.32	7.00
PI 422413	CNT 1	Brazil	13.86	6.50
VIR 63915	Flame	Great Britain	15.72	6.50
PI 404115	Timson	Australia	12.03	6.25
PI 494096	Tadinia	USA	13.35	6.25
PI 168724	Benvenuto	Argentina	11.60	5.50
PI 306551	2944	Romania	13.30	5.50
PI 355706	69Z5.715	Azerbaijan	13.70	5.50
Cltr 15645	II-62-4	USA	16.56	5.50
PI 344468	Piamontes Inta	Argentina	16.88	5.50
Cltr 17904	Owens	USA	17.13	5.50
PI 355560	SK 1B	Switzerland	10.60	5.30
PI 94743	290	Russia	12.15	5.25
		G r o u p II		
PI 190974	Aristato Blanco	Italy	11.00	4.50
PI 191353	Novokrymka 204	Ukraine	15.20	4.50
PI 341803	WIR43065	France	15.50	4.50
PI 345242	241-VII/4	Macedonia	12.90	4.30
PI 300991	Kurazim	Israel	13.40	4.30
PI 427867	G2697	Iraq	12.83	4.25
PI 418584	WIR 38555	Georgia	18.40	4.00
PI 290518	01.01.2004	Hungary	19.13	4.00
		G r o u p III		
Cltr 12536	Anderson	USA	33.50	7.30
VIR 64130	Warigal Dagger	Australia	36.50	7.30
PI 168725	Benvenuto 3085	Argentina	48.00	7.30
PI 338913	Azteca	Mexico	66.40	7.30
PI 168724	Benvenuto INCA	Argentina	56.50	6.80
PI 278222	Gambridge Rivet	Great Britain	29.00	6.50
PI 604225	KS96WGRC40	USA	29.10	6.30
Cltr 13113	Kansas № 26365	USA	42.20	6.30
Cltr 15378	Piamontes	Argentina	24.58	6.25
Cltr 14465	Kansas № 594-2	USA	34.00	6.00
Cltr 14492	Azteca	Mexico	22.16	6.00
PI 214401	Veranopolis	Brazil	33.67	6.00
PI 520552	Bobwhite 'S'	Mexico	28.66	5.50
Cltr 12053	Cadet	USA	30.58	5.50
PI 422273	Alondra 'S'	Mexico	24.28	5.50
Cltr 11765	Chinese 166	Germany	39.28	5.50
PI 348702	69Z6.886	Spain	26.68	5.00
PI 479692	K-20	Republic of South Africa	35.00	5.00
Cltr 64135	IW 562	Australia	52.00	5.00
LSD$_{95}$			13.86	0.92

N o t e. Laboratory testing results. The groups are described in the *Technique* section.

3. Wheat cultivars from the collection of the Germplasm Resources Information Network (GRIN, the USA) partially resistant to *Septoria tritici*

№ as per the GRIN catalog	Cultivar	Origin	Spot size, mm^2	Latent period, days
		G r o u p II		
Cltr 15378	Piamontes	Argentina	20.00	14.50
PI 433753	Gambridge 010	Mexico	16.60	8.00
PI 648860	BW 28154	Mexico	18.30	8.00
PI 191204	Rubiao	Portugal	13.50	7.75
Cltr 11765	Chinese 166	Germany	11.50	7.25
PI 189631	Trareano	Brazil	12.90	7.75
PI 352061	Klein Inpacto	Argentina	15.00	7.25
PI 422413	CNT 1	Brazil	14.00	7.00
		G r o u p III		
PI 338913	Azteca	Mexico	25.00	15.00
PI 520555	Alondora 'S'	Mexico	36.30	15.00
LSD$_{95}$			21.10	1.94

N o t e. Laboratory testing results. The groups are described in the *Technique* section.

Group III included the cultivars that provided slower development of the disease due to longer latent period. Cultivars Azteca and Alondora 'S' belonged to them by resistance to *S. tritici*, and 19 cultivars were included due to resistance to *St. nodorum* (see Table 2). Susceptible cultivars, the latent period and size of infectious spots in which were consistent with or exceeded the similar parameters in the susceptible control, were referred to group IV. This group included 30 forms that demonstrated weak susceptibility to *S. tritici* pathogen in field experiments, and 20 forms showed weak susceptibility to *St. nodorum*.

Thus, as a result of multi-year studies of samples from the GRIN collection (Germplasm Resources Information Network, USA), wheat cultivars ensuring partial resistance of plants to Septoria blotch due to the extended latent period and reduced size of infectious spots, as well as capable of limiting the epiphytotic development of the disease, have been selected. They include 10 cultivars partially resistant to *Septoria tritici* pathogen, and 40 cultivars partially resistant to *Stagonospora nodorum*. The varieties most valuable for selection of cultivars with a durable resistance to Septoria blotch are Tadinia (PI 494096), Owens (Cltr 17904), II-62-4 (Cltr 15645) originating from the USA, Flame (VIR 63915) from Great Britain, Azteca (Cltr 14492), Alondora 'S' (PI 520555) from Mexico, Timson (PI 404115) from Australia, Chinese 166 (Cltr 11765) from Germany, CNT 1 (PI 422413) from Brazil, Benvenuto (PI 168724), Piamontes (Cltr 15378), Piamontes Inta (PI 344468) from Argentina, 2944 (PI 306551) from Romania, 69Z5.715 (PI 355706) from Azerbaijan, SK 1B (PI 355560) from Switzerland, and 290 (PI 94743) from Russia.

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INFLUENCE OF PHOSPHOMIDE, A CHEMICAL MUTAGEN, ON AGROBIOLOGICAL SIGNS OF SOFT SPRING WHEAT *Triticum aestivum* L.

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A b s t r a c t

The method of chemical mutagenesis allows in a relatively short time to obtain a material with new features and properties, including completely new mutations. The effectiveness of the application of the method for the creation of selection valuable forms of plants in various soil and climatic conditions is shown in the works of a number of authors. The purpose of this work was to study the effect of the mutagen namely phosphomide in different concentrations on the agrobiological characters at the hybrid form and the initial cultivars of spring soft wheat (*Triticum aestivun* L.). Two domestic cultivars (Scant 1, Scant 3, var. *lutescens*) and three foreign cultivars (Cara, var. *erythrospermum*; Hybrid, var. *ferrugineum*; Lutescens 70, var. *lutescens*) were the source material. A preliminary study of the varieties was carried out in 2006-2008. In 2009, hybrid combinations involving these varieties were obtained using incomplete diallel crosses and forced pollination. The efficacy of phosphomide, a chemical mutagen, at concentrations of 0.002 and 0.01 % was studied in two varieties (Cara and Scant 3) and F_4 hybrid (Cara × Scant 3). For the treatment, the seeds were soaked in phosphomide solutions for 3 hours, and the control seeds were soaked in distilled water. Germination energy and morphometric parameters of the seedlings were evaluated in laboratory tests to calculate the growth inhibition indices. In the spring 2014, 25 mutagen-treated seeds were sown in the field (Biostation of Tyumen State University, Tyumen Province) in 4-fold replication separately for each variant of treatment to obtain M_1 generation. From individual M_1 plants were separately derived M_2 generation. Resistance of the specimens toward leaf fungus diseases (powdery mildew, leaf rust and spotting) was assessed under natural infection development (Biostation of Tyumen State University, Tyumen Province) during the entire vegetation period, from appearance of the symptoms till the leaves dried up, using laboratory tests for specific pathogen identification. The results obtained suggest that the use of phosphomide promotes diversity of the breeding material. In the first generation (M_1), there was an inhibitory effect of phosphomide on field wheat germination and the morphometric parameters of seedlings (the length of roots and shoots) which depended on the mutagen concentration. The seedlings showed the least tolerance to phosphomide as to the number of germinal roots. The effect of stimulation compared to the control occurred in the hybrid combination Cara × Scant 3 as to seed germination energy indices (by 5.9 %) in the laboratory tests and plant viability in the field during the growing season (by 14.0-80.0 %). The mutagen increased significantly the grain weight per 1 m^2 in the hybrid (by 16.0 %) while the grain yield in the varieties Cara and Scant 3 decreased by 67.0 and 57.0 %, respectively. In high concentration (0.01 %) the mutagen reduced resistance to powdery mildew and brown rust in the varieties whereas in the hybrid at the same concentration the susceptibility to powdery mildew decreased, and to brown rust — increased. The phenotypic changes in M_2 were assessed according to the morphology of the ear, stem, leaves (color, pubescence, shape, size) and biological properties (late ripening, early ripening, winter type plants). In total, there were 12 types described. Plants with a large ear, strong stems, stunted and

dwarfs were more common in the hybrid (13.6-20.0 % of the total number of modified forms). The varieties often produced early ripening forms (16.4-24.2 %). The proportion of families with altered plants in the varieties was 5.3 % less than in the hybrid. The highest rate of modifications in the varieties and the hybrid was influenced by 0.01 % concentration. Thus, it is shown that phosphomide at concentrations of 0.002 and 0.01 % is effective to improve agronomically important characteristics in the varieties and intraspecies hybrid of soft spring wheat.

The basis of food and bioresource safety is the genetic resources of plants. By the middle of the 21st century, up to 60 % of their species composition can be lost [1, 2]. The loss of plant resources is considered as one of the ecological crisis factor of the biosphere [3]; therefore, a strategy for the conservation and sustainable use of the biodiversity is necessary, including its expansion by classical and modern methods.

The traditional method of chemical mutagenesis allows organisms with new characteristics and properties to be obtained in a relatively short time [4]. It is still widely and effectively used to create valuable plant forms in various soil and climatic conditions, including in Western Siberia [5-8]. The development of mutational selection can be associated both with the use of known chemical supermutagens (N-nitrosomethylurea, N-nitrosoethylurea, ethyleneimine) and with the discovery of new highly active substances [9]. These include chiral stereoisomers used in the herbicide, insecticide and fungicide production industry [10]. The mutagenic effect is also shown by alkylating agents, nitrous acid, nitrogen oxide, analogues of nitrogenous bases and related compounds, antibiotics, intercalating agents and topoisomerases of poisons [11].

Diethylenimid-2-amidopyrimidylphosphoric acid (phosphomide) consists of two groups of ethyleneimine combined with phosphorus and a pyrimidine base. Ethyleneimine causes mutations; the pyrimidine base is incorporated into the chromosome during DNA synthesis, determining effect specificity. The mutagenic effect of phosphomide [12] was studied earlier on the *Crepis capillaris* L. model. Studies with *Triticum aestivum* L. have not been carried out. The necessary working step in experimental mutagenesis is determining the ranges of concentrations in which the desired effects are possible.

The specificity of the mutagenic effect of phosphomide on varieties (Cara and Scant 3) and the hybrid form of spring soft wheat has been studied for the first time in this work; and the effect of a chemical mutagen gene on the variability of population and individual characteristics has been determined.

Our goal was to study the effect of phosphomide in different concentrations on the agrobiological traits of spring soft wheat.

Technique. Soft wheat (*Triticum aestivum* L.) was represented by five varieties from VIR World collection (N.I. Vavilov All-Russia Institute of Plant Genetic Resources, St. Petersburg) of Russian origin (Scant 1 and Scant 3, var. *lutescens*, Russia, Tyumen Province), and of foreign origin (Cara, var. *erythrospermum*, Mexico; Hybrid, var. *ferrugineum*, Mexico; Lutescens 70, var. *lutescens*, Kazakhstan). A preliminary study of the varieties was carried out in 2006-2008 (Tyumen VIR Reference Station) [13]. In 2009, hybrid combinations involving these varieties were obtained using incomplete diallel crosses and forced pollination [14].

Comparative field evaluation of hybrid and parental forms was carried out in 2010-2014 when planting in blocks with 10×20 cm feeding area of each plant in the first stage (F_1) and then in subsequent sowing (F_2, F_3, etc.) by families (offspring of one plant). For each hybrid combination in F_1, more than 50 plants were analyzed, in F_2 and subsequent generations 200 plants and more were tested.

The efficacy of phosphomide at concentrations of 0.002 and 0.01 % was studied in two varieties (Cara and Scant 3) and in F_4 hybrid (Cara × Scant 3). The seeds were submerged in phosphomide solutions for 3 hours. The control was the seeds submerged in distilled water. A total of 300 seeds of each variety and a hybrid combination (100 in each variant of the experiment, i.e. the control, 0.002 % mutagen, 0.01 % mutagen) for laboratory and 300 seeds for field studies were used.

The germination energy and the morphometric parameters of the seedlings were evaluated in laboratory conditions [15]. Growth inhibition indices were calculated as the ratio of the length of the roots, shoots and number of roots of the seedlings under the action of the mutagen and in the control.

The generation M_1 was studied in field experiments (Lake Kuchak Biostation of Tyumen State University, Tyumen region). In the spring of 2014, 25 seeds were sown in 4-fold replication separately for each mutagen treatment. The M_2 progeny were obtained individually from each plant. Resistance of specimens to fungal diseases (powdery mildew, leaf rust and leaf spotting) was assessed under natural infection development during the entire growing season (from the appearance of the first symptoms of the disease till the leaves dried up) [16]. The pathogen was identified using microscopy and moist chamber method [17]. In M_2, phenotypic changes were assessed according to the morphological features of the ear, stem and leaves (color, pubescence, shape and size) and biological properties (late ripening, early ripening and winter type plants).

Data processing was carried out using Microsoft Excel spreadsheet and STATISTICA 6.0 software (StatSoft Inc., USA). For quantitative traits, the mean values (X_m) and the error of the mean (S_x) are presented in the tables; for qualitative traits, the value (X) and the indicator of traits variability (S) are shown. The significance of the differences between the mean values of the variants was estimated using the Student t-test.

Result. The variety Cara belonged to the group of undersized varieties (plant length of 54.9 cm), and was resistant to lodging (7-9 points). The vegetation period was 77 days like that in Hybrid, but was 5-6 days less then in Scant 1, Scant 3 and Lutescens 70. This variety was resistant to powdery mildew and, according to GRIS (Genetic Resources Information System for Wheat and Triticale), carries *Lr13* gene for resistance to rust a stable allelic state. The Hybrid variety showed high adaptive properties in the extremely unfavorable meteorological conditions of 2008. During the seeds germination and the shoots formation, the amount of precipitation was 68.6 % of the norm, and the average daily air temperature was 0.5 °C above the multiyear average; there was an active fly-out of the *Osinosoma frit* L. The variety was characterized by high drought resistance (7 points) and medium resistance to pest damage (5 points). The varieties Scant 1, Scant 3 and Lutescens 70 are included in the State Register of Selection Achievements approved for use in the 10th West Siberian region of Russia. These varieties are medium-ripening (82-83 days), with high field germination of seeds and productive tillering, as well as moderate susceptibility to diseases.

Based on the study of 10 combinations of intraspecific crosses (F_1-F_4), the hybrid Cara × Scant 3 was selected according to combinational ability, the character of gene interaction and dominance in F_1, transgression and heritability in F_2. Its high productivity was confirmed by ecological testing of hybrids F_4 and F_5 in three geographical locations essentially differing in climatic conditions: the experimental field of Lake Kuchak Biostation (Tyumen State University, Tyumen region, Russia), the Land of Baden-Württemberg (experimental field of the Waldorf School, Germany), and Lower Saxony (Experimental Station Waldhof, Germany).

In 2013-2014, biological productivity of the best line from Cara × Scant 3 hybrid combination exceeded the standards by 62.2 % in Tyumen region (Novosibirskaya 15 and Irgin varieties), and by 44.1 % in Baden-Württemberg (Ashby and Scirocco varieties). In Lower Saxony, no advantages appeared compared to Eminent and Granus varieties [18]. The hybrid was characterized by high resistance to spotting (*Alternaria* spp. and *Helminthosporium* spp.) and brown rust [*P. recondita* Rob. ex. Desm f. sp. *tritici* Eriks. (=*P. triticina* Eriks.)].

The possibility of using recombination and mutational variability was considered to expand the biodiversity of *T. aestivum* L. One of the main criteria for the successful use of the mutagen of phosphomide was the proportion of modified plants. In total, 1238 M_2 families for Cara and Scant 3 varieties and 636 families of the Cara × Scant 3 hybrid were analyzed (Table 1).

1. Effectiveness of phosphomide mutagenesis of spring soft wheat (*Triticum aestivum* L.) estimated in M_2 generation ($X_m \pm S_x$, Biostation of Tyumen State University, Tyumen region, 2014; field tests)

Concentration of mutagen, %	Cara and Scant 3 varieties, families			Hybrid, families		
	total number		mutants, %	total number		mutants, %
	tested	mutant		tested	mutant	
Control	329	12	3.6±1.03	120	9	7.5±2.40
0.002	550	72	13.1±1.44	272	27	9.9±3.28
0.01	359	64	17.8±4.08	244	74	30.3±2.95

The genotypic features of varieties and a hybrid on the relative output of valuable forms could be established based on response to different doses of the mutagen. The greatest number of mutants, both in the parental varieties and in the hybrid, was found at a mutagen concentration of 0.01 %.

The hybrid significantly differed from the varieties on spontaneous mutation frequency (3.6 % for the populations of the varieties, while among hybrid families mutant plants were 2 times more frequent and reached up to 7.2 %).

Phenotypic mutations in variants with the use of a chemical mutagen proved to be quite diverse. The following valuable types were distinguished in M_2: tall, low-growth, dwarfs, large-sized, winter-type plants, late ripening, early ripening, with a wide flag leaf, with altered shape and color of spike (spelled, pyramidal, yellow) and with strong straw. In the hybrid form, 8 mutation types of 12 detected were observed. The greatest frequency of the mutagen forms (30.33 %) was found in the hybrid at 0.01 % phosphomide concentration, and in Cara variety (15.31 %) at 0.002 % phosphomide concentration. Tall plants with a large ear, early or late maturation were recorded more often, plants with a modified shape of the ear were less often. Hybrid plants were mostly large-sized (ear length 16.5-21.8 cm) (20.0 %); tall (89.3-103.5 cm in height) (18.2 %); with a solid straw (resistance to lodging 9 points) (13.6 %); undersized and dwarfs (39.8-61.2 cm in height) (16.4 %). Early-maturing forms (3-5 days earlier than in the control) appeared in a larger number in the Cara and Scant 3 varieties (24.2 and 12.3 %, respectively). A speltoid spike was found in five M_2 plants grown from seeds treated with 0.01 % phosphomide and tested in M_3. These mutations were not revealed in M_1 despite the fact that they are dominant and semi-dominant. Grain in speltoid spikes is characterized by increased protein content [19]; speltoids have been cytogenetically studied in the mutants in detail [20]. The observed differences in the frequency and diversity of the mutant forms in the hybrid and the initial varieties may be related to their susceptibility to the mutagen.

To differentiate the varieties and the hybrid on their response to mutagen, we estimated root and shoot growth in the laboratory experiment and calculated the indices of growth inhibition. The indices, as a criterion of plant re-

sponse to stressors (low temperatures, aluminum ions, fluorides, lead nitrates, oil pollution, etc.), are widely described [21-23]. In our experiment, the number of germinal roots in seedlings reflects their tolerance to phosphomide (Table 2). Significant differences from the control were found only in Scant 3 variety at 0.01 % mutagen concentration when the root number index amounted to 0.47, while in other variants of mutagenesis it reached 0.85-0.99.

2. Morphometric parameters of spring soft wheat (*Triticum aestivum* L.) seedlings in M_1 generation after seed treatment with different concentrations of phosphomide (laboratory experiment**)**

Concentration, %	Samples	Number of germinal roots		Shoot length		Root length	
		$X_m \pm S_x$	RNI	$X_m \pm S_x$, mm	SLI	$X_m \pm S_x$, mm	RLI
Control	Cara	2.84±0.12	1.00	8.21±0.78	1.00	21.42±1.09	1.00
	Cara × Scant 3	3.82±0.32	1.00	11.35±0.80	1.00	20.09±1.40	1.00
	Scant 3	3.82±0.21	1.00	9.18±0.85	1.00	13.19±0.99	1.00
0.002	Cara	2.80±0.12	0.99	3.22±0.13**	0.39	12.95±0.87**	0.60
	Cara × Scant 3	3.56±0.22	0.93	11.17±1.34	0.98	15.07±1.41*	0.75
	Scant 3	3.64±0.27	0.95	8.56±1.00	0.93	12.57±1.24	0.95
0.01	Cara	2.81±0.14	0.99	4.83±0.21**	0.59	8.75±0.63**	0.41
	Cara × Scant 3	3.25±0.31	0.85	6.44±1.42	0.57	11.69±1.81**	0.58
	Scant 3	1.80±0.37**	0.47	4.80±0.77**	0.52	8.44±2.56*	0.64

N o t e. RNI — root number index, SLI — shoot length index; RLI — roots length index.
*, ** Differences to the control are statistically significant at P < 0.05 and P < 0.01.

The strongest toxic effect of phosphomide on the primary root system appeared at a concentration of 0.01 %, when Cara variety showed the maximum sensitivity to the mutagen (RLI = 0.41). In this variety, the shoot length reduced by 61.0 % at a low mutagen concentration (0.002 %), while 0.01 % concentration led to only a 41.0 % decrease. In Scant 3 variety and Cara × Scant 3 hybrid, a higher toxic effect was observed at 0.01 % mutagen concentration. Phosphomide did not stimulate root and shoot growth. The greatest inhibition of growth in the parental varieties and the hybrid was noted at a 0.01 % mutagen concentration.

The germination energy in laboratory experiments and field germination are the criteria for assessing the sowing qualities of seeds. Mutagen at a concentration of 0.002 % had a stimulating effect on seed germination rate in the hybrid form, and also contributed to germination energy in Cara which was 5.9 % higher as compared to that of the best parent variety (Table 3).

3. Seed sensitivity (compared to control, %**) to different phosphomide concentrations in spring soft wheat (*Triticum aestivum* L.) parent varieties and F_4 hybrid estimated in M_1 generation (**Biostation of Tyumen State University, Tyumen region, 2014; field and laboratory experiments**)**

Concentration, %	Samples	Seed germination energy	Field seed germination	Biological sustainability
0.01	Cara	75.0*	37.0*	27.0*
	Cara × Scant 3	47.1*	90.0	180.0*
	Scant 3	52.9*	64.9*	54.6*
0.002	Cara	100.0	97.0	90.0*
	Cara × Scant 3	105.9*	67.0*	114.0*
	Scant 3	94.1*	82.4*	82.5*

* Differences to the control (seeds treated with distilled water) are statistically significant at P < 0.05.

The control seeds of the varieties and hybrid were characterized by high field germination (97-100 %). A decrease in this index was noted in all variants with phosphomide, which may be due to violation in embryo development. Cara variety showed a high sensitivity to the mutagen at a concentration of 0.01 %. Biological sustainability of plants in this variant, calculated as the ratio of the number of plants that survived to harvest to the number of sown seeds, was very low (27.0 %). In the hybrid form, in contrast to the parental varieties, the toxic

effect of high concentration on field germination was less pronounced, and the effect of stimulation was observed for biological stability. The increased resistance of F_4 hybrid to the chemical mutagen could be due to heterozygosity associated with the hybrid origin or induced by phosphomide.

Quantitative trait expression, in the opinion of many researchers [24-27], depends on duration of vegetative period, plant length, resistance to lodging, and the sensitivity of photosynthetic apparatus to unfavorable environmental factors. The yield is the result of the complex interaction of the genotype with the environment [28-30].

We revealed an ambiguous plant response to the mutagen by seed production in M_1. When the seeds were treated with phosphomide at a concentration of 0.01 %, the stimulatory effect in Cara × Scant 3 hybrid was manifested in a statistically significant increase of grain weight per 1 m^2 by 16.0 % compared to the control. Also, there was a significant decrease in seed production in Cara and Scant 3 varieties (by 67.0 % and 57.0 %, respectively). The hybrid, which is more resistant to the chemical mutagen, as indicated by plant survival, also had higher grain weight compared to the original forms.

Parental and hybrid forms differed significantly in the prevalence (P) of fungal diseases under natural infection. In 2013, wheat plants had no leaf damage caused by mildew pathogen *Erysiphe graminis* DC. and brown rust pathogen *Puccinia recondita* Rob. ex Desm. f. sp. *tritici* Eriks. (= *P. triticina* Eriks.). The prevalence of powdery mildew reached a maximum (100 %) in 2014.

The highest activity of spotting caused by *Alternaria* spp. and *Helminthosporium* spp. was recorded in 2013 (P = 2-50 %). In this case, the Cara × Scant 3 hybrid, when seeds were treated with phosphomide at a concentration of 0.01 %, had the lowest prevalence of spotting (Table 4).

4. Resistance of M_1 and M_2 generations of spring soft wheat (*Triticum aestivum* L.) parental varieties and F_4 hybrid to fungal diseases depending on seed treatment with different phosphomide concentrations (Biostation of Tyumen State University, Tyumen region, 2013-2014; field trial**)**

Samples	Concentration, %	Powdery mildew		Brown rust		Spotting	
		P, %	Res	P, %	Res	P, %	Res
			M_1 (2013)				
Cara	Control	0	–	0	–	30	Very high
	0.002	0	–	0	–	5	Very high
	0.01	0	–	0	–	10	High
Cara × Scant 3	Control	0	–	0	–	40	High
	0.002	0	–	0	–	40	Very high
	0.01	0	–	0	–	2	Very high
Scant 3	Control	0	–	0	–	40	High
	0.002	0	–	0	–	30	High
	0.01	0	–	0	–	50	High
			M_2 (2014)				
Cara	Control	100	High	50	Very high	30	Very high
	0.002	100	High	50	Very high	30	Very high
	0.01	100	Low	100	Low	5	Very high
Cara × Scant 3	Control	100	Medium	100	Very high	5	Very high
	0.002	100	Low	50	Very high	10	Very high
	0.01	100	High	100	Low я	3	Very high
Scant 3	Control	100	Medium	100	Low	5	Very high
	0.002	100	Medium	100	Low	5	Very high
	0.01	100	Low	100	Low	10	Very high

N o t e. P — prevalence (disease occurrence); Res — resistance to disease; a dash indicates the absence of a disease.

A full picture of plant resistance to leaf phytopathogens can be obtained by determining the damage and disease development indices (R), calculated based on accounting sick and healthy plants and the damage score. Four groups were distinguished by disease development indices: very high resistance (R = 0-20 %), high resistance (R = 21-40%), medium resistance (R = 41-60%), low

resistance (R = 61-80%). In M_2 generations of parental varieties, there was a decrease in resistance to powdery mildew and brown rust pathogens from high and medium in the control to low at 0.01 % mutagen concentration. Under the influence of a high phosphomide concentration the hybrid susceptibility to powdery mildew decreased and to brown rust increased. The plants in the control and after the application of phosphomide showed high and very high resistance to spotting (Table 4).

In general, our laboratory and field experiments showed that phosphomide had activity similar to that of nitrosoethylurea and dimethyl sulfate supermutagens at 0.01, 0.02; 0.03 and 0.05 % concentrations, which we studied earlier on Truggve, WW 5530 and WW 6110 winter wheat varieties from Sweden [8]. The data obtained suggest that the phosphomide can be used in mutagenesis and spring soft wheat breeding. The role of experimental mutagenesis is confirmed by the creation in the world of more than 3000 varieties of different crops [31].

Thus, 0.002 and 0.01 % phosphomide concentrations effectively generated mutant plants in the spring soft wheat varieties and the intraspecies hybrid. Treating seeds with phosphomide led to a strong inhibitory effect on germination and morphometric parameters in seedlings which depended on the mutagen dose, and increased plant viability during the growing season. The hybrid Cara × Scant 3 had an advantage over the parents on seed production. Mutagen concentration of 0.01 % generated an increase in plant resistance to powdery mildew. A lower sensitivity to the mutagen in M_1 and a greater frequency and variety of mutants in M_2 were characteristic of the hybrid as compared to the parental varieties. There was no clear correlation between the susceptibility of varieties and the hybrid to phytopathogenic fungi and phosphomide concentrations. Differences in response to the mutagenic factor between the initial varieties and the hybrid form were due to the genotypic features of the varieties and to heterosis and heterozygosity of the hybrid. This can positively result in high adaptability which provides increased adaptation to unfavorable environmental conditions in newly created wheat varieties.

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PRESOWING TREATMENT OF SEEDS OF SPRING WHEAT WITH LOW-FREQUENCY ELECTROMAGNETIC FIELD

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A b s t r a c t

Seeking for effective natural stimulants that enhance crop productivity is relevant to ensure high quality yield production. The influence of physical factors (e.g. electric and magnetic fields, ultraviolet, infrared, laser irradiation) on seeds which contributes to an increase in sowing properties, plant photosynthetic activity, survival and yield is in the focus for researchers. However, a more detailed study of the mechanism of energy influence on the internal seed structure, plant growth and development sill remained relevant. Besides, it is necessary to develop effective, simple, reliable and low cost devices for agrophysical stimulation. The Federal Scientific Agroengineering Center VIM together with the Kazakh National Agrarian University has developed low frequency electromagnetic radiation construct which is mounted directly on combine-harvester to expose seeds to electromagnetic field during harvesting. The purpose of this study is to assess the sowing qualities of seeds and biometric indicators of the derived plants of spring wheat (*Triticum aestivum* L.) variety Omskaya 18, as influenced by low frequency electromagnetic fields depending on intensity and time of irradiation. The seeds were harvested in September 2015 (Republic of Kazakhstan). The construct used was developed on the basis of the magnetotherapy apparatus Almag-02, placed on a combine-harvester Enisey 1200 NM (Russia). We compared different modes of seed exposure. These were flow treatment (a dynamic mode) at magnetic induction B = 6 mT and frequency f = 10 Hz; static treatment for 3, 6, and 9 min at 6 mT and 3 Hz/16 Hz, and flow treatment at 6 mT and 3 Hz/16 Hz. Irradiated and non-irradiated seeds (control) were used in further studies. Seed germination parameters were estimated in laboratory tests. The seeds were germinated on filter paper in Petri dishes in darkness in a thermostat LP-113 (Labor Muszeripari Muvek Esztergom, Hungary). To assess the growth and development of seedlings and plants we used a phytotron Vic-Terra (FSAC VIM, Russia). Treatment with low frequency electromagnetic field for 9 min in static mode increased seed germination energy and germination rate by 12-13 %. When irradiating seed flow, germination was below the control, e.g. at 6 mT, 10 Hz this parameter decreased by 4.3 % in the laboratory test and by 3.5 % in the phytotron. Plant weight and height were higher in the irradiated samples. Under static irradiation for 9 min at 6 mT, 3 Hz and 16 Hz the plant weight was 0.56 and 0.59 g, respectively. The smallest weight (0.46 g per plant) resulted from flow treatment at 6 mT, 16 Hz. After storage of treated seeds at laboratory conditions for 3 and 7 months the indicators (germination energy and germination rate) remained satisfactory. The best result was noted for seeds, processed in static mode for 9 min at 6 mT, 3 Hz/16 Hz. A decline in sowing properties did not exceed 6 %, indicating the preserving effect of the electromagnetic field.

Keywords: wheat, *Triticum aestivum*, seeds, low-frequency electromagnetic field, germination rate, germination energy, phytotron

Seed accumulate genetic and biological potentials of a plant variety and determine considerably the quantity and quality of the yield that defines the role of seed production in the sustainable plant growing as a whole [1-3]. Germination of seeds when their nutrients undergo significant qualitative changes is one of the critical periods of ontogenesis affecting all the growth and development stages. These are seeds where physicochemical and physicobiological processes as

well as morphological modifications leading to increased permeability of coverings occur, the activity of hydrolytic and oxidation-reduction enzymes rises, the cell division speeds up, and reactions maintaining normal germ functions are activated [4].

The bioenergy potential of seeds when stored is depleted that may have adverse effects on the growth, development and disease-resistance of the resulting plants. To restore the potential, various pre-sowing seed treatment methods are applied which improve seed properties and increase plant yields [5-7].

In recent years, electrophysical treatment methods for plants and seeds of grain, vegetable and legume crops have been introduced extensively into agricultural practices to intensify plant growing [8, 9]. Physical factors influencing seeds may include electromagnetic fields of various ranges (from γ-radiation to UHF radio-frequency range), X-ray radiation, ultraviolet and optical radiation (particularly, laser red radiation with λ= 632.8 nm), infrared radiation, electric corona field, and ultrasonic exposure [10, 11].

There are about 170 published works where results of using different seed irradiation sources are reflected [8]. Also, there are studies in which the mild exposure of physical factors stimulated the crop yield and improved the quality of products [12, 13]. For example, the laser treatment increased electrical conductivity of plant tissues by 10-14 %, improved ion-exchange processes, enhanced permeability of biological membranes, water adsorption, spare capacities and protective reactions of seeds. The leaf surface area, stooling and disease-resistance of plants increased [8].

Using the electromagnetic exposure of different frequencies may result in yield gain by 10-12 %, kill a seed infection, increase germination energy and germinating ability of seeds [14-16], enhance plant resistance to fungal and bacterial diseases [17, 18]. The research paper of I.V. Egorova et al. [19] contains data that the high-frequency electromagnetic field treatment (UHF EMF) enhances the biological value of wheat grains due to an increase in some water-soluble vitamin contents, and influences biometric indicators of radicles. A good number of experimental works are dedicated to the UV irradiation of seed materials [20-23]. It has been noted that plants are highly sensitive to irradiation conditions [24, 25]. So, growth processes are inhibited in the short-wave region (λ = 254 nm), while in the mid-wave (λ = 313 nm) and long-wave regions (λ = 365 nm) they are stimulated.

In studies of grain, vegetable and forage crops, it was found that the short-term exposure to high-voltage corona discharge resulted in accelerating seed germination both in the laboratory and field conditions. The germination energy increases by 5-22 %, there is a higher number of plants survived until crops. It has been noted that, on day 5-7 of their germination, plants derived from the treated seeds outgrow those from untreated seeds, and such outgrowing persists throughout the vegetation period. It is generally believed that, during such treatment, the germination energy and increase in the field germination rate of seeds are positively influenced by their surface disinfection [26] which restricts the spread and development of powdery mildew, brown rust, root rots and other diseases.

In this work, we have shown for the first time that the static low-frequency electromagnetic irradiation of spring wheat seeds (*Triticum aestivum* L.) during the combine harvesting improves sowing properties and biometric indicators of plants as well as increases the seed quality stored for 7 months (under laboratory conditions). In the dynamic irradiation mode, sowing qualities of seeds and morphological parameters of plants were somewhat worse.

Our purpose was to assess the impact of the low-frequency electromag-

netic field of various intensity and time of irradiation on the germination energy, germinating ability of spring wheat seeds and biometric indicators of spring wheat plants while growing and developing under the phytotron conditions.

Technique. During combine harvesting, seeds of spring wheat (*Triticum aestivum* L.) variety Omskaya 18 (Ulanskaya MTS, Republic of Kazakhstan, September 2015) were exposed to the low-frequency electromagnetic field using radiators of Almag-02 magnetotherapy device (Yelatma Instrument-Making Enterprise, Russia) installed on Enisei 1200 NM combine-harvester (Krasnoyarsk Combine Harvester Plant, Russia).

The system allowed irradiation of both moving flow and stationary mass of seeds varying the electromagnetic field exposure time and characteristics. The following programs of the magnetotherapy device were used: flow treatment of seeds (dynamic mode) at the magnetic induction $B = 6$ mT and frequency $f = 10$ Hz (Program № 7); static treatment for 3; 6; 9 min at $B = 6$ mT and $f = 3$ Hz or $f = 16$ Hz (Programs № 22 or № 23, respectively); flow treatment in Programs № 22 and № 23. Flow irradiated seeds were sampled from beneath the top of the elevator that delivers the seeds from a grain screw conveyor to a tank. During the static treatment mode, samples taken from the non-irradiated thrashed heap in a tank were spread in a thin layer on a fixed surface and radiators were placed on them.

Irradiated and non-irradiated seeds sampled from the tank (control samples) were stored under laboratory conditions and used in studies. In 3 and 7 months, germination energy and germinating ability of the seeds were identified in accordance with State Standard GOST 12038-84 [27]. The seeds were germinated on a filter paper in Petri dishes under darkroom conditions in LP-113 thermostat (Labor Muszeripari Muvek Esztergom, Hungary) (in 4 replicates). Each sample contained 100 seeds. The germination energy was identified on day 4, the germinating ability — on day 7-8.

The formation of seedlings, growth and development of plants were studied on a real-time basis in Vic-Terra vegetation and growth chamber (phytotron with the overall dimensions of 3100×1700×1400 mm) (FSAC VIM, Russia) under controlled conditions [28]. Twelve containers (850×1300 mm) were placed in the isolated box of the phytotron. The unit was complete with lighting, computer-based air temperature and relative humidity control as well as irrigation systems. The seeds were sowed in 12 containers filled with soil (pH 6.7-7.0) on December 4, 2015. The depth of seeding was 3-4 cm. There were two furrows spaced 15 cm in each container. Biometric indicators of the plants (shoot weight and height, root length) were identified in 31 days.

The experimental findings are represented as the arithmetic mean (*X*) and its standard error ($\pm x$). They were calculated using Statistica 8.0 (Dell Software Inc., USA) and Microsoft Excel 2007.

Results. The static low-frequency electromagnetic irradiation of wheat seeds increased the germination energy and germinating ability by 12.7 % (Fig. 1, laboratory test). The seeds irradiated in flow in all the programs had lower germination energy and germinating ability as compared to the control samples.

The maximum germinating ability of the seeds in the phytotron (89-90 %) was observed in the static irradiation for 9 min (6 mT, 16 Hz). The earliest seedlings have appeared on day 8 out of the seeds treated in Program № 23 for 6-9 min. Upon exposure, a stem shoot was overgrown with 2 to 5 true green leaves and stopped to grow for a while. At this time, secondary roots and new stem shoots branching the leading shoot were formed. The root system developed, the tillering stage started (Fig. 2). It should be noted that the root system of plants derived from the statically irradiated seeds was more extensive as compared to that

of the control plants and plants grown out of the flow irradiated seeds.

Fig. 1. Germination energy (a) **and germinating ability** (b) **of seeds of spring wheat (*Triticum aestivum* L.) variety Omskaya 18 exposed to the low-frequency electromagnetic field in the static and flow mode, at the magnetic induction B = 6 mT and frequencies f = 3 Hz** (A) **and f = 16 Hz** (B) **for different time intervals:** 1 — control (untreated); 2 — 3 min, 3 — 6 min, 4 — 9 min (static mode); 5 — flow (laboratory experiment).

Fig. 2. Spring wheat (*Triticum aestivum* L.) variety Omskaya 18 plants grown to the initial stage of tillering in the phytotron after seed exposure to the low-frequency electromagnetic field): 1, 2, 3 — control (non-irradiated), 4 and 5 — static irradiation at B = 6 mT and f = 3 Hz for 3 and 9 min, respectively.

The exposure of wheat seeds to the low-frequency magnetic field affected biometric indicators of the plants derived (Table 1). The plant weight changed depending on the impulse frequency and time of irradiation. When using Program № 23, the plants had greater weight, longer roots and were higher. So, the total weight of freshly harvested plants was 0.10-0.15 g higher than that of the plants derived from the seeds irradiated in Program № 22. In the dynamic flow treatment, there were no significant differences between the two programs. The maximum weights of a plant, i.e. 0.56 g and 0.59 g, were obtained upon seed irradiation for 9 min in Programs № 22 and № 23, respectively. This was 20-25 % higher than for the control plant. The minimum total weights (2.30-2.40 g) of plants were observed upon flow treatment of seeds in all the programs (see Table 1).

1. Biometric parameters of spring wheat (*Triticum aestivum* L.) variety Omskaya 18 plants at tillering depending on the mode of low-frequency electromagnetic field irradiation of seeds (laboratory experiment)

Показатель	C	P № 22				P № 23				P № 7 (f)
		irradiation time, min								
		3 (s)	6 (s)	9 (s)	(f)	3 (s)	6 (s)	9 (s)	(f)	
Total weight of plants, g	2.35	2.55	2.50	2.80	2.50	2.60	2.60	2.95	2.30	2.40
Δ, %		0.25	0.15	0.45	0.15	0.25	0.25	0.60	0.05	0.05
Weight of a plant, g	0.47	0.51	0.50	0.56	0.50	0.52	0.52	0.59	0.46	0.48
Δ, %		0.04	0.03	0.09	0.03	0.05	0.05	0.12	−0.01	0.01
Root length, cm	2.12	2.24	3.28	2.50	3.70	3.90	3.00	4.90	3.80	3.30
Δ, %		0.12	1.16	0.36	1.58	1.78	0.88	2.78	1.68	1.18
Plant height, cm	29.0	30.2	36.6	38.5	38.0	34.7	35.0	36.4	30.2	32.7
Δ, %		1.2	7.6	9.5	9.0	5.7	6.0	7.4	1.2	3.7

N o t e. C — control, P — irradiation program (see the program description in the *Technique* section); Δ, % — difference between indicators of plants derived from the irradiated and non-irradiated seeds; f — flow irradiation, s — static mode. The relative error of the mean values did not exceed 5 %.

It was important to know whether the stimulating effect of the electromagnetic field was preserved when storing seeds under laboratory conditions. It was found out that the exposure of seeds to the low-frequency magnetic field was good for their quality after storage (Table 2).
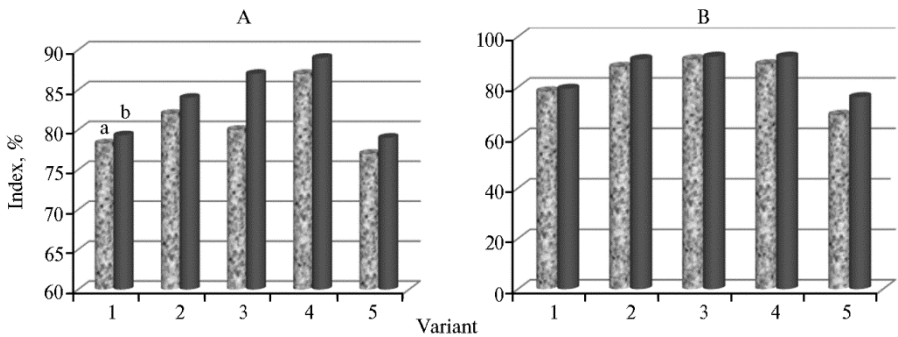
2. Germination energy and germinating ability of seeds in spring wheat (*Triticum aestivum* L.) variety Omskaya 18 after storage depending on the mode of low-frequency electromagnetic field irradiation (*X*±*x*, laboratory experiment)

Parameter	C	P № 22				P № 23				P № 7 (f)
		irradiation time, min								
		3 (s)	6 (s)	9 (s)	(f)	3 (s)	6 (s)	9 (s)	(f)	
					Upon storage for 3 months					
Germination energy (December 2, 2015), %	78.3±1.2	82.0±0.9	80.0±1.1	87.0±0.8	77.0±1.2	88.0±1.2	91.0±0.8	89.0±0.9	69.0±1.3	74.0±1.4
Δ, %		3.7	1.7	8.7	–1.3	9.7	12.7	10.7	–9.3	–4.3
Germinating ability (December 9, 2015), %	79.3±1.2	84.0±0.8	87.0±0.9	89.0±1.1	79.0±1.3	91.0±0.9	92.0±1.2	92.0±0.9	76.0±1.5	75.0±1.3
Δ, %		4.7	7.7	9.7	–0.3	11.7	12.7	12.7	–3.3	–4.3
					Upon storage for 7 months					
Germination energy (April 15, 2016), %	75.7±1.1	80.0±0.9	81.0±0.9	85.0±1.2	61.0±2.0	83.0±1.3	83.0±1.2	86.0±0.9	68.0±1.1	75.0±1.6
Δ, %		4.3	5.3	9.3	–14.7	7.3	7.3	10.3	–6.7	–0.7
Germinating ability (April 19, 2016), %	77.7±1.3	84.0±1.2	85.0±0.9	88.0±1.1	66.0±1.4	86.0±0.9	86.0±0.8	89.0±1.2	69.0±1.4	76.0±1.3
Δ, %		6.3	7.3	10.3	–11.7	8.3	8.3	11.3	–8.7	–1.7

N o t e. C — control, P — irradiation program (see the program description in the *Technique* section); Δ, % — difference between indicators of plants derived from the irradiated and non-irradiated seeds; f — flow irradiation, s — static mode.

The maximum increase of the seed germinating ability as compared to the control samples (by 12.7 %) was observed after 3 months of storage for the seeds treated in Program № 23 (6 mT, 16 Hz, 6 and 9 min). There was some decline in sowing qualities of seeds in 7 months of storage as compared to those after 3 months (see Table 2). For example, the difference of both germination energy and germinating ability in the static treatment of seeds in Program № 22 (3-9 min) did not exceed 2.0 %. The germinating ability of the seeds treated in Program № 23 decreased by 3-6 %. It should be noted that the indicators were significantly lower for the seeds exposed to the electromagnetic field in the flow in Programs № 22 and № 23.

Thus, the exposure of wheat seeds to the low-frequency electromagnetic field during harvesting contributes to the increase in their germination energy, germinating ability, and, thereafter, in the weight, root length and height of the plants derived. The electromagnetic exposure effectiveness depends on the impulse frequency and time of irradiation of seeds. In our experiments, the maximum stimulating effect on the sowing qualities of seeds and biometric indicators of plants was observed in the treatment for 9 min at the electromagnetic induction of 6 mT and the frequency of 16 Hz. The seed germination energy and germinating ability have increased by 12-13 %, and the plant weight at the tillering stage has increased by 20-25 % as compared to the control samples (non-irradiated seeds). When treating the seed flow, the germinating ability of the seeds in the laboratory conditions decreased. It was found out that the magnetic field had positive impact on the seed properties after 3 and 7 months of storage under laboratory conditions.

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Events

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DYNAMICS OF CHERNOZEM MICROBIAL COMMUNITY DURING BIODEGRADATION OF CELLULOSE AND BARLEY STRAW

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Abstract

Study of cellulose decomposition is an extremely important for the agricultural sciences, as straw is one of the most affordable organic fertilizers. In this area, a large number of research works devoted to biochemical mechanisms of cellulose destruction, as well as study of the cellulolytic microorganisms' taxonomic diversity were conducted. However, the composition of the microbial community was estimated only by the methods based on cultivation and thus describing only a very small part of the soil microbiome. With the advent of «new generation sequencing» methods the analysis of whole microbial communities found in the soil became possible. The main objective of this work was the implementation of an integrated approach, combining agrochemical techniques of biodegradation processes intensity estimation with modern molecular methods (soil metagenome analysis of 16S rRNA) in the process of cellulose decomposition in two substrates (straw and filter paper). It was first shown by the method of high-throughput sequencing that the introduction of a straw in soil caused dipper changes in the structure of the microbial community than the introduction of chemically pure cellulose. The model experiment was carried out on typical chernozem, sampled in the Voronezh region in fallow areas from a depth of 2-15 cm. Chemically pure cellulose (very fine-chopped filter paper) and crushed straw of barley were added at 1 g per 100 g of soil. Analyzes were performed on days 0, 7, 14, 21 and 28. CO_2 emission, the content of nitrates, ammonium nitrogen, labile carbon, and microbial biomass were estimated. During the first 7 days, microbial mass and respiration increased when straw was added. For cellulose, an increase in biomass was not significant, and respiration was activated with a delay. The introduction of cellulose and straw reduced the soil level of nitrate nitrogen compared to control, and with straw, the indicator lowered to a lesser extent. In this work, some taxa were found, the proportion of which increased (*Chthoniobacteraceae, Xanthomonadaceae, Chitinophagaceae*), and decreased (*Gaiellaceae*). Among the microorganisms whose proportion increased after the introduction of cellulose, we found classic cellulose destructors (*Chitinophaga* and representatives of the families *Streptosporangiaceae* and *Micromonosporaceae*), and microorganisms whose ability to decompose cellulose had not previously been reported yet (*Chthoniobacter, Chitinophaga*). Community homeostasis was found (i.e. significant changes in the composition of microbiome on day 14 of the experiment returned to the original state on day 28). Agrochemical analysis (dynamics of nitrate nitrogen content, the rate of release of carbon dioxide, dynamics of bacterial biomass) fully agreed with the analysis of metagenomic data in fact that the microbial community actively respond to the introduction of straw as opposed to the introduction of pure cellulose.

Keywords: microbiome, soil, metagenome, straw, cellulose, biodegradation

Destruction of plant residues in soil is one of the most important biogeo-

chemical functions of soil microorganisms. Global climate changes directly affecting the temperature and water regimes alongside with increasing human-induced adverse impact may change significantly the biodegradation tendency and intensity for organic substrates. A hypothesis of that a final result of substrate destruction depends not only on its chemical composition but also on a structure of destructing microbial community gains increasingly strong confirmations. However, regularities of biodiversity formation and microbial community functioning during the straw decomposition remain unstudied [1]. It is particularly associated with the multifunctionality of microorganisms, i.e. changes in the microbial community structure do not necessarily depend on a type of substrate applied [2].

One of the most important components of plant residues, including those by their weight, is cellulose. Straw is one of the most affordable crop residues in agriculture, which are recommended to be left on fields and added to soil. It is considered a good resource for humus accumulation. According to different sources, its isohumic coefficient equals to 0.10-0.25, i.e. leaving of 2-4 t of straw causes the formation of 0.2-1.0 t of humus per 1 ha [3]. We have assumed that pure cellulose and straw will be decomposed in different ways due to their chemical peculiarities. Straw consists of 90 % of fiber, which is composed of 35-45 % of cellulose, 14-20 % of lignin, 20-30 % of pentosans, 2-3 % of kutin, and 3-5 % of silica salts [3]. As a consequence, the destruction of different substrates will involve different microorganisms, first of all, cellulose-decomposing ones, that is of real practical and academic interest [4, 5].

The study of a microbial community when adding straw and pure cellulose in soil by molecular methods will enable to better demonstrate a role of the functional structure of the community, trace its relationship with a taxonomic composition, space-time variability of the mentioned indicators, and agrochemical parameters of soil. This approach will make it possible to establish a probable role of microorganisms which are not taken into account by traditional methods. It is also interesting to consider differences in the taxonomic composition of microbial communities that decompose straw and pure cellulose over time, and compare microbiomes formed by the end of the process.

It was first shown by the high-throughput sequencing method that the introduction of straw in soil caused deeper changes in the structure of the microbial community than the introduction of chemically pure cellulose.

The objective of the work was to implement an integrated approach that combines conventional biochemical techniques for estimation of intensity of biodegradation processes with modern molecular methods in order to study microbial communities decomposing two cellulose-containing substrates (straw and filter paper) over time.

Technique. In a model experiment, chemically pure cellulose (as a very fine-chopped filter paper) and shredded straw of barley (ash 7.6 %, N_{total} 1.95 %, P_{total} 1.1 %, K_{total} 2.47 %) were added to typical chernozem sampled in Voronezh region (Kamennaya Steppe conservation area, 51°01′41.6″N, 40°43′39.3″E) in fallow areas from a depth of 2-15 cm (C_{total} 4.48±0.06 %, C_{humus} 4.37±0.05 %, N_{total} 0.394±0.002 %, pH_{water} 6.31±0.01, pH_{salt} 7.35±0.0, total exchangeable bases 44.17±0.43 mg-equ/100 g, labile phosphorus and potassium content according to Machigin 67.5±8.0 and 12.6±0.8 mg/100 g). Soil without any cellulose-containing substrates added was used as a control.

The substrate was added at 1 g per 100 g of soil. The experiment was carried out in Petri dishes, in 5 replicates for a period of the experiment, and 7 replicates for the last measurement. Dry soil (50 g) was placed into each dish and 30 g of dry soil was places into each 500 ml vial (to measure carbon dioxide

emissions, in 5 replicates). The soil was wetted up to 50 % of the full field moisture capacity, placed under a glass dome together with a water container in order to maintain the constant humidity level and incubated in a dark room at 23-25 °C. CO_2 emission, contents of nitrates, ammonium nitrogen, labile carbon, microbial biomass were estimated on the day when the substrate were introduced (day 0), as well as on days 7, 14, 21 and 28. The taxonomic structure of a microbiome was identified based on the total DNA analysis. All the data was obtained for a mixed sample, in 3-5 replicates.

Agrochemical soil analyses were conducted by generally accepted methods [6]. Content of labile carbon in an extract, according to Shults [7], was identified using Ultraspec spectrophotometer (LKB, Sweden; $\lambda = 340$ nm) [8]. Soil respiration was measured by Tsvet gas chromatograph (ZAO Tsvet, Russia) equipped with a thermal-conductivity detector and using helium as a carrier gas. Microbial biomass in soil was measured by the substrate-induced respiration method [9], with identifying the total biomass and fungal biomass (treatment with streptomycin and rifampicin, 16 mg of antibiotic per 1 g of soil).

DNA was extracted from 0.5 g of soil in 3 replicates for each time period (a total of 45 samples). The samples were exposed to mechanical breakdown for 1 min in an extraction buffer with glass beads at the maximum power in Precellys apparatus (Bertin Technologies, France). The extraction buffer consisted of 350 µl of solution A (200 mM sodium phosphate buffer, 240 mM guanidine isothyocyanate; pH 7.0), 350 µl of solution B (500 mM Tris-HCl, 1 % SDS, w/v; pH 7.0), 400 µl of phenol:chloroform mixture (1:1). The preparation was centrifuged at the maximum speed for 5 min. Then, the aqueous phase was sampled and the re-extraction with chloroform was conducted. DNA was precipitated by adding an equal volume of isopropyl alcohol. After centrifugation, the precipitate was washed by 70 % ethanol and dissolved in water at 65 °C for 5-10 min. It was purified by 1 % agarose gel electrophoresis with further DNA extraction from the gel by silicon oxide sorption method [10, 11].

The purified DNA preparation (10-15 ng) was used as a matrix for the PCR (30 s at 95 °C, 30 s at 50 °C, 30 s at 72 °C; a total of 30 cycles) with applying Encyclo polymerase (Evrogen, Russia) and universal primers to variable region V4 of 16S rRNA gene: F515 (5′-GTGCCAGCMGCCGCGGTAA) and R806 (5′-GGACTACVSGGGTATCTAAT) [12]. Besides, oligonucleotide identifiers for each sample and auxiliary sequences required for pyrosequencing as per Roche protocol (Switzerland) were introduced in primers. Sample preparation and sequencing was carried out in GS Junior apparatus (Roche, Switzerland) in accordance with the manufacturer's recommendations.

The resulting sequences were analyzed in QIIME 1.8.0 [13]. Libraries were classified by identifiers, sequencing quality was checked and nucleotide sequences were filtered, sequences were combined into operational taxonomic units (OTU, analogue of species) by de novo method based on 97 % threshold of similarity of genes 16S rRNA [14], representative sequences were sampled (one from each OTU), representative nucleotide sequences were aligned by Uclust method, matrix of genetic distances and phylogenetic tree were constructed by Fasttree method. The sequences presented in all samples less than twice as well as those belonging to chloroplasts were deleted from the data obtained. Taxonomic identification of OTUs was carried out using RDP algorithm (http://rdp.cme.msu.edu/) and Greengenes data bank [15].

Based on the table of OTU representation in samples, Shannon's biodiversity indices (ChaoI) were calculated: S_{est} (ChaoI) $= S_{obs} + a^2/2b$, where, S_{est} is the estimated number of OTUs; S_{obs} is the observed number of OTUs; a is the number of OTUs found once; b is the number of OTUs found twice. Moreover,

cumulative curves that characterize the OTU number expansion over time depending on the quantity of sequences experienced sequencing have been plotted. Statistical processing was carried out in QIIME (http://qiime.org/). In recording the bacterial biomass, nitrate nitrogen content in soil and CO_2 emission, the mean values and standard errors of mean ($\pm$SEM) were calculated. Significance of differences was estimated by the Student's *t*-test (significance level $p < 0.05$).

Results. One of the parameters reflecting an increment in microbiological activity of soil during the destruction of complex organic compounds is an amount of bacterial biomass. The maximum biomass was registered after the straw had been added to soil (Fig. 1, A). The total bacterial biomass accumulated after introduction of cellulose in soil showed no statistical difference from the control. The reason for this was probably a lack of nitrogen accessible for microorganisms as there was no nitrogen in the chemically pure cellulose, while in the straw it was present with carbon at the ratio of C:N = 23.7. In addition, the tested straw contained a considerable amount of labile organic substances that resulted in the increase of their proportion in soil by 40 % as compared to the control.
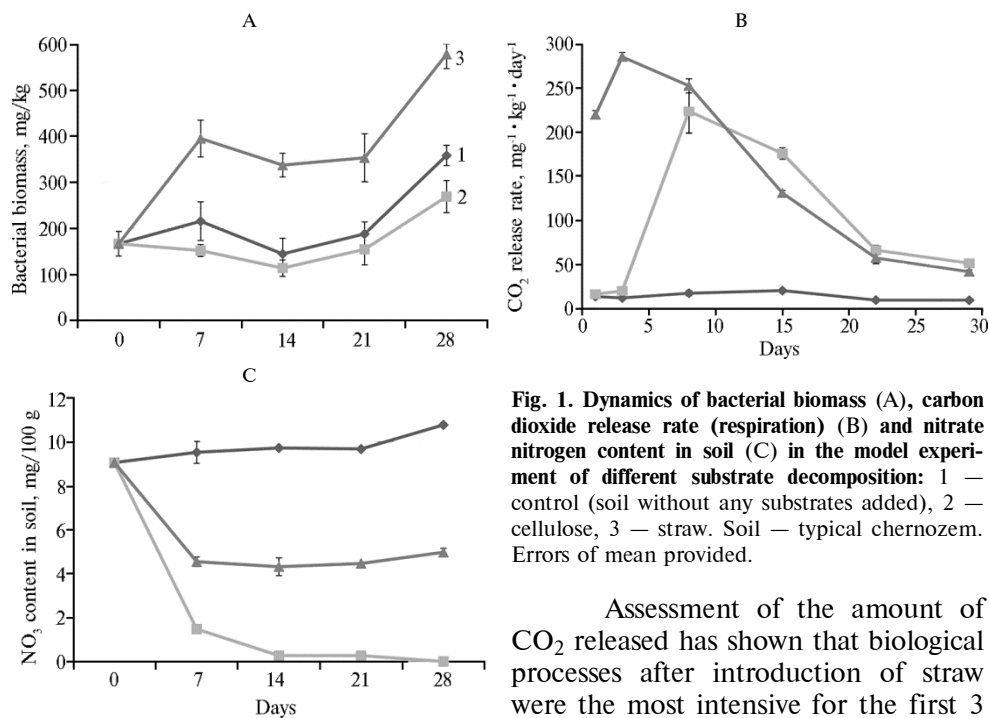
Fig. 1. Dynamics of bacterial biomass (A), carbon dioxide release rate (respiration) (B) and nitrate nitrogen content in soil (C) in the model experiment of different substrate decomposition: 1 — control (soil without any substrates added), 2 — cellulose, 3 — straw. Soil — typical chernozem. Errors of mean provided.

Assessment of the amount of CO_2 released has shown that biological processes after introduction of straw were the most intensive for the first 3 days (see Figure 1, B) due to the use of water-soluble organic compounds that is consistent with the literature data [16]. For cellulose, there was a delay in activation of microorganisms followed by carbon dioxide emission (lag-phase was observed) [17, 18]. Starting from day 8, the intensity of carbon dioxide release from soil decreased.

The introduction of cellulose and straw decreased the content of nitrate nitrogen in soil as compared to the control (see Fig. 1, C), and the decrease for straw was lesser than for pure cellulose. It is explained by the already mentioned absence of nitrogen in the filter paper that causes the immobilization of mineral nitrogen from soil during the decomposition process [3]. For straw, the substrate was decomposed with the use of nitrogen contained both in soil and the substrate itself.

Our data obtained by conventional methods are consistent to each other. During the first 7 days we observed the gain in biomass and activation of respire-
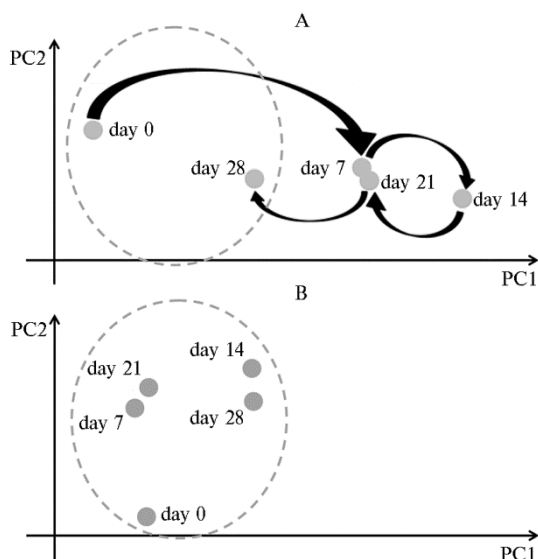
Fig. 2. **Results of the similarity analysis of microbial communities (β-diversity analysis) by the principal component method** (PC) **when introducing straw** (A) **and cellulose** (B) **in soil** (model experiment, typical chernozem soil)**.** Samples averaged for replicates (3 replicates for each day) are ringed. Locations of control samples (soil without substrates introduced) are dotted.

tion processes related thereto when the straw was added. For cellulose, there was no significant gain in biomass, and the respiration intensified with delay. It may be assumed that the straw as a substrate has stimulated a considerable part of the bacterial community, including R-strategists which actively utilize easily-accessible nutrients from plant residues [18]. Cellulose has activated a small number of microorganisms specialized only in the destruction of this substrate. The observed dynamics of amounts of nitrogen-containing compounds was associated with that soluble forms of nitrogen in the experiments with cellulose-containing substrates were involved in the biodegradation of the latter. Since the straw contains organic nitrogen in itself, bacteria will, first of all, consume this nitrogen.

As a result of sequencing DNA extracted from soil, 87,563 sequences belonging to 408 OTUs have been obtained. There were OTUs from 22 bacterial phyla and 1 archaea phyla, as well as 265 procaryotic families. Biodiversity of the bacterial community in soil remained unchanged irrespective of the substrate introduced (indices of Shannon and ChaoI varied within 7.4-6.9 and 611-528, respectively).
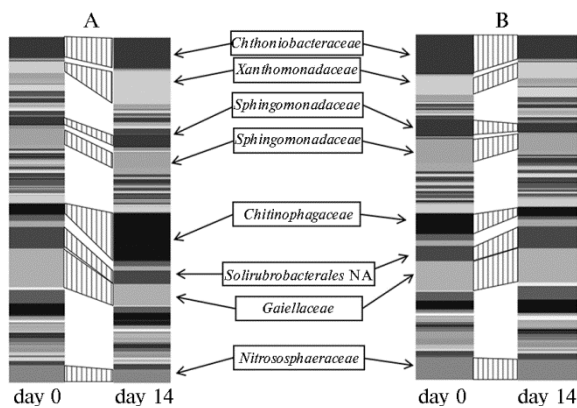
Fig. 3. **Bacterial community composition in soil on day 0 and day 14 of the experiment when introducing straw** (A) **and cellulose** (B) (model experiment, typical chernozem soil).

Straw added to soil had greater impact on the bacterial community composition than the filter paper did. According to the high throughput sequencing and the agrochemical analysis, the most significant changes in the community composition were observed on day 14 with its further recovery to the original state (control, Fig. 2) that was indicative of the community homeostasis, the maintenance of which resulted in the original structure recovery in the decomposition processes of cellulose-containing substrates. It should be noted that bacteria families was found whose proportion in the community either decreased (*Gaiellaceae*) or increased (*Chthoniobacteraceae*, *Xanthomonadaceae*, *Chitinophagaceae*) (Fig. 3). On the plot constructed by the principal component method for the cellulose introduction, all the samples (irrespective of sampling

time) were close to the control ones. Hence, cellulose had a slight impact on the microbial community in soil at initial biodegradation stages.

On day 28 of the experiment with straw, we observed a significant increase in a number of bacteria (88 times as much in some cases) belonging to genus *Chitinophaga*, families *Streptosporangiaceae* and *Micromonosporaceae* (Fig. 4). According to the present-day data, representatives of these taxa are able to destroy cellulose [19-21]. On day 28 after introducing the filter paper in soil, an increase in abundance of bacteria of families *Promicromonospora* and *Devosia* was registered (Fig. 4). They also can produce cellulosolytic enzymes [22, 23]. Only one OTU has been revealed there, its number increased both for the straw and for filter paper. This OTU belonged to genus *Chthoniobacter* phylum *Verrucomicrobia*. Bacteria of the phylum are understudied yet (one cultivated representative), therefore, there is no data of their ability for cellulose decomposition [24]. All the found archaea belonged to genus *Nitrososphaersceae* widely spread in soils of Russia [25]. However, this OTU showed no significant dynamics throughout the experiment (see Fig. 2).
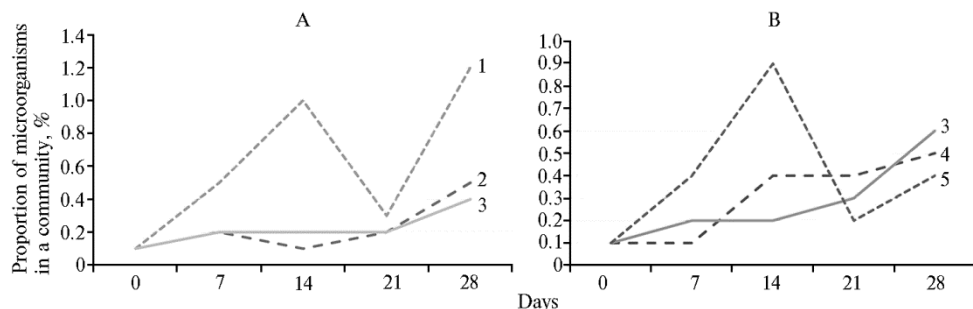
Fig. 4. Dynamics in number of bacteria of *Streptosporangiaceae* (1) and *Micromonosporaceae* families (2) and genera *Chtoniobacter* (3), *Promicromonospora* (4), *Devosia* (5) when introducing straw (A) and cellulose (B) in soil (model experiment, typical chernozem soil).

As we are the first who applied the metagenomic analysis of gene 16S rRNA, it is difficult to compare the data obtained with any results of other works. The most authors studied the species identity of cellulosolytics revealed [26-28], while we used taxonomic units. The data obtained earlier has been confirmed in our study on the basis of higher taxonomic ranks (families and orders), as the majority of works indicate that actinobacteria are actively involved in the cellulose decomposition. However, we have also identified OTUs belonging to genera whose ability to decompose cellulose in soil had not been reported yet (*Chthoniobacter*, *Chitinophaga*).

It should also be noted that all the regularities revealed have been tested only for the soil concerned (typical chernozem) and are subject to confirmation for other types of soils (sod-podzolic, grey forest soil, etc.). Further study of cellulose decomposition processes in soil will be associated with an analysis of cellulose hydrolysis enzyme system which is represented in soil bacteria by complex aggregates of proteins that can break β-1,4-glucosidic bonds inside of a cellulose molecule (endoglucanase), from the reducing and nonreducing ends (cellobiohydrolase), as well as in the glucose dimer cellobiose (cellobiase). Molecular genetic testing of microorganisms (including genomic sequencing) responsible for the destruction of cellulose-containing substrates is required as well.

Thus, we have obtained similar results using both conventional agrochemical methods and high throughput sequencing. The decomposition of the straw added to soil involved a considerable part of the bacterial community as this substrate was more well-balanced in the composition of primary biogenic elements and more natural for the soil. As a result, we observed the increased

respiration of microorganisms, gain in their biomass through day 14 and further reduction in these parameters by day 28. Only a small part of the bacterial community appeared to be able to destruct pure cellulose, no significant differences in the composition of microbial communities for this variant over time were revealed. The obtained data on changes in a microbiome after introducing the straw and chemically pure cellulose may serve as a basis for developing predicative dynamics models for microbiomes during the organic substance decomposition process depending on a type of substrate to be decomposed and physicochemical properties of soil. Predicative models to be developed in future will allow studying relationships between the taxonomic and functional structures of the soil microbiome, as well as improving the composition of microbial preparations and their application methods.

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Genotypes: analysis and selection

PEA (*Pisum sativum* L.) CULTIVARS WITH LOW ACCUMULATION OF HEAVY METALS FROM CONTAMINATED SOIL

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A b s t r a c t

Heavy metals are among the most common contaminants of agricultural lands. Cleaning (remediation) of such territories is extremely difficult or impossible. A promising approach for the production of environmentally friendly products of crop industry in the contaminated soils can be a selection of varieties with reduced accumulation of heavy metals. The aim of this work was to study the variability of pea in accumulation and transport of heavy metals from shoots to seeds and to identify varieties with low accumulation of heavy metals from contaminated soils. The objects of research were 30 varieties of pea (*Pisum sativum* L.) from the collection of Federal Research Center N.I. Vavilov All-Russia Institute of Plant Genetic Resources (St. Petersburg). The pot experiment was carried out in summer in a greenhouse with natural light and temperature (All-Russian Research Institute of Agricultural Microbiology, St. Petersburg). Pea seeds were surface-sterilized and scribbled with concentrated H_2SO_4 for 30 minutes and germinated for 3 days at 22 °C in Petri dishes with wet filter paper. The seedlings were planted in pots (5 seedlings per pot, 3 pots for each genotype) containing 5 kg of sod-podzolic fallow soil. Ten days before seed sowing the soil was enriched with heavy metals in the form of chlorides (mg/kg): Cd — 5, Co — 25, Cr — 60, Cu — 10, Ni — 15, Pb — 100, Sr — 50, Zn — 50. At the same time, fertilizers were applied (mg/kg): NH_4NO_3 — 15, KNO_3 — 200, KH_2PO_4 — 200, $MgSO_4$ — 30, $CaCl_2$ — 20, H_3BO_3 — 3, $MnSO_4$ — 3, $ZnSO_4$ — 3, Na_2MoO_4 — 1.5. The plants were grown until the seed maturing phase, dried and ground to a powder. Samples (separately shoots and seeds) were digested in a mixture of concentrated nitric acid and 38 % H_2O_2. The content of heavy metals and nutrients was determined using ICPE-9000 spectrometer (Shimadzu, Japan). The studied samples differed significantly in the content of heavy metals in shoots and seeds that indicated a high variability of pea in the accumulation of heavy metals and their transport from vegetative to reproductive organs. The variability values for shoots and seeds were comparable in magnitude, but did not correlate with each other. The shoots or seed contents of various heavy metals, as well as nutrients, in many cases positively correlated, which could be due to the diversity of molecular transport channels in plants and their low specificity. There was positive correlation between the content of elements in shoots and seeds for Cd, Co, Cr, Ni, P, Sr, and negative was found between the shoot and seed contents of Zn and K. The results indicate specific mechanisms of transport of individual elements from shoot to seed and the barrier for abiogenic metal transport from vegetative to reproductive organs. The effectiveness of these mechanisms depends significantly on the plant genotype. The possibility of selection of pea varieties with a low content of many heavy metals simultaneously is shown. Pea varieties k-188, k-1027, k-1250, k-2593, k-3445, k-4788, k-5012, k-6468, k-8093 and k-8543 are recommended for use in selection programs for obtaining ecologically safe crop production.

Keywords: biodiversity, pea, soil contamination, heavy metals, environmentally friendly

products

Environmentally friendly and energy-saving approaches for developing sustainable agriculture and providing with quality food products is becoming especially important because of growing anthropogenic pollution of the environment. Heavy metals (HM) (Cd, Co, Cr, Cu, Ni, Pb, Zn) of the highest class of hygienic hazard are among the most common soil pollutants. The total area of HM-contaminated agricultural land in Russia is about 150 thousand hectares [1]. Basically, these are soils with medium or low HM contamination, which does not have a toxic effect on plant growth, but leads to exceeding the maximum permissible content of HM for agricultural products. The remediation of such territories is extremely difficult, expensive or impossible. The selection of varieties with reduced accumulation of heavy metals [2-4] is a promising approach for obtaining environmentally friendly products, in contrast to phytoextraction technologies [5].

It is known that many crops, including legumes [6-8], oilseeds [7-8] and cereals [9-11], differ significantly in HM accumulation. A high intraspecific polymorphism in HM content in several crops, including legumes, was also described. Significant varietal differences have been shown in soybean [6, 12], peanut [6] and beans [6, 13] for Cd accumulation, in soybeans [14] for Zn accumulation, and in beans [13] for Pb and Zn accumulation from contaminated soil. However, many experiments compared a limited number of varieties (from 2 to 20) that did not reflect the diversity, domestication and history of cultivation of the species, which reduces the value of such studies in terms of trait polymorphism.

We showed for the first time a significant intraspecific variability of garden pea (*Pisum sativum* L.) in the accumulation of HM in shoots by comparing 99 samples (mostly primitive and local varieties) from VIR collection of (N.I. Vavilov All-Russia Institute of Plant Genetic Resources, St. Petersburg), grown on soil enriched with a mixture of heavy metals (Cd, Cr, Cu, Ni, Pb, Sr, Zn) [15]. It was found that the accumulation of these metals in the shoots differed multiply depending on the plant genotype. As a result, samples with a low content of several HM in the shoots were detected, which were involved in the presented study.

The choice of initial pea seed material for selection of varieties tolerant to accumulation of heavy metals in biomass on contaminated soils was carried out in this work for the first time.

Our goal was to study the intraspecific variability of garden pea according to accumulation and transport of heavy metals from the shoot to the seeds and to identify the relationship of these processes to the consumption of nutrients by the plants.

Technique. In the study, 30 samples of garden pea (*Pisum sativum* L.) from VIR collection were involved, some of which (k-188, k-1027, k-1250, k-1693, k-2593, k-3445, k-4788, 5012, k-6468, k-6883, k-7131, k-8093, k-8543) were characterized by low accumulation of HM from contaminated soil [15]. In addition, varieties and lines from different countries with valuable agronomic traits (high productivity and protein content, fruit abounding, etc.) were selected based on VIR collection evaluation databases, i.e. k-6935, k-8861, k-8862, k-9283, k-9384, k-9385, k-9386, k-9389, k-9465, k-9509, k-9510, k-9526, k-9540 and k-9566. Control samples with high HM content in shoots when growing on contaminated soil were k-1658, k-1930, and k-8274 [15].

The pot experiment was carried out in summer in a greenhouse under natural light and temperature (All-Russian Research Institute for Agricultural Microbiology, St. Petersburg). Pea seeds were surface-sterilized and scarified with concentrated H_2SO_4 for 30 min, after which they were germinated for 3 days at 22 °C in Petri dishes with wet filter paper. The seedlings were planted in

pots (5 seedlings per pot, 3 pots per genotype) containing 5 kg of sod-podzolic fallow soil (C_{com} 2.34±0.05%, N_{com} 0.18±0.1%, N_{NO} 1.5±0.2 mg N/100 g, P_{mob} 7.7±0.6 mg P/100 g, K_{mob} 13.5±0.9 mg K/100 g; pH_{KCl} 6.6±0.1). The agrochemical soil parameters were determined by standard methods [16]. Ten days before sowing, the soil was enriched with heavy metals (as chlorides): Cd (5 mg/kg), Co (25 mg/kg), Cr (60 mg/kg), Cu (10 mg/kg), Ni (15 mg/kg), Pb (100 mg/kg), Sr (50 mg/kg), and Zn (50 mg/kg). Simultaneously, fertilizers were applied, i.e. NH_4NO_3 (15 mg/kg), KNO_3 (200 mg/kg), KH_2PO_4 (200 mg/kg), $MgSO_4$ (30 mg/kg), $CaCl_2$ (20 mg/kg), H_3BO_3 (3 mg/kg), $MnSO_4$ (3 mg/kg), $ZnSO_4$ (3 mg/kg), Na_2MoO_4 (1.5 mg/kg). The soil moisture was maintained at 60-70 % of the total moisture capacity during the experiment by adding water (to the same weight of vessels). The plants were grown until seed maturation, dried and ground to a powder; samples (separately shoots and seeds) were burned in a mixture of concentrated nitric acid and 38 % H_2O_2 at 70 °C in a DigiBlock graphite furnace (LabTech, Italy). The content of heavy metals and nutrients was determined using an ICPE-9000 spectrometer (Shimadzu, Japan) according to the manufacturer's protocol.

The statistical processing was carried out by dispersion methods, correlation and cluster analysis using Statistica 8.0 software (StatSoft Inc., USA) and DIANA software [17]. The cluster analysis of standardized values (SV) of elements content was carried out using the Ward method with squares of Euclidean distances. The heavy metal content index was calculated as the average of the standardized content values of all heavy metals (Cd, Co, Cr, Cu, Ni, Pb, Sr, Zn) in shoots and seeds.

Results. The studied pea samples (Table 1) differed significantly in the content of heavy metals in the shoots (Table 2).

1. Samples of garden pea (*Pisum sativum* L.) estimated for accumulate and transport of heavy metals from shoots to seeds (VIR collection, N.I. Vavilov All-Russia Institute of Plant Genetic Resources, St. Petersburg)

№ in VIR catalog	Name	Origin	Use
k-188	Mahorg	Pamir	Fodder
k-1027	Untitled	Germany before1986	Fodder
k-1250	Local	Tajikistan, Pamir, Yarchych	Fodder
k-1658	Weibull	Sweden	Fodder
k-1693	Untitled	United Kingdom	Vegetable
k-1930	Local	Italy	Grain
k-2593	Local	Cyprus	Grain
k-3445	Untitled	Azerbaijan	Grain
k-4788	Untitled	Mongolia	Fodder
k-5012	Ojo negro especial	Argentina	Grain and vegetable
k-6468	Local	Sudan	Grain
k-6883	Local	Uzbekistan	Fodder
k-6935	Preclamex	France	Vegetable
k-7131	Local	Tunisia	Vegetable
k-8093	Telephone	Madagascar	Vegetable
k-8274	Vendevil	France	Grain and vegetable
k-8543	Fillbaskit	Sri Lanka	Grain and vegetable
k-8861	Effulgent	Russia, the Kirov region	Grain
k-8862	Agate	Belarus	Fodder
k-9283	Nikolka	Russia, the Tyumen region	Fodder
k-9384	Falcon mustachioed	Russia, the Kirov region	Grain
k-9385	Boreas	Russia, the Kirov region	Fodder
k-9386	G-16992	Russia, the Kirov region	Grain
k-9389	D-13560	Russia, the Kirov region	Fodder
k-9465	Tyumenets	Russia, the Tyumen region	Grain
k-9509	Vlad	Belarus	Vegetable
k-9510	Azure	Belarus	Grain
k-9526	Azur	Germany	Grain
k-9540	ID 29001914	Australia	Fodder
k-9566	Kazar	France	Vegetable

The samples k-1027, k-4788 and k-9540 showed the minimum values of

this index for Cd, Co and Cr, respectively. Low content of Co, Cr, Ni, Sr, Zn was characteristic of k-1027, and low Pb was found in k-4788. In the shoots of k-3445, Cu, Ni, Pb, Sr, Zn amounts were minimal, and Cd, Co, Cr were low. In k-6468 Cd, Cu and Sr content was low, in k-8093 Cu, Ni, Pb and Zn were low. The minimum and maximum levels of heavy metals in the shoots differed 12-fold for Cd (in k-1027 and k-9389), 2.5-fold for Co (in k-1027 and k-6935), 2.5-fold for Cr (in k-1027 and k-6935), 7.3-fold for Cu (in k-3445 and k-9385), 2.8-fold for Ni (in k-3445 and k-6935), 3.9-fold for Pb (in k-3445 and k-9566), 2.9-fold for Sr (in k-3445 and k-9566), 4.3-fold for Zn (in k-3445 and k-9566) (Table 2). The obtained results are consistent with data of a high variability of garden pea on HM accumulation in shoots [15]. The samples (k-1658, k-1930, k-8274), taken as control, showed a high content of heavy metals in shoots, but it was slightly lower or close to that in the samples k-6935, k-9389, k-9566.

2. Shoot biomass and heavy metal content in garden pea (*Pisum sativum* L.) shoots grown on contaminated soil (vegetation experience)

№ in VIR catalog	Dry shoot weight, g/plant	Content, mg/kg dry weight							
		Cd	Co	Cr	Cu	Ni	Pb	Sr	Zn
k-188	2.0	4.4	9.3*	7.5	29	9.8*	5.2*	449*	156
k-1027	2.2	1.1*	6.4*	6.3*	26	7.7*	5.5*	323*	108*
k-1250	2.2	6.4	9.3	7.4	25*	10.3	8.1	407*	170
k-1658a	2.5	6.7	12.4	7.8	29	15.0	10.5	557	239
k-1693	1.6	1.9*	7.0*	7.8	25*	9.5*	5.7*	467	169
k-1930a	1.1	6.9	13.3	9.1	30	11.7	10.3	561	180
k-2593	2.0	4.0	7.9*	6.7*	21*	9.4*	5.1*	392*	140
k-3445	2.2	2.0*	6.2*	6.0*	14*	6.7*	3.5*	288*	60*
k-4788	2.1	2.0*	6.0*	6.5*	26	9.8*	4.4*	411*	140
k-5012	1.5	3.5*	8.9*	6.6*	31	10.6*	6.1*	471	192
k-6468	1.8	2.5*	9.7	7.1	17*	8.8*	5.7*	326*	135*
k-6883	1.3	2.5*	8.5*	7.7	29	12.1	6.3*	440*	154
k-6935	1.5	8.5	15.9	14.6	28	18.6	9.9	680	180
k-7131	2.0	5.2	9.5	8.0	26	11.2	10.3	547	213
k-8093	1.9	2.8*	7.9*	6.5*	15*	7.6*	5.4*	375*	83*
k-8274a	0.8	7.8	10.8	10.6	30	11.2	7.4	674	153
k-8543	1.9	3.5*	7.6*	6.8*	26	8.9*	4.5*	320*	131*
k-8861	1.9	6.0	10.5	7.4	58	12.6	7.0	418*	143
k-8862	1.8	3.9*	11.2	9.5	36	14.4	6.8	556	99
k-9283	2.0	4.5	10.8	8.0	67	14.2	6.9	407*	191
k-9384	1.6	8.8	11.8	7.0	63	14.8	6.7	520	121*
k-9385	1.6	6.5	9.7	6.6*	74	13.5	5.9*	421*	150
k-9386	1.3	9.9	13.0	7.3	18*	15.0	7.7	657	159
k-9389	1.7	13.2	12.9	6.5*	14*	14.8	10.2	652	174
k-9465	1.5	6.6	11.2	6.2*	50	12.9	8.1	578	171
k-9509	1.9	5.6	12.6	6.6*	38	15.9	8.7	640	222
k-9510	1.8	10.2	12.2	6.4*	26	13.7	10.0	572	215
k-9526	1.1	9.4	17.3	7.7	55	16.8	10.4	734	171
k-9540	2.0	5.7	11.9	5.9*	27	11.9	7.0	550	147
k-9566	2.2	10.2	14.7	8.6	22*	18.6	13.8	827	257
Average	1.8	5.7	10.5	7.5	33	12.3	7.4	496	161
Cv, %	28	53	26	22	60	27	32	27	31
LSD$_{0.05}$	0.47	1.52	1.31	1.05	7,3	1.93	1.51	64	12,3

N o t e. Samples from VIR collection (N.I. Vavilov All-Russia Institute of Plant Genetic Resources, St. Petersburg): a — samples with high heavy metals content used as a control; an asterisk for each element denotes values that were less than the difference between the average for all samples and the confidence interval when analyzing the average presented in the table; *Cv* is the variation coefficient.

The analysis of element composition in seeds also showed a high variability in HM content (Table 3). The minimum amount of Cd was found in the seeds of the sample k-1693, which, however, had a near-average content of other metals. The sample k-3445 was characterized by a minimum content of Co and Cr with low Cd, Ni, Sr; the sample k-9566 had minimum content of Cu and low Pb and Zn; the sample k-188 had minimum content of Ni and low Pb and Sr; for the sample k-8274 minimum content of Pb was characteristic at low Co, Ni, Sr and Zn level. In the seeds of other samples (k-1250, k-1658, k-2593, k-6468, k-8543, k-9389), several heavy metals was also low. The minimum and maximum accu-

mulation of heavy metals in seeds differed 9 times for Cd (samples k-1693 and k-9384), 4.2 times for Co (k-3445 and k-9283), for 10 times Cr (k-3445 and k-1693), 3.3 times for Cu (k-9566 and k-9384), 3.2 times for Ni (k-188 and k-9283), 6.8 times for Pb (k-8274 and k-9526), 4.1 times for Sr (k-8543 and k-9509), and 4.3 times for Zn (k-8861 and k-8093). Previously, high variability in heavy metal level in seeds, mainly in Cd, was noted for soybean [12], peanut [6, 18], maize [19], rice [10], wheat and barley [9].

3. Seeds weight and heavy metal content in garden pea (*Pisum sativum* L.) grown on contaminated soil (vegetation experience**)**

№ in VIR catalog	Seed dry weight, g/plant	Content, mg/kg dry weight							
		Cd	Co	Cr	Cu	Ni	Pb	Sr	Zn
k-188	1.3	0.50	2.8	0.35	23	3.4*	0.54*	16*	14
k-1027	1.1	0.37*	2.5*	0.56	25	4.1*	0.67*	24	20
k-1250	1.8	0.64	2.1*	0.24*	19	5.8*	0.57*	17*	15
k-1658a	1.5	0.49*	1.9*	0.23*	16*	6.7	0.83	21	13
k-1693	1.2	0.22*	3.0	0.89	26	7.2	1.55	36	13
k-1930a	1.2	0.89	2.8	0.28	28	5.5*	0.36*	22*	13*
k-2593	1.3	0.65	2.3*	0.18*	23	5.1*	0.47*	17*	23
k-3445	1.8	0.36*	1.5*	0.09*	20	5.2*	0.79	12*	31
k-4788	1.4	0.36*	1.9*	0.40	21	6.7	1.28	16*	9
k-5012	1.4	0.40*	2.8	0.30	16*	7.0	0.99	32	18
k-6468	1.0	0.28*	1.8*	0.21*	15*	5.8*	1.02	19*	25
k-6883	1.0	0.43*	2.5*	0.38	21	4.4*	0.68*	20	15
k-6935	1.2	1.12	4.8	0.61	24	8.3	1.27	88	12*
k-7131	1.1	0.41*	3.0	0.44	23	7.8	0.64*	28	16
k-8093	1.2	0.35*	2.6*	0.49	22	8.7	0.83	25	26
k-8274a	0.9	0.73	2.0	0.58	18	3.9*	0.29*	16*	12*
k-8543	1.1	0.32*	2.0	0.23*	20	5.2*	1.10	11*	15
k-8861	2.0	0.61	2.7	0.31	14*	7.5	0.89	31	6*
k-8862	2.1	0.99	3.9	0.36	18	7.3	1.09	39	10*
k-9283	1.6	0.86	5.3	0.54	24	10.9	1.33	47	14
k-9384	1.5	1.99	5.7	0.54	39	8.4	1.76	70	14
k-9385	1.7	0.98	4.2	0.31	22	6.3	0.95	37	10*
k-9386	1.1	0.57	3.7	0.28*	17*	8.1	0.82	30	9*
k-9389	1.9	0.87	3.2	0.18*	14*	6.3	0.69	23*	9*
k-9465	1.7	0.49*	4.1	0.27*	15*	7.4	1.35	33	8*
k-9509	1.6	0.63	5.3	0.32	17*	7.9	0.87	45	13
k-9510	1.5	0.44*	4.4	0.27*	18	8.0	1.10	35	17
k-9526	1.3	0.69	5.0	0.26*	20	6.9	1.98	25	12*
k-9540	2.0	0.67	3.9	0.25*	14*	6.9	1.31	28	9*
k-9566	1.4	1.21	3.1	0.28	12*	7.3	0.69*	34	8*
Average	1.4	0.65	3.2	0.36	20	6.7	0.96	30	14
Cv, %	23	56	38	51	27	24	42	56	43
HCP$_{0.05}$	0.54	0.150	0.81	0.096	6.1	1.83	0.32	7.8	3.6

N o t e. Samples from VIR collection (N.I. Vavilov All-Russia Institute of Plant Genetic Resources, St. Petersburg): a — samples with high heavy metal content used as a control; an asterisk for each element denotes values that were less than the difference between the average for all samples and the confidence interval when analyzing the average presented in the table; *Cv* is the variation coefficient.

The variability in the content of elements in pea seeds was higher or comparable to that of shoots, as evidenced by high coefficients of variation (Tables 2, 3). However, we did not find a correlation between *Cv* in shoots and seeds. This indicated the differences in the mechanisms of transport of metals from shoots to seeds in the studied samples. The content of heavy metals in seeds was several times less than in shoots, which is consistent with the description of legumes as species with low metal translocation in the root—shoot—seed system [2, 5, 7, 20].

A negative correlation was found between shoot biomass and the content of Cd (*r* = –0.44; P = 0.015), Co (*r* = –0.51; P = 0.004), Cr (*r* = –0.40; P = 0.026), Ni (*r* = –0.41; P = 0.024) and Sr (*r* = –0.63; P = 0.001), which probably was due to the dilution of the metal with biomass at limited accessibility from pots. However, the observed effect could also be due to the peculiarities of the samples, since the shoot weight did not correlate with the accumulation of other heavy metals (Cu, Zn) and nutrients (P, K, Fe, Mn), and we also failed to detect correlations between seed weight and HM or nutrient levels.

In many cases, there was a positive interdependence between the accumulation in the shoots of various heavy metals, as well as nutrients (Table 4), for example, Cd, Co, Mg, Ni, Pb, S, Sr and Zn. The same pattern was manifested in seeds (see Table 4). Thus, the amount of Sr in seeds correlated with the content of all elements, with the exception of B, Fe, S. The presence of such correlation was first discovered by us [15]. This paper confirms this phenomenon and supplements it with a description of the correlation relationships for Co and nutrients, the content of which has not previously been measured. The obtained results are consistent with the data of W. Cheng et al. [10], which found a positive correlation between the content of As, Cd, and Pb; As and Zn; Cr and Ni in grain in different varieties of rice grown on contaminated soils. Probably, this phenomenon is connected with the variety of molecular transport mechanisms and their low specificity in plants (the same channels provide transportation of both nutritional elements and HM) [21-23].

4. Correlation coefficients between the chemical element content in seeds (1) **and shoots** (2) **of garden pea (*Pisum sativum* L.) grown on contaminated soil** (vegetation experience)

1 \ 2	B	Ca	Cd	Co	Cr	Cu	Fe	K	Mg	Mn	Ni	P	Pb	S	Sr	Zn
B		0,30	0,13	0,11	0,45*	0,44*	0,30	–0,05	0,10	0,19	–0,01	0,19	0,07	0,17	0,26	0,12
Ca	0,32		0,61*	0,65*	0,46*	0,47*	–0,24	0,51*	0,36*	0,64*	0,58*	–0,42*	0,47*	–0,09	0,97*	–0,10
Cd	0,30	0,77*		0,57*	0,11	0,40*	–0,41*	0,46*	0,15	0,20	0,29	–0,46*	0,21	0,06	0,65*	–0,36*
Co	0,45*	0,81*	0,81*		0,24	0,28	–0,49*	0,87*	0,49*	0,45*	0,68*	–0,46*	0,58*	–0,04	0,75*	–0,37*
Cr	0,48*	0,45*	0,23	0,46*		0,53*	0,05	0,15	0,59*	0,23	0,22	0,11	0,25	0,30	0,46*	–0,11
Cu	0,49*	0,03	0,14	0,19	–0,03		0,23	–0,07	0,56*	0,40*	0,07	0,41*	0,24	0,27	0,38*	0,21
Fe	0,17	–0,27	–0,30	–0,18	0,32	0,01		–0,63*	–0,05	0,29	–0,33	0,69*	–0,30	–0,16	–0,36	0,64*
K	–0,41*	–0,25	–0,38*	–0,32	–0,06	–0,37*	–0,09		0,34	0,34	0,75*	–0,66*	0,53*	–0,16	0,65*	–0,52*
Mg	0,39*	0,88*	0,71	0,75*	0,52*	0,06	–0,05	–0,24		0,44*	0,40*	0,26	0,20	0,10	0,37*	0,02
Mn	–0,13	0,14	–0,05	–0,05	0,19	–0,26	0,47*	0,08	0,32		0,56*	–0,06	0,38*	–0,39*	0,55*	0,32
Ni	0,55*	0,72*	0,75*	0,88*	0,46*	0,34*	–0,03	–0,46*	0,68*	–0,08		–0,43*	0,53*	–0,23	0,63*	–0,21
P	–0,28	–0,23	–0,45*	–0,36	0,03	–0,11	0,28	0,52*	0,01	0,58*	–0,48*		–0,35	0,20	–0,52*	0,64*
Pb	0,26	0,79*	0,75*	0,81*	0,37*	–0,02	–0,09	–0,29	0,79*	0,27	0,76*	–0,25		–0,12	0,48*	–0,20
S	0,20	0,74*	0,43*	0,52*	0,47*	–0,01	–0,26	0,03	0,68*	0,12	0,40*	0,07	0,44*		–0,03	–0,22
Sr	0,29	0,94*	0,78*	0,85*	0,44*	0,01	–0,29	–0,29	0,81*	0,06	0,83*	–0,35	0,83*	0,64*		–0,27
Zn	0,17	0,61*	0,50*	0,54*	0,20	0,04	0,02	–0,14	0,57*	0,31	0,62*	–0,17	0,78*	0,36*	0,62*	

* Statistically significant correlation coefficients (r) (for r > 0.35 $P \le 0.05$; for r > 0.45 $P \le 0.01$; for r > 0.56 $P \le 0.001$; $n = 30$).

The samples were grouped into clusters. The individual clusters included those that had a low content of Cd, Co, Cr, Cu, Ni, Pb, Sr, Zn HM and B, Ca nutrients in the shoots (Fig. 1, A, cluster № 3), low heavy metals Cd, Co, Ni, Pb, Sr and Ca, K nutrients in seeds (Fig. 1, B, cluster № 3), low Cr, Cu, Zn in seeds (Fig. 1, B, cluster № 3). The composition of clusters that combined samples with a low content of most heavy metals was similar in shoots and seeds. This was consistent with a positive correlation of metal amount in shoots and seeds for Cd ($r = +0.55$, P = 0.002), Co ($r = +0.57$, P = 0.001), Cr ($r = +0.41$; P = 0,023), Ni ($r = +0.48$, P = 0.007), Sr (r = + 0.37, P = 0.048), and also for the nutrient P ($r = +0.67$, P < 0.001). At the same time, we found a negative correlation between the accumulation of the element in shoots and seeds for Zn ($r = –0.45$; P = 0.012) and K ($r = –0.59$; P < 0.001). In the control samples k-1658, k-1930 and k-8274, HM content in shoots was high (see Table 2), in the seeds it was low (see Table 3), and they were grouped in cluster № 3 (see Fig. 1, B). Samples that were included in clusters with low HM accumulation in shoots or seeds had a high content of K and P or Fe, P and Zn, respectively. The obtained results testify to the specificity of the mechanisms ensuring transport of individual elements from shoot to seeds, and a barrier for the transport of abiogenic metals from the vegetative organs to the reproductive ones. The effectiveness of these mechanisms also depends significantly on the plant genotype. Such biodiver-

sity and wide practical use of peas (fodder, grain and vegetable varieties) indicate the need to take into account the heavy metal content simultaneously in shoots and seeds when implementing breeding programs.
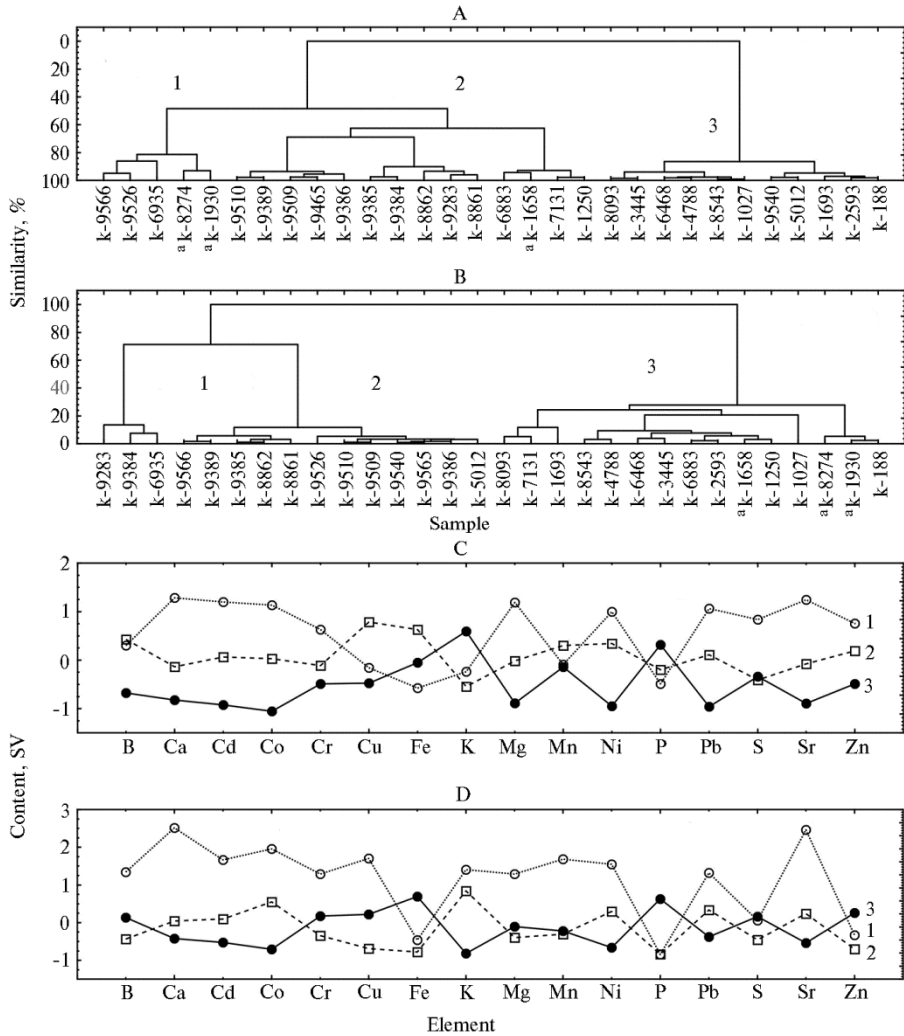
Fig. 1. Cluster diagrams showing the grouping of the samples of garden pea (*Pisum sativum* L.), grown on contaminated soil by the content of heavy metals and nutrients in shoots (A) and seeds (B), as well as the mean values of element abundance in shoots (B) and seeds (D): 1 — cluster 1, 2 — cluster 2, 3 — cluster 3; a — samples with a high heavy metals content used as a control, SV — standardized values of the content of elements (vegetation experiment).

To generalize the obtained results, the index was calculated of the content of all heavy metals in shoots and seeds, which made it possible to reveal samples with minimum average values of this parameter (Fig. 2). Most samples with a low index were related to previously characterized forms with low accumulation of heavy metals in shoots [15], i.e. k-188, k-1027, k-1250, k-2593, k-3445, k-4788, k-5012, k-6468, k-8093, and k-8543. They were old local varieties, and their comparison with modern varieties and lines showed that the latter actively accumulate heavy metals in shoots and transport them to seeds. This may be the result of selection for intensive technologies with large yield gain at a high agricultural background (that is, effectively using mineral fertilizers). Probably, intensive assimilation and transport of nutrition mineral elements predeter-

mine active accumulation of abiogenic elements.

It should be emphasized that many heavy metals (Co, Cu, Ni and Zn) are necessary for the plant in low concentrations. In our experiment, they were introduced in doses exceeding the MAC for agricultural use soils. However, these concentrations turned out to be lower than those for peas, as the plants developed normally and showed no signs of injury. In addition, there was a positive correlation between the content of certain nutrients (Ca, K, Mg, P and S), HM of biogenic nature (Co, Cu, Ni, Zn) and abiogenic elements (Cd and Pb) in shoots and/or seeds (see Table 4).
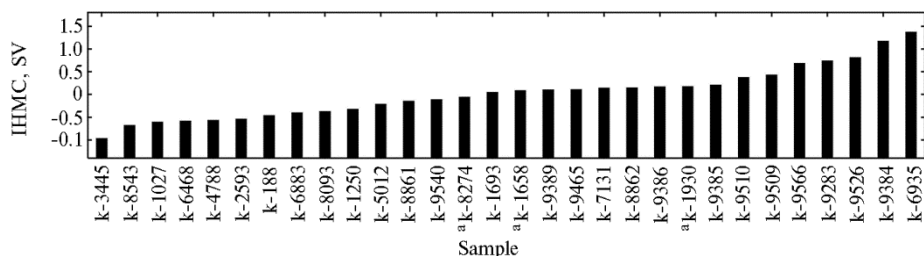

Fig. 2. The index of heavy metals content (IHMC) **in shoots and seeds of** *Pisum sativum* **L., grown on contaminated soil:** a — samples with a high heavy metals content used as a control, SV — standardized values of elements content (vegetation experiment).

Thus, valuable information on pea plant polymorphism on accumulation of heavy metals has been obtained and correlations between the content of various elements (nutrient macro-, micro- and abiogenic elements) in shoots and/or seeds have been found. The results demonstrate the complexity and multicomponent mechanisms and processes of nutrient and toxic elements consumption by plants, as well as high intraspecific variability of peas according to these features. It is shown that the parameters characterizing the activity of plants nutrient intake can serve as additional criteria for the search for genotypes with a reduced content of heavy metals. It should be taken into account that selection for the effective use of mineral nutrition elements can enhance the accumulation of toxic elements by plants on contaminated soils. At the same time, it is possible to select for low simultaneous accumulation of many heavy metals. For these, the old local varieties of peas from VIR collection (N.I. Vavilov All-Russia Institute of Plant Genetic Resources, St. Petersburg) k-188, k-1027, k-1250, k-2593, k-3445, k-4788, k-5012, k-6468, k-8093, k-8543, and also the modern selection material (lines) k-9386, k-9389 (Kirov region) and k-9465 (Tyumenets, Tyumen region) are prospective.

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EVALUATION OF THE SYMBIOTIC EFFECTIVENESS OF PEA (*Pisum sativum* L.) GENOTYPES IN POT EXPERIMENT

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A b s t r a c t

The successful use of plant-microbe systems based on legumes in agriculture requires creation of new cultivars of crop legumes effectively interacting with microbes. For the selection of cultivars of crop legumes, the effectiveness of interaction with beneficial soil microorganisms (EIBSM) trait has been proposed. EIBSM is estimated as a percentage of increment in a number of agriculturally important parameters (plant biomass, number and total biomass of seeds, as well as weight of 1000 seeds) after applying the microbial inoculum compared to untreated control. The aim of this study was to evaluate the EIBSM trait in a pot experiment involving two pea (*Pisum sativum* L.) genotypes that have shown high and low effectiveness of interaction with beneficial soil microflora in a three-year field experiment. The highly effective in symbiosis with nodule bacteria and arbuscular-mycorrhizal fungi genotype k-8274 (France, cv. Vendevil) and ineffective genotype k-3358 (Saratov region, Russia) from the VIR Collection of cultivated peas of N.I. Vavilov All-Russian Institute of Plant Genetic Resources were used in the study. Plants were grown during the summer season of 2012 in a greenhouse in the 5-liter pots on sod-podzolic light loamy soil. Treatment variants were as follows: control (untreated soil); nodule bacteria + arbuscular mycorrhizal fungi (NB + AMF); nodule bacteria (NB); mineral nutrition (NPK). Variants of soil moisture were as follows: low moisture (watering with 30 % of full moisture capacity, FMC) and optimal moisture (watering with 60 % of FMC). Mineral fertilizers (NPK) were applied in the form of pure salts in a dose of 0.1 mg a.i. per 1 kg of soil. For NB inoculation, the effective strain *Rhizobium leguminosarum* bv. *viciae* RCAM1026 was used. AM fungi for inoculation were propagated on the sorghum (*Sorghum* sp.) roots. A mixed inoculum containing three AMF isolates, *Rhizophagus irregularis* (syn. *Glomus intraradices*) RCAM8 (= BEG144), *R. irregularis* BEG53 and *Glomus* sp. ST3, was applied. Samples were gathered at the budding stage, after 1 month of vegetation (to determine the amount of nodules and the dry plant biomass) and at the end of vegetation about 3 months after planting (to determine the dry plant biomass, seed weight and amount). When grown in pots, highly effective and ineffective genotypes demonstrated the expected differences in EIBSM. The dry weight after 1 month of vegetation of highly effective k-8274 genotype showed a statistically significant increase in the NB and NPK variants compared to the control. At the same time, this parameter in the ineffective genotype k-3358 significantly increased only under the influence of mineral nutrition, but not nodule bacteria. Also, under treatment of NB, k-8274 genotype increased 1000 seed weight, while in k-3358 under the same treatment this parameter was slightly decreased. Statistically significant negative effect of low soil moisture on all estimated parameters in k-3358 genotype and the majority of the estimated parameters in k-8274 genotype was also revealed. Thus, the plants grown in the pot experiment are equivalent to the material grown in the field, and therefore can be used to determine physiological and biochemical markers of functioning of symbiotic systems in order to identify the molecular basis of EIBSM trait. For modeling the pea EIBSM trait in a pot experiments it is recommended to use at least 6 replicas for each genotype. Also, it is necessary to carefully control the soil moisture.

Keywords: legume-rhizobial symbiosis, arbuscular mycorrhiza, symbiotic effectiveness,

garden pea, *Pisum sativum* L.

To minimize the chemical impact on the environment (reducing application of chemical fertilizers and agents for protection against pathogens) [1, 2] and the adverse effect of environmental factors (unfavorable weather conditions, impact of pathogens, etc.) [3], it is advisable to use microbilogical preparations, for example, those based on nodule bacteria or arbuscular mycorrhizal fungi that have positive influence on plants [4, 5]. There are also preparations containing a complex of beneficial soil microorganisms [6, 7].

Legume plants are crops suitable for cultivation within the modern concept of adaptive plant growing that was shown for lentils [8], soya [9], peas [10, 11], beans [12, 13], chickpeas [14], and other legumes [15-17]. The successful use of plant-microbe systems based on legumes requires creation of entirely new cultivars capable of effectively interacting with beneficial soil microorganisms [17, 18]. One of the methods to achieve the purpose is to mobilize plant genetic resources, i.e. search for valuable gene alleles that can improve symbiotic properties and height parameters of a plant when introducing into a genotype.

The nitrogen fixation effectiveness trait is traditionally used when selecting legume crops for the increased symbiotic potential [19-21]. It has also been proposed to consider the integral trait of the effectiveness of interactions with beneficial soil microorganisms (EIBSM) [17, 22] which is estimated as an increment in a number of economically important parameters (plant biomass, seed number and total weight, as well as weight of 1000 seeds) after applying microbiological preparations as compared to untreated control. Earlier, the field experiments on 25 pea genotypes allowed estimating EIBSM variability and identifying contrast genotypes (highly effective and ineffective when interacting with beneficial soil microorganisms) [10, 12].

In this work, we have considered for the first time the possibility of EIBSM modelling in a pot experiment as well as assessed the impact of soil moisture content on the trait emergence.

The aim of the study was to evaluate the effectiveness of interactions of two pea genotypes (that had shown high and low effectiveness in a field experiment) with beneficial soil microorganisms in the model pot experiment.

Technique. The highly effective in symbiosis with nodule bacteria k-8274 genotype (France, Vendevil cultivar) and ineffective k-3358 genotype (Saratov Province, Russia) of edible peas (*Pisum sativum* L.) from the VIR collection of cultivated peas (N.I. Vavilov All-Russian Institute of Plant Genetic Resources, St. Petersburg) were used in the study [23]. The plants were grown during the summer season of 2012, in greenhouses, in the 5-liter pots (3 plants in each pot) on sod-podzolic light loamy soil (Leningrad Province, area of Belogorka Science and Production Association), C_{humus} 1.27 % and N_{total} 0.11%, pH_{salt} 4.92. Agrochemical soil studies were conducted using generally accepted methods [24]. Calcium carbonate was used to chalk the soil.

Treatment variants were as follows: control (untreated soil); nodule bacteria + arbuscular mycorrhizal fungi (NB + AMF); nodule bacteria (NB); mineral fertilizers (NPK). The effect of low (30 % of the full moisture capacity, FMC) and optimal (60 % of the FMC) moisture content was compared as well (there were 6 replicates for the optimal watering and 3 replicates for 30 % FMC watering). Mineral fertilizers (NPK) were applied in the form of pure salts at a dose of 0.1 mg of a.i. per 1 kg of soil ($N_{0.1}P_{0.1}K_{0.1}$). Ammonium nitrate NH_4NO_3, monobasic calcium phosphate $Ca(H_2PO_4)_2 \cdot H_2O$, and potassium chloride KCl were applied. For the NB inoculation, the effective strain of *Rhizobium leguminosarum* bv. *viciae* RCAM1026 from the collection of All-Russian

Research Institute for Agricultural Microbiology was used [25, 26]. The bacteria were grown for 3 days with aeration in the liquid medium № 79 (K_2HPO_4 0.5 g/l; $MgSO_4 \cdot 7H_2O$ 0.2 g/l; NaCl 0.1 g/l; $CaCO_3$ traces; mannitol 10 g/l; yeastrel 0.4 g/l). Then, sterilized seeds of edible peas were added to the suspension of bacteria having 10^7-10^8 cell titer and placed on a shaker for 12 hours [27, 28]. The mycorrhizal fungi for inoculation were propagated on sorghum (*Sorghum* sp.) roots in the soil mixed with washed filter cake (WFC) [6, 29]. A mixed inoculum contained three AMF isolates from the collection of All-Russian Research Institute for Agricultural Microbiology, the *Rhizophagus irregularis* (syn. *Glomus intraradices*) RCAM8 (= BEG144), *R. irregularis* BEG53 and *Glomus* sp. ST3. Before planting, the AMF inoculum was mixed with soil in the amount of 20 g per a pot, and 1.5 g of inoculum was added under each pea seed.

Plants were sampled at budding—blooming after 1 month of vegetation (to count the number of nodules and determine the dry plant weight) and at the end of vegetation about 3 months after planting (to determine the dry plant weight, seed weight and number).

Statistical processing was carried out in SigmaPlot 12.0 (Systat Software, USA). The effect of moisture and treatment factors on the traits was assessed by the two-way analysis of variance for each genotype. The Student's *t*-test was used to compare treatment variants to each other. The weight of 1000 seeds calculated as (weight of plant seeds/number of plant seeds) × 1000 was estimated using the nonparametric Mann-Whitney U-test.

Results. The two-way analysis of variance has revealed the statistically significant negative effect of the low soil moisture on all estimated parameters in ineffective k-3358 genotype and the majority of those in highly effective k-8274 genotype (Table, Fig.).

Effect of various factors and their interactions on productivity parameters in pea (*Pisum sativum* L.) genotypes under optimal and insufficient moisture conditions, when treated (NB, NB + AMF, NPK) and untreated (two-way analysis of variance)
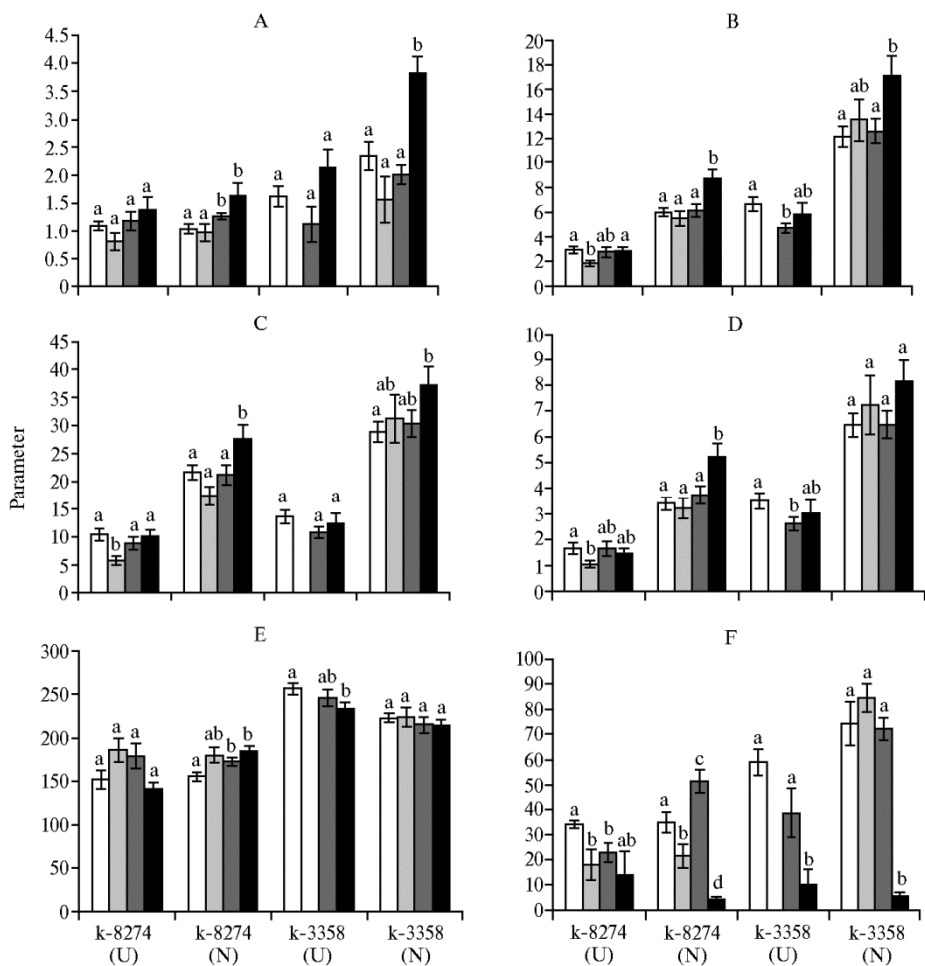
Genotype	Factor	Aboveground dry biomass		Seeds		Number of nodules
		in 1 month	in 3 months	number	weight	
k-8274	Moisture content	0.347	< 0.001*	< 0.001*	< 0.001*	0.107
	Treatment	0.004*	0.010*	0.005*	0.034*	< 0.001*
	Interaction of the two factors	0.809	0.090	0.354	0.073	0.002*
k-3358	Moisture content	< 0.001*	< 0.001*	< 0.001*	< 0.001*	0.011*
	Treatment	< 0.001*	0.0770	0.243	0.291	< 0.001*
	Interaction of the two factors	0.118	0.076	0.212	0.248	0.025*

N o t e. Optimal and insufficient moisture content is 60 and 30 % of the full moisture capacity, respectively; NB — nodule bacteria *Rhizobium leguminosarum* bv. *Viciae* RCAM1026; AMF — arbuscular mycorrhizal fungi, mixed inoculum of *Rhizophagus irregularis* (syn. *Glomus intraradices*) RCAM8 (= BEG144), *R. irregularis* BEG53 and *Glomus* sp. ST3; NPK — $N_{0.1}P_{0.1}K_{0.1}$. P-values reflecting the significance of each factor and interaction of two factors provided.
* P < 0.05.

At the same time, the treatment variant had no expressed effect (see Table, Fig.). Therefore, EIBSM in limited volume pots was strongly affected by the soil moisture that surpassed the impact of symbiotic microorganisms and mineral fertilizers.

At the low moisture, the aboveground dry biomass of k-8274 plants gathered in 1 month of vegetation did not change depending on the treatment variant (see Fig., A). The significant gain in biomass in the NB and NPK variants as compared to the control was observed under the optimal moisture conditions. As for k-3358, the estimated parameter did not change in the lack of moisture, and the significant gain was revealed at the optimal moisture when treated with mineral fertilizers, but not nodule bacteria.

Aboveground dry biomass of plants in 1 (A) **and 3 months of vegetation** (B), **g; number of plant seeds, pcs** (C); **seed weight per plant, g** (D); **1000 seed weight, g** (E); **number of nodules, pcs** (F) **in pea** (*Pisum sativum* **L.) genotypes k-8274 (highly effective when interacting with beneficial soil microorganisms in the field experiment) and k-3358 (ineffective) at various soil moisture and different treatment variants:** 1 — control (untreated soil), 2 — nodule bacteria + arbuscular-mycorrhizal fungi (NB + AMF), 3 — NB, 4 — mineral fertilizers (NPK). U — insufficient soil moisture, N — optimal soil moisture. No available data on NB + AMF variant for k-3358 under the insufficient moisture conditions. The lettered values (a, b, c, d) have statistically significant (P < 0.05) differences within a single treatment variant (a differs from b, and ab does not differ from a and does not differ from b).

The low moisture amplified the effects of NB + AMF treatment on the aboveground dry biomass of k-8274 genotype plants gathered upon completion of vegetation (see Fig., B). No data on this treatment variant was available for k-3358. Probably, the formation and support of the arbuscular mycorrhizal symbiosis under the moisture deficit in a confined pot volume were energetically unfavorable for the plants. The NB treatment against the low moisture resulted in the decrease of k-3358 plant biomass that evidenced of its low effectiveness when interacting with symbionts. In the optimal moisture conditions, the statistical significance was characteristic of mineral fertilizers, but not the treatment with microorganisms. Perhaps, the positive effect of microorganisms was abolished during long vegetation due to the stress experienced by plants because of limited volume pots.

The number of seeds of k-8274 (see Fig., C) decreased in the NB + AMF

variant under the low moisture. The treatment with NB only had no considerable impact on the parameter in both genotypes at either optimal or low moisture. The positive effect of mineral fertilizers was noted for k-8274 and k-3358 at the optimal moisture, but not when there was a lack of moisture.

The NB + AMF treatment had adverse effect on the weight of seeds for k-8274 genotype (see Fig., D) in the low moisture conditions. The same effect was observed when treating k-3358 seeds with NB at moisture deficiency. The weight of seeds increased only due to mineral fertilizers applied at the optimal soil moisture.

The 1000 seed weight in k-8274 genotype did not depend on the control moisture and, however, increased when treating with NB (statistically significant only in the optimal moisture conditions) (see Fig., E). For k-3358 genotype, the 1000 seed weight, to the contrary, decreased slightly after the NB treatment (statistically significant in the lack of moisture conditions). It is consistent with the results of field experiments where the weight of 1000 seeds increased after treatment with microbiological preparations in the highly effective genotypes and did not change or decreased slightly in the ineffective ones [10, 22].

The application of mineral fertilizers had adverse effects on the number of nodules (see Fig., F) that was associated with activation of autoregulation systems (negative control over the energy-consuming nodule formation when there was sufficient amount of bound nitrogen in soil) [30, 31]. The lack of moisture led to decreased number of nodules in k-3358 genotype in any treatments. The NB inoculation (including NB + AMF) against the lack of moisture inhibited the nodule formation in both genotypes because of autoregulation. The optimal moisture variant showed the decrease in the number of nodules in k-8274 genotype in case of NB + AMF that was probably associated with the operation of common autoregulation system of nodular and arbuscular mycorrhizal symbioses [30-32]. The number of nodules when using only NB was higher in k-8274 compared to the untreated control that may be a genetically reduced ability for the nodule formation in this genotype.

Thus, the pea genotypes which showed the high and low effectiveness when interacting with nodule bacteria in the field experiments had similar differences in the greenhouse trial. For example, the statistically significant gain in plant biomasses 1 month after planting and in 1000 seed weight, when inoculated with nodule bacteria (as compared to the non-inoculated control), was observed only in highly effective k-8274 genotype, while ineffective k-3358 genotype demonstrated no positive relation with inoculation by these parameters. On the contrary, the weight of plant seeds when inoculated with nodule bacteria in the insufficient moisture conditions decreased significantly as compared to the non-inoculated control. Hence, still unknown genetic programs underlying EIBSM are implemented similarly in pot experiments and under field conditions. The material grown in the pot experiment is equivalent to that obtained in field and may be used to identify molecular underpinnings of genetic control of EIBSM trait through the transcriptomic sequencing.

So, for modeling the integral EIBSM trait (effectiveness of interactions with beneficial soil microorganisms) in peas in pot experiments, at least 6 replicates for each genotype should be used. It is also necessary to control the soil moisture since the drought stress masks the beneficial action of microorganisms reducing differences between the highly effective and ineffective genotypes. The budding—blooming stage at which first distinctions between genotypes are noted can be recommended to collect materials for analyzing differential expression of genes, enzymatic activity and identifying other physiological and biochemical markers of symbiotic system functioning.

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EVALUATION OF FLAX (*Linum usitatissimum* L.) GENOTYPES ON ENVIRONMENTAL ADAPTABILITY AND STABILITY IN THE NORTH-EASTERN BELARUS

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A b s t r a c t

Flax is the only industrial crop cultivated in Belarus for fiber on the area over 50 thousand ha. An environmental adaptation is of special interest for innovative flax varieties. We focused on selection of genetic sources of adaptability and stability and the search for environmentally adapted genotypes among the extensive flax hybrids. For this, we have studied a response of the parent forms and 36 hybrid populations of fiber flax to abiotic factors and estimated the influence of environment on economically important traits. The field trials (Orshanskii Region, Belarus, 2012 to 2014) were carried out under weather conditions which varied in precipitations (16.7-27.1 mm) and daily air temperature (12.4-16.4 °C). Plant height, stem technical length, weight, and fiber content were studied in 10 parental varieties different in geographic origin (i.e. derived from Belarus, Russia, France, Ukraine, Lithuania), and also in their combinations. Morphological and biological screening identified 11 early ripening combinations, 15 forms with tall stem, 10 forms with a significant technical stem length, 4 ones with high stem weight, and 3 ones with high fibre content. A statistically significant impact (at 95 and 99 % level) of genotypes ($F_o > F_e$), environment factor and their interactions (genotype × environment) have been found. The estimation of stability and responsiveness to environmental conditions of each of the studied flax genotypes by Eberhart and Russell method revealed that high responsiveness to the improved growing conditions was characteristic of Alizee ($b_i = 1.12$), Upite 2 ($b_i = 1.22$), Lvovskiy 7 ($b_i = 1.02$), Lvovskiy 7 × Yarok ($b_i = 1.79$), Lvovskiy 7 × Voskhod ($b_i = 3.07$) for plant height, K-65 ($b_i = 1.33$), Velich ($b_i = 1.12$), Upite 2 ($b_i = 1.22$) Lvovskiy 7 ($b_i = 1.32$), Yarok × K-65 ($b_i = 1.87$), K-65 × Laska ($b_i = 1.37$), Yarok × Velich ($b_i = 2.09$) for technical stem length; Voskhod ($b_i = 2.38$), Laska ($b_i = 1.85$), K-65 ($b_i = 1.76$), Voskhod ($b_i = 2.32$), Yarok × Laska ($b_i = 1.87$), Laska ×Voskhod ($b_i = 2.16$), Voskhod × Alizee ($b_i = 1.17$), K-65 × Laska ($b_i = 1.31$) for stem weight; Laska ($b_i = 1.14$), Voskhod ($b_i = 1.27$), Drakkar ($b_i = 1.59$), K-65 ($b_i = 1.32$), Vasilek ($b_i = 1.39$), Yarok × Laska ($b_i = 2.54$), Yarok × Voskhod ($b_i = 1.78$), Velich × Voskhod ($b_i = 3.11$), Yarok × Lvovskiy ($b_i = 1.31$) for fiber content. Low levels of variance stability was found in Velich ($S^2d_i = 2.33$), Lvovskiy 7 ($S^2d_i = 4.62$), Drakkar ($S^2d_i = 6.18$) for plant height; in Velich ($S^2d_i = 5.37$), Lvovskiy 7 ($S^2d_i = 4.62$), Drakkar ($S^2d_i = 6.20$) for technical stem length; in Upite 2 ($S^2d_i = 1438.68$), Yarok ($S^2d_i = 3465.94$), Lvovskiy 7 ($S^2d_i = 2268.62$) for stem weight; in Vasilek ($S^2di = 1.47$), Upite 2 ($S^2d_i = 0.85$), Drakkar ($S^2d_i = 3.98$), Voshod ($S^2d_i = 3.65$), Yarok ($S^2d_i = 2.29$) for the fiber content. High adaptable, stable and productive genotypes were few thereby necessitating further researches to create new breeding material.

Keywords: flax, varieties, hybrids, environmental adaptability, stability, productivity

Nowadays, the fiber flax selection is focused on improving resistance to biotic and abiotic factors while preserving the high productivity and quality of the feedstock. The main method in generating the parent material is still the intravariety hybridization which allows combining important traits of parents [1].

In the literature, there is sufficient information on the environmental study of varieties and hybrids of many crops, such as grains [2-7], corn [8, 9), lupine [10], rice [11], beet [12], clover [13], trees [14], sorghum [15], ornamental plants [16], sunflowers [17], potatoes [18], and tomatoes [19]. At the same time, there are extremely little papers dedicated to the environmental assessment of samples,

varieties and, moreover, hybrid forms of fiber flax that actualizes these studies. A.B. D'yakov [20] highlights qualitative differences among flax ecotypes by their adaptability to soil and climate conditions, the specificity of plant competition in fiber and oil flax agrocoenosis, as well as the peculiarities of flax interactions with other biocenosis components. The agro-environmental assessment [21] has shown that the mid-ripening varieties of fiber flax selected in Pskov are the most adapted to natural conditions of the Northern Altai low-mountain terrain; therefore, the fiber flax has been considered a promising variety for the region. Detection of individual *L. perenne* plants in the arctic conditions of lower reaches of the Ob, Yenisei, Lena and Indigirka rivers, as well as in the Polar and Middle Ural mountains, Yakutia and Altai (where the northern populations of perennial flax *L. perenne* grow as well) bears evidence of homologous parallelism between these species on their adaptability to arctic and high-mountain conditions and defines the possibility to select cultivated flax varieties adaptable to the northern territories [22]. The most adapted samples which are recommended to be selected for productivity, quality, resistance to lodging and diseases under various growing conditions have been identified in the fiber flax derived from Lithuania [23].

The flax productivity and yield depend on plant genetic diversity and the genotype × environment interaction [24]. Donors of a number of economically important traits have been found when studying the samples and highly productive forms adaptable to the conditions of the Nonchernozem Belt Central Area [25, 26]. The flax growth and development intensity is also associated greatly with the pre-sowing soil treatment and the interaction between this factor and genotype. The emergence of flax seedlings depended considerably on temperature: the maximum germination (87 %) was observed at 30 °C, the minimum one — at 10 °C [27]. Under controlled conditions, the emergence of flax seedlings was even and quick at 30 °C, while a photoperiod had no significant effect on the process [28].

V.Z. Bogdan [29] and L.M. Polonetskaya [30] were the first who studied the oil flax collection using statistical approaches and determined the impact which several factors had on trait formation. As a result, the authors have identified the genotypes adapted to the conditions of North-Eastern Belarus. L.V. Ivashko [31] and K.P. Korolev [32], having analyzed the variation of parmeters in different varieties, have found out the high environmental stability sources, i.e. Grant, Yarok, Vasilek, Vesta, Laska (Belarus), Drakkar, Alizee (France), Suzanne (the Netherlands).

In this work, we have identified the responsiveness of parent and hybrid forms of fiber flax to abiotic factors and the extent of their impact on the formation of traits such as plant height, technical stem length, stem weight and fiber content, for the first time in Belarus.

The purpose of our study was to assess forms of fiber flax by their environmental adaptability and stability in the conditions of North-Eastern Belarus.

Technique. Observations were made in 2012-2014, on the trial field (Orshansky Region, Vitebsk Province, Republic of Belarus). Flax plants were grown in sod-podzolic, light loamy soil underlied with moraine loam at the depth of 1 m (humus content 1.6-1.9 %, P_2O_5 193.0-228.0 mg/kg, K_2O 107.0-219.0 mg/kg, pH_{KCl} 5.2-5.9) after grain crops as precursors. The study included the domestic varieties (Yarok, Laska, Vasilek, K-65, Velich) and those derived from France (Drakkar, Alizee), collection samples of Upite 2 (Lithuania), Voskhod (Russia), Lvovskiy 7 (Ukraine) (gene pool of the Flax Institute, Republic of Belarus), as well as 36 F_2-F_4 hybrid populations of fiber flax. Varieties of Yarok, Alei and Mogilevsky from Belarus served as standards. Hole sowing was carried out in the hybrid nursery (feeding area 2.5×2.5 cm) upon the optimum conditions. Parent forms were sown in 20 sets, with several guard rows at the beginning

and end. Test arrangement, records and observations were carried out in accordance with the fiber flax selection guidelines [33].

Weather conditions differed in the years of trials. In 2012, the vegetation season was dry (lack of moisture at seedling—budding period), with high average daily temperature in the decade III of June—decade I of July, while in 2013 precipitations and air temperature were generally favorable. In 2014, precipitation fell out unevenly and temperature fluctuated: rains were 33 % of the norm in rapid growth—blooming period, while during ripening when the total precipitations were 0.8 mm (3 % of the norm) the average temperature reached 23.5 °C (6.2 °C above the climate norm).

Statistical processing was performed according to B.A. Dospekhov [34] using software packages of Microsoft Excel 2007 and Statistica 7 (StatSoft, Inc., USA). Plasticity index (b_i) and stability variance (S^2d_i) were assessed by S.A. Eberhart and W.A. Russell [35].

Results. The viability of genotypes selected as parent forms was associated with their high productivity, quality, resistance to lodging and fusarium wilt. They were preliminary studied under the conditions of North-Eastern Belarus. The standards were varieties Yarok, Alei and Mogilevsky adopted for commercial growing in Belarus which showed for a long time a lesser dependence on weather conditions as to principal economically important traits. Assessment of the whole set of studied traits using a two-way analysis of variance has proved the consistency of the genotype factor influence. For genotype × environment ineraction, the significance of mean square (MS) variation (linear) for fiber content was statistically unconfirmed, indicating similar responsiveness of genotypes to varied growing conditions, while for other traits the reliable interaction of the factors was found out (Table). At 95 and 99 % confidence levels, genotypes maximally contributed to stem weight (MS = 19361.41) and plant height (MS = 7347). Environment mostly contributed to two traits, the stem weight (MS = 343543.12) and technical stem length (MS = 8343.12).

Two-way analysis of variance on four target traits in fiber flax (*Linum usitatissimum* L.) varieties and F_2-F_4 intervariety hybrids (*n* = 46, Orshansky Region, Belarus, 2012-2014)

Factor	Mean square (MS)			
	plant height	technical stem length	stem weight	fiber content
Genotype	73.47*	59.24*	19361.41*	10.79*
Environment	22.70	17.41	5865.55	2.82
Environment (linear)	3644.52*	8343.12	343543.12	150.16
Genotype × environment (linear)	25.83*	17.96*	9231.85*	5.96
Pooled deviation from regression	3.71	1.29	2214.41	1.41
* Differences among values are statistically significant at p > 0.05.				

The most favorable growth conditions for fiber flax were in 2013 when the plant heights of K-65, Lvovskiy 7 varieties and Vasilek × K-65, Yarok × Voskhod, Vasilek × Drakkar hybrids reached 98.2 cm. The high average daily air temperature during blooming in 2012 negatively affected generative organs and fertilization, but the year was the most favorable for the technical stem length: the maximum lengths we observed in Vasilek × Drakkar (81.4 cm), Yarok (79.3 cm) and Voskhod (77.3 cm) varieties. The maximum stem weight was in 2014 as evidenced by parameters in the hybrid combinations involving Voskhod and Drakkar varieties.

The morphological analysis of hybrid forms has identified early ripening genotypes (in the combinations Yarok × Laska, Laska × K-65, Laska × Velich, Yarok × Upite 2, Yarok × Drakkar, Yarok × Vasilek, Yarok × Voskhod, Voskhod × Lvovskiy 7, Voskhod × Laska, Voskhod × Velich; Yarok × Alizee) and tall-growing genotypes (Yarok × Velich, Vasilek × K-65, Laska × Yarok,

Yarok × Velich, Alizee × Upite 2, Upite 2 × Drakkar, Laska × Drakkar, Upite 2 × Vasilek, Upite 2 × Yarok, K-65 × Yarok, K-65 × Drakkar, Velich × Alizee, Voskhod × Yarok, Laska × Voskhod, Voskhod × Alizee); genotypes having the maximum technical stem length (Upite 2 × Laska, Upite 2 × Drakkar, Yarok × Velich, Voskhod × Yarok, Voskhod × Alizee, Velich × Alizee, Voskhod × K-65, Laska ×Yarok, Yarok ×Velich, Alizee × Upite 2) and the maximum stem weight (Alizee × Upite 2, Upite 2 × Drakkar, Laska × Yarok, Yarok × Velich), the maximum fiber weight (Laska × Yarok, Yarok × Velich, Alizee×Upite 2, Voskhod × K-65, Upite2 × Drakkar), and the maximum fiber content (Yarok ×Velich, Upite2 × Drakkar, Alizee × Upite 2).

Adaptability and stability of parental varieties of fiber flax (*Linum usitatissimum* L.) on plant height (A), **technical stem length** (B), **stem weight** (C) **and fiber content** (D): 1 — Vasilek, 2 — Lvovskiy 7, 3 — Upite 2, 4 — Velich, 5 — K-65, 6 — Drakkar, 7 — Alizee, 8 — Voskhod, 9 — Laska, 10 — Yarok; plasticity index b_i is provided by diagrams (left scale), stability variance S^2d_i is shown by curves (right scale) (Orshansky Region, Belarus, 2012-2014).

Among all parental forms (Fig.), the minimum variability for plant height was observed in Yarok ($b_i = 0.45$, $S^2d_i = 16.21$), Voskhod ($b_i = 0.25$, $S^2d_i = 10.92$), K-65 ($b_i = 0.67$, $S^2d_i = 11.90$), Vasilek ($b_i = 0.23$, $S^2d_i = 15.78$) varieties, for technical stem length in Voskhod ($b_i = 0.47$, $S^2d_i = 6.23$), Vasilek ($b_i = 0.23$, $S^2d_i = 15.78$), Velich ($bi = 0.89$, $S^2d_i = 2.45$), Drakkar ($b_i = 0.89$, $S^2d_i = 2.45$), for stem weight in Upite 2 ($b_i = 0.76$, $S^2d_i = 1553.86$), Lvovskiy 7 ($b_i = 0.98$, $S^2d_i = 2268.64$), for fiber content in Velich ($b_i = 0.48$, $S^2d_i = 31.2$), Upite 2 ($b_i = 0.35$, $S^2d_i = 41.5$). Among hybrid combinations, the adaptability on plant height was identified in Yarok × Laska ($b_i = 0.67$, $S^2d_i = 12.33$), Yarok × Voskhod ($b_i = 0.87$, $S^2d_i = 12.33$), Voskhod × Yarok ($b_i = 0.83$, $S^2d_i = 32.31$), Voskhod × Laska ($b_i = 0.83$, $S^2d_i = 32.31$), Velich × Yarok ($b_i = 0.23$, $S^2d_i = 12.34$), on technical stem length in Velich × Alizee ($b_i = 0.53$, $S^2d_i = 34.43$), Voskhod × Yarok ($b_i = 0.54$, $S^2d_i = 2.45$), Laska × Voskhod ($b_i = 0.31$, $S^2d_i = 56.78$), Laska × Drakkar ($b_i = 0.12$, $S^2d_i = 11.43$), on stem weight in Yarok × Upite 2 ($b_i = 0.78$, $S^2d_i = 3243.12$), Upite 2 × Drakkar ($b_i = 0.14$, $S^2d_i = 2256.78$), Yarok × Lvovskiy 7 ($b_i = 0.43$, $S^2d_i = 1237.01$), on fiber content in Yarok × Velich ($b_i = 0.76$, $S^2d_i = 4.56$), Laska × Velich ($b_i = 0.56$, $S^2d_i = 3.23$), and K-65 × Upite 2 ($b_i = 0.32$, $S^2d_i = 2.34$).

Currently, due to climate changes, screening for adaptability and stability is considered an important step in selection. As L.V. Ivashko [31] notes, Yarok and Vasilek varieties surpass foreign analogues in productivity and tolerance to unfavorable environment factors in a number of cases. However, foreign varieties possessing both high productivity and lodging resistance should be involved in

crossings to enhance the genetic diversity of novel varieties. Adaptable forms among collection samples of fiber and oil flax was reported by K.P. Korolev [32], V.Z. Bogdan et al. [29] and L.M. Polonetskaya [30] based on environmental studies in Belarus. It is consistent with the findings of foreign researchers [26] who identified trait variability in 1117 collection samples of flax.

It should be noted a local variety in the parentage of varieties bred in Belarus (Yarok, Vasilek, Laska, Velich, K-65) that makes it possible to obtain hybrids with both productivity and resistance to unfavorable environment factors without a decrease in the trait expression.

Thus, the varieties developed in Belarus and the majority of their hybrids appeared to be more stable in the varied environment conditions than foreign varieties. The stability in plant height and technical stem length was characteristic of Voskhod, Yarok, K-65 while technical stem length, stem weight and fiber content stability were observed in Vasilek, Velich, Drakkar and Upite 2. Upite 2, Velich, Drakkar, Lvovskiy 7, Laska, and Alizee varieties may be referred to genotypes well responded to improved environmental conditions ($b_i > 1$) on plant height, stem weight, and fiber content. High adaptability on all the studied traits was characteristic of Laska and Alizee varieties. As a result of our trials conducted in North-Eastern Belarus, the varieties Yarok, Vasilek, Velich, K-65, Upite 2, and hybrid combinations Laska × Yarok, Yarok × Velich, Alizee × Upite 2, Voskhod × K-65, Upite 2 × Drakkar, Laska × Velich, K-65 × Upite 2 may be used to develop novel flax varieties having high productivity and resistance to unfavorable environmental factors.

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POLYPHENOLS OF RED GRAPES IN WINE AND CONCENTRATES FOR USE IN REHABILITATION TECHNOLOGIES

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A b s t r a c t

In recent years, as cardiovascular mortality is increasing, the interest in studying the influence of polyphenol-rich grape products (PRGP) on cardiovascular risks is constantly growing. The estimation of a safe and effective dose of PRGP deserves special attention, because an excessive consumption can lead to endothelial dysfunction and oxidative stress. The hygienic and curative properties the young branches of grapevine, leaves, berries, juice and wine are used in traditional medicine for a long time. The curative properties of grapes are known to be due to the presence of biologically active grape polyphenols, which are accumulated in grapeskin, pulp, and seeds, etc. Polyphenols are extracted during alcoholic fermentation and determine the antioxidant status and biological activity of wines and other grape products. Here our objective was to analyze polyphenols in red wine (RW) and extracts from red grapes (EPG) and to compare the influence of fixed concentration of polyphenols on cardiovascular parameters, lipid peroxidation (LPO) and lipid metabolism at ischemic heart disease (IHD) and essential hypertension (EH) in the course of the SPA and resort-based treatment of 259 patients. The clinical trials of therapeutic and prophylactic properties of the experimental samples of red wine and extracts of polyphenols from red grapes showed that the use of these products as part of complex therapy contributes to the significant improvement of lipid metabolism, i.e. total cholesterol was reduced by 22 % and the atherogenic index decreased by 10 %, to a reduced free radical oxidation (end products of LPO decreased by 30.3 % when using the RW, and by 32.3 % in case of EPG), as compared to the patients from control groups who were not provided with PRGP in addition to a standard rehabilitation. The normalization of cardiovascular indexes also occurred. In the most patients, the tolerance to physical activity increased by 22.4 % compared to the control group. The clinical effect was achieved during a 14-day course at a daily dose of 3.6 ml/kg for RW, and of 0.45 ml/kg for EPG. The mechanisms of action of these polyphenolic products and the possibility of their use for primary and secondary prevention of disorders in patients with cardiovascular disease are discussed.

Keywords: grape polyphenols, antioxidant activity, ischemic heart disease, hypertension, red wine, extract of polyphenols from red grapes

Products of grape processing with a high content of polyphenols obtained from grape pulp exhibit unique bioactive properties and may be used in pharmacology [1-3]. Red grape wines containing on average 2.5 g/dm^3 of poly-

phenols, are recommended at a dose of 150-300 ml/day by American nutrition experts for reducing risk of cardiovascular diseases [4] that is explained by a beneficial effect on cardiometabolic factors [5-7]. Cardioprotection effect of polyphenols is associated with the vitamin P activity of the tannin-catechin complex that enhances the strength of capillary walls [3], the similar effect of anthocyans [3] and fortifying effect of procyanidins which inhibit collagen-destructive enzymes and reduce aortic elastin-associated cholesterol [3]. Antioxidative activity of wine correlates to the content of polyphenols in grapes, and there is a synergy of effects of polyphenols in the product [8, 9].

Controlled and regular wine consumption is considered favorable for mitigating a threat of cardiovascular accidents [5-7, 10], although alcohol at doses of more than 31 ml/day may negatively affect cardiovascular system [11-13]. Therefore, it is necessary to identify effective and safe doses of wines and other polyphenol-enriched products of grape processing. It should be noted that the content of polyphenols in these products is not controlled by domestic and foreign standards. Such an uncertainty makes it difficult to assess grapes and determine quality requirements for primary producers and breeders, as well as elaborate regulations for manufacturing and introducing the functional products in rehabilitation.

Polyphenols of red grapes in wines and concentrates are composed of two primary groups of flavonoid and non-flavonoid substances [2, 8, 14-16]. Monomeric flavonoids are glycosides of anthocyans (delphinidin, malvidin, cyanidin, petunidin), quercetin and its glycoside, (+)-D-catechin, (−)-epicatechin. Oligomeric flavonoids (procyanidins) consist of several (up to six) condensed catechin units (catechin, epicatechin and their dimers); polymeric procyanidins contain seven and more catechin units. Oligomeric and polymeric forms of flavonoids constitute a greater labile part of total polyphenols in bioactive red wines and concentrates. Among non-flavonoid polyphenols, there are hydroxybenzoic acids (gallic, syringic) and hydroxycinnamic acids (caftaric, cautaric), chlorogenic acid and trans-resveratrol [16].

Clinical studies of patients with chronic diseases have revealed some differences in the effectiveness of enotherapy [17, 18]. In particular, it has been found that the cardioprotection activity depends on the structure and concentration of polyphenols, their bioavailability, and patients' diet and individual characteristics. Thus, there is grate necessity for more representative clinical studies of effects of polyphenols contained in red wines and concentrates.

We are the first who developed formulae and manufacturing technologies for grape wines and concentrates having identical compositions and amounts of polyphenols for therapeutic application and estimated experimentally their cardioprotection activity.

The objective of the study was to obtain products for enotherapy and concentrates with standardized contents of polyphenols using red grapes, and to investigate their efficacy in the sanatorium-and-spa treatment (SST) of cardiovascular diseases.

Technique. Red table wine Zdorovye was produced from mature Saperavi grape with total sugars of at least 170 g/dm^3. Fermentation with floating cap was performed in a vinificator at 25-27 °C for 4.5 days with a control for concentration of total polyphenols in the substrate ready for bottling (at least 2.5 g/dm^3). In preparing an extract of polyphenols, fermented Cabernet Sauvignon grape pulp undergone a water-alcohol extraxtion with further dealcoholization under vacuum to the ethanol content of 10.5-15.0 vol.% and the concentration of total phenolic substances of at least 20.0 g/dm^3. Experimental products were produced from grapes harvested in 2015. Samples of commercial wines from red

grapes (Cabernet Sauvignon, Merlot and Saperavi; vintage of 2014) were used to compare the polyphenol composition.

The composition and quantity of polyphenols were analyzed by the high-performance liquid chromatography (HPLC) using an Agilent 1100 chromatograph with a diode array detector (Agilent Technologies, USA). Chromatographic column Zorbax SB-C18 (2.1 × 150 mm) filled with silica gel with bonded octadecylsilyl phase (sorbent particle size of 3.5 μm) was used for separation. The eluent consisted of A (methanol) and B (0.6 % trifluoroacetic acid aqueous solution). Chromatography was performed in the gradient mode with varying the content of component B as follows: 0 min — 8 %; 0-8 min — 8-38 %; 8-24 min — 38-100 %; 24-30 min — 100 %; eluent flow rate was 0.25 ml/min; injection volume 1 μl. Separation of fractions was recorded at 280 nm for gallic acid, (+)-D-catechin, (–)-epicatechin and procyanidins, at 313 nm for derivatives of hydroxycinnamic acids, at 371 nm for quercetin, and at 525 nm for anthocyans. For identification of substances, retention time was compared with that of standards [19, 20]; for quantitation we used calibration curves for individual substance solutions. Aanthocyan contents were expressed as equivalent of malvidin-3-O-glucoside chloride, caftaric acid — as equivalent of caffeic acid, polymeric and oligomeric procyanidins — as equivalent of (+)-D-catechin. Gallic acid, caffeic acid, (+)-D-catechin, malvidin-3-O-glucoside chloride, quercetin dihydrate, isoquercitrin (Fluka Chemie AG, Switzerland) and trans-resveratrol, (–)-epicatechin, syringic acid (Sigma-Aldrich, Switzerland) served as standards. All measurements were made in 3 replicates. For the consistency of results, amounts of substances were expressed in milligrams per 1 kg of dry husk weight.

Mass concentration of phenolic substances in the products studied was identified colorimetrically. To an aliquot of 1 cm^3 of a sample diluted to 10^{-2} the Folin-Ciocalteu reagent (1 cm^3) and sodium carbonate (10 cm^3) were added, then the mixture was diluted to 100 cm^3 with distilled water and allowed at room temperature (20±0.5 °C) for 30-40 min. Optical density was measured against the control in a cuvette with 10 mm optical path length at $\lambda = 670$ nm. The mass concentration of phenolic substances (C, mg/dm^3 expressed as equivalent of gallic acid) was calculated using the calibration curve as $C = F \times OD$, where, C is mass concentration of phenolic substances, F is dilution factor, OD is optical density. The calibration curve was constructed as described [21]. The arithmetical mean of two measurements with the allowable discrepancy of no more than 33 mg/dm^3 for the range of 3000-20000 mg/dm^3 was taken as a final result. The measurement error limit for mass concentration of phenolic substances at P = 0.95 for this range was ±39 mg/dm^3. The measurements were made in 3 replicates. Antioxidative activity of samples was assessed amperometrically with trolox antioxidant as standard (Tsvet Yauza 01-AA apparatus, Khimavtomatika, Russia) [22].

Clinical studies (Ai-Petri sanatorium, May-October 2016; protocol approved by a local ethics committee) involved 259 patients aged from 30 to 80 years old with ischemic heart disease (IHD, $n = 96$) and hypertension (HT, $n = 163$). Among them, 40 patients without carbohydrate metabolism disorders, comparable in age and sex, were control groups (IHD, $n = 20$; HT, $n = 20$). The diseases were diagnosed for 2-15 years, in IHD there was a stable effort angina of no more than Functional Class III (FC) in a treadmill test (as recommended by the Canadian Cardiovascular Society), chronic heart failure (CHF) of no more than Stage IIa (according to the classification of Vasilenko-Strazhesko), CHF FC I-III. All enrolled patients signed the informed participation consent forms. The treatment group of patients with IHD included 34 (44.7 %) males and 42 (55.3 %) females, with a median age of 61.0 years old (46.0 and 77.0, respectively). A total of 47 pa-

tiens with IHD had stable angina without myocardial infarction; the average FC of angina was 1.98±0.60; 24 patients with IHD had HT as a concomitant disease. The treatment group with HT (grade I-II arterial hypertension) included 105 (73.4 %) males and 38 (26.6 %) females, with a median age of 57.5 years old (37.0; 75.0); 23 patients with HT had IHD as a concomitant disease. Initially, the treatment and control groups with the same disease had no differences in significant parameters ($p < 0.05$).

All the groups were prescribed with similar non-drug treatment (climatotherapy, exercise therapy, massage, balneotherapy, instrumental physiotherapy, etc.) [18] with regard to individual indications and medicines according to the standards approved: isosorbide dinitrate 20 mg twice daily (in the IHD group), lisinopril 20 mg once daily (in the HT group), acetylsalicylic acid 75 mg once daily, amlodipine 5 mg once daily, bisoprolol 2.5-5.0 mg once daily, atorvastatin 10 mg once daily. In the treatment groups, daily intake of polyphenolic products of grape processing (PPGP) (10 mg of total polyphenols per 1 kg of body weight) as wine Zdorovye (3.6 ml/kg per day) or polyphenol concentrate (0.45 ml/kg per day) were added to dinner and supper diets for a 14-day course. No PPGPs were given to the patients in the control groups.

Examinations at the beginning and end of the treatment course (day 0 and day 14) included the assessment of complaints, arterial blood pressure (ABP) dynamics by N.S. Korotkov's method, functional status of cardiovascular and respiratory systems (spirographic, rheographic, electroencephalographic and electrocardiographic indices) using DKh-NT Poli-SPEKTR+ diagnostic suite (Spektromed-Ukraine, Kharkov), hemodynamic cardiac function, i.e. stroke (SVB) and minute volume of blood (MVB), total peripheral resistance (TPR) and exercise tolerance (using the treadmill test). Hematologic study included complete blood count, glucose analysis, blood chemistry, i.e. C-reactive protein, lipid profile, total bilirubin, diene conjugates (DC), determination of prothrombin index (PTI) and fibrinogen. Concentration of C-reactive protein was determined by the enzyme-linked immunosorbent assay [23], for other hemostatic profile Technology-Standard kit (Russia) was used. Lipid peroxidation and blood antioxidant activity of were estimated [24].

The data was analyzed by variation statistics methods using the Mann-Whitney U-test. The mean (*M*), standard deviations (±σ), medians (*Me*) and quartiles ($Q_{25\%}$, $Q_{75\%}$) are provided. Differences among the values were considered statistically significant at $p < 0.05$.

Results. Polyphenols in wines and concentrates produced from Cabernet Sauvignon, Merlot and Saperavi red grapes (originated from commercial vineyards in various southern regions of Russia) were represented by groups of flavonoid and non-flavonoid substances in the monomeric, oligomeric and polymeric forms that was consistent with the literature data [2, 4]. Trans-resveratrol was found in neither commercial nor experimental samples, or it was detected in concentrations less than 1.0 mg/dm^3 that was likely associated with seasons of 2014-2015 good for winegrowing and winemaking. Integrated indices for polyphenols determined by the HPLC and using the Folin-Ciocalteu reagent, were different, however, the antioxidant activity characterizing the potential of biological activity varied in proportion to the content of polyphenols in wines and concentrates, as noted previously [8].

Experimental samples of red wine used in the clinical studies contained at least 2.5 g/dm^3 of polyphenols, and those of concentrate (extract of grape polyphenols) contained at least 20.0 g/dm^3 of polyphenols, i.e. the samples were identical to commercial products in composition and concentration of polyphenols (Table 1).

1. Composition of polyphenols in commercial and experimental samples of the medicinal wine and concentrates produced from red grapes ($M\pm\sigma$)

			Commercial					Experimental	
1	2	3	4	5	6	7	8	9	10
Anthocyans, g/dm³									

Anthocyans, g/dm³
Total anthocyans

1	2	3	4	5	6	7	8	9	10
20.3±0.4	23.8±0.5	23.4±0.6	133.3±2.7	167.5±3.8	556.2±12.4	18.9±0.4	28.5±0.6	22.8±0.6	235.1±7.5

Flavones, g/dm³
Quercetin-3-O-glycoside

8.5±0.2	15.9±0.3	11.5±0.3	15.7±0.3	36.9±0.8	9.8±0.2	3.1±0.1	3.5±0.1	19.0±0.1	17.4±0.6

Quercetin

2.8±0.1	1.6±0.03	1.2±0.03	0.3±0.1	4.1±0.1	0.7±0.2	49.6±1.1	81.2±1.1	3.4±0.1	5.8±0.2

Flavan-3-ols, g/dm³
(+)-D-catechin

34.7±0.7	44.8±0.9	26.8±0.6	60.8±1.2	83.5±1.9	58.6±4.0	177.6±4.0	208.5±5.1	18.4±0.4	880.3±28.1

(–)-Epicatechin

34.5±0.7	47.4±0.9	29.7±0.7	52.9±1.1	78.8±1.8	71.2±1.6	118.4±2.7	127.3±3.1	28.7±0.7	435.2±13.9

Oxycinnamic acids, g/dm³
Caftaric acid

45.6±0.9	58.0±1.1	44.3±1.1	29.9±0.6	52.7±1.1	69.6±1.6	11.7±0.3	16.9±0.4	149.8±3.7	12.1±0.4

Cautaric acid

7.5±0.2	10.0±0.2	7.4±0.2	3.5±0.1	5.4±0.1	11.8±0.3	1.8±0.0	2.4±0.1	5.6±0.1	3.4±0.1

Oxybenzoic acids, g/dm³
Gallic acid

39.3±0.8	42.6±0.8	33.8±0.8	78.1±1.6	67.8±1.5	63.8±1.4	341.1±7.7	465.2±11.3	66.0±1.6	127.3±4.1

Syringic acid

7.0±0.1	5.3±0.1	9.0±0.2	8.0±0.2	4.0±0.1	4.3±0.1	22.6±0.5	26.2±0.6	11.7±0.3	5.3±0.2

Proanthocyanidins, g/dm³
Oligomeric proanthocyanidins

187±4	222±4	200±5	221±5	222±0	212±5	603±14	1614±39	319±8	1625±52

Polymeric proanthocyanidins, g/dm³

3045±61	3723±73	3525±84	2068±42	2072±47	2380±53	28155±634	38436±932	4670±114	39943±127

Integrated indices
Total phenolic substances (by HPLC), g/dm³

3.43±0.10	4.20±0.10	3.91±0.10	2.67±0.50	2.79±0.06	3.44±0.08	29.50±0.70	41.01±1.00	5.30±0.10	43.30±1.40

Total phenolic substances (by Folin-Ciocalteu), g/dm³

4.35±0.11	4.56±0.11	4.25±0.10	3.89±0.10	3.85±0.08	4.13±0.08	18.51±0.49	21.81±0.59	3.02±0.10	21.50±0.80

Antioxidant activity (trolox), g/dm³

2.36±0.06	2.75±0.07	2.38±0.70	2.38±0.70	2.49±0.06	2.69±0.06	24.72±0.73	36.48±0.92	1.72±0.10	33.74±1.20

N o t e. Wine brands and appellations (grape variety geographical indication): 1 — Cabernet, 2 — Merlot, 3 — Saperavi (Massandra); 4 — Cabernet, 5 — Merlot, 6 — Saperavi (Kuban); concentrates: 7 — Enoant, 8 — Enoant Premium; 9 — red table wine Zdorovje (Saperavy variety), 10 — extract of grape polyphenols (Cabernet Sauvignon variety); the samples are described in the *Technique* section. HPLC — high-performance liquid chromatography.

The experimental samples have passed clinical testing in the sanatorium. Initially, complaints in the IHD groups generally included typical angina attacks (78.7 %), decrease in memory (68.2 %), rapid fatigue (62.4 %), palpitation (36.1 %), headache (34.6 %), dizziness (30.5 %). After the SST course with PPGP intake, the number of angina attacks reduced almost twice in 84.4 % of patients ($p < 0.05$). In 24.3 % of patients the angina FC changed from II to I by the end of the treatment course. The majority of patients (87.3 %) showed decreased fatigue and increased exercise tolerance: the permissible exercise stress increased by 22.4 % ($p = 0.002$), and the recovery time decreased by 16.4 % ($p = 0.01$). Positive dynamics and clinical effectiveness of the concentrate and the medicinal wine did not differ. Efficacy of the basic treatment course was somewhat lower: cardiac pains decreased only in 52.7 % of patients, palpitation complaints decreased in 34.2 % of patients, fatigue reduced in 55 % of patients. A number of clinical symptoms reduced or disappeared, with improving general condition, in the majority (83 %) of patients with HT in the treatment group who received the PPGP. In the control group (basic SST course), the clinical symptoms and complaints decreased only in 44 % of patients.

Positive effects of PPGP were observed in IHD and HT by objective parameters characterizing the cardiovascular system status (Table 2).

2. Dynamics of heart rate (HR)**, systolic and diastolic blood pressure** (SBD and DBD) **in patients with ischemic heart disease and hypertension after the sanatorium-and-spa treatment course with administration of products of red grape processing with high content of polyphenols**

Group	HR, beats/min		SBD, mm Hg		DBD, mm Hg	
	at entry	at discharge	at entry	at discharge	at entry	at discharge
I s c h e m i c h e a r t d i s e a s e						
Control	72±8; 72	69±5; 68	138±17; 143	128±14; 130**	87±8; 85	83±7; 80
($n = 20$)	(67; 77)	(67; 71)	(134; 146)	(118; 140)	(80; 95)	80; 85)
RTW	71±9; 69	66±5; 67**	137±12; 140	123±11; 120**	87±7; 85	81±7; 80**
($n = 30$)	(65; 78)	(63; 70)	(131; 144)	(115; 130)	(80; 95)	(80; 85)
EGP	73±7; 75	66±7; 63**	137±12; 135	124±9; 125**	88±8; 88	81±5; 80**
($n = 30$)	(67; 778)	(60; 69)	(131; 140)	(116; 130)	(80; 95)	(80; 85)
H y p e r t e n s i o n						
Control	78±8; 79	74±6; 74	156±18; 160	137±8; 138**	94±10; 95	87±6; 95**
($n = 20$)	(74; 83)	(70; 79)	(140; 175)	(135; 140)	(90; 100)	(84; 91)
RTW	75±8; 78	71±7; 73**	157±13; 155	131±16; 133**	94±10; 95	82±9; 80**
($n = 30$)	(70; 80)	(65; 78)	(146; 170)	(116; 140)	(85; 99)	(75; 90)
EGP	77±6; 78	70±6; 69**	156±19; 163	129±11; 130*, **	96±10; 95	84±7; 85**
($n = 30$)	(74; 80)	(65; 74)	(136; 170)	(120; 135)	(90; 105)	(80; 90)

N o t e. RTW — red table wine, EGP — extract of grape polyphenols (the experimental products are described in the Procedure section). Values of $M\pm\sigma$, Me ($Q_{25\,\%}$; $Q_{75\,\%}$) provided.
*, ** $p < 0.05$ (differences to control groups and differences between parameters at entry and at discharge, respectively).

Furthermore, the IHD and HT groups reported the positive effect of PPGP on metabolism. So, the decrease in total cholesterol after administration of PPGP was more significant ($p < 0.05$) than that in the control group. The fact that the administration of PPGP allowed the improvement of some lipid metabolism indices which were especially disturbed in patients with IHD was an additional confirmation of the efficacy of polyphenols in its normalization ("French paradox").

Blood chemistry parameters (glucose level, total bilirubin, PTI) in the IHD and HT groups did not differ significantly at entry and at discharge and were essentially independent of the PPGP administration. SST in conjunction with PPGP contributed to reduction in C-reactive protein concentration (to less than 2.6 mg/l in all the groups) that was indicative of the suppression of sub-clinical inflammation and reduced risk of vascular complications. Efficacy of the medicinal wine and the extract of grape polyphenols was comparable to and exceeded that of the basic SST course.

3. Some lipid peroxidation indices in patients with ischemic heart disease and hypertension after the sanatorium-and-spa treatment course with administration of products of red grape processing with high polyphenol content

Group	Parameter	TBA-AP, nmole MDA/ml		CP, mg/l	
		at entry	at discharge	at entry	at discharge
I s c h e m i c h e a r t d i s e a s e					
Control ($n = 20$)	M	194.52	158.79	301.42	268.14
	σ	26.04	29.53	57.29	50.60
RTW ($n = 30$)	M	186.97	143.49	325.03	233.13
	σ	34.82	23.59	55.27	38.83
EGP ($n = 30$)	M	185.15	139.97	317.74	223.58
	σ	31.23	24.40	72.61	60.28
H y p e r t e n s i o n					
Control ($n = 20$)	M	178.00	149.26	241.41	224.86
	σ	28.21	22.65	39.67	27.64
RTW ($n = 30$)	M	177.17	141.26	229.75	242.30
	σ	27.03	23.19	22.59	33.18
EGP ($n = 30$)	M	184.00	148.10	244.14	231.84
	σ	26.79	26.48	30.50	46.81

N o t e. RTW — red table wine, EGP — extract of grape polyphenols (the experimental products are described in the Procedure section); TBA-AP — active products of thiobarbituric acid, MDA — malondialdehyde, CP— ceruloplasmin. Differences with the control are statistically significant at $p < 0.05$.

As to oxidant-antioxidant homeostasis (Table 3), in IHD and HT,

PPGPs helped reduce free-radical oxidation that was evidenced by the decrease in number of primary and secondary lipid peroxidation products and the reactivation of antioxidative enzymes.

Hence, the PPGPs as a part of a combined therapy for patients with HT and IHD both have the hypotensive action and improve the lipid metabolism, as well as the activity of the antioxidant system. It should be noted that the PPGPs helped improve the health status in patients with IHD to a greater extent than in those with HT. Central hemodynamics was improved in patients with both diseases.

Thus, the developed technology for manufacturing products from red grapes provides a standardized content of biologically active polyphenols. It has been found out that the products (red table wine Zdorovye and extract of polyphenols) have a curative effect in ischemic heart disease and hypertension and may be used in rehabilitation in addition to the basic sanatorium-and-spa treatment. The therapeutic dose shall correspond to known long-term performance of the total polyphenols and be about 3.6 ml/kg per day in enotherapy, and about 0.45 ml/kg per day for the extract of polyphenols (14-day course). For optimization of technologies for sanatorium-and-spa treatment and rehabilitation, the dosages should be further specified.

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