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Polymorphism of the available potato gene pool for resistance to abiotic and biotic factors of the environment and its practical use

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The article presents the results of 1954-2021 studies on the potato polymorphism as a consequence of species evolution and practical application of these investigations. The issue of adaptability of valuable genes of potato, which contributed to its preservation in nature for a long time, is covered, and the potato evolution is traced on living material. It is noted that some species of this crop do not exist any longer and, given this, the importance of the plant genetic bank as a depository of material and intellectual assets of the people of Ukraine is stressed. The studies allowed for identification of valuable potato accessions, analysis of the genealogy of individual cultivars, which provided an opportunity to investigate the relationships between modern cultivars and their ancestors. The identified or newly created valuable potato accessions found further practical use, as they were involved in breeding, scientific and educational programs. Due to multi-year research aimed at targeted involvement, effectively use and preservation of the authenticity of the valuable gene pool of potatoes as well as at optimization of the qualitative and quantitative composition of the National Bank of Plant Genetic Resources of Ukraine, a pedigree database on 301 accessions from 18 countries with certificates for 35 valuable unique accessions was formed and registered with the National Center for Plant Genetic Resources. We also built-up collections of 6 different types, specifically, a trait collection for yield including 46 accessions from 8 countries; a trait collection for starch content and technological scores (61 accessions from 5 countries); a working trait collection for large tubers (121 accessions from 16 countries); a working trait collection for resistance to viral diseases (31 accessions from 7 countries); and a trait collection for distinctness traits (568 accessions from 15 countries). The above collections and accessions of the potato gene pool are a concentrated reserve of valuable genes as well as material and an intellectual asset of the people of Ukraine; they play an important role in improving the welfare of the nation and the strength of the state, increase its scientific, intellectual and spiritual potentials and need preserving in a viable state and genetic integrity.

Key words: *potato, polymorphism, gene pool, genealogy, cultivars, breeding.*

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Introduction

Among agricultural crops, the potato is favorably distinguished due to an extraordinary diversity of species, subspecies, variety groups, varieties, forms, accessions, cultivars, and hybrids. All the potato diversity is its gene pool. The gene pool of a crop is the totality of all genes of its taxonomic units, which are characterized by certain frequencies. The potato gene pool consists of an assortment of cultivated and aboriginal cultivars and endemic forms, numbering about 200 wild and domestic species, which differ significantly one from another. The potato diversity is a continuous polyploid series, from di- to hexaploids. Most of the potato species (about 70%) are diploids (Gorbatenko, 1989).

The tropical origin of the potato explains its significant polymorphism, as the tropical flora is generally noticeable for a great range of forms. Polymorphism, as a consequence of the evolution of a species, is of great biological importance, as it contributes to the existence of the species under very different conditions and to the emergence of new species. The trait expression polymorphism within a species (Zhukovskiy, 1971; Pushkaryov, 1962; Podhaietskyi 1995) requires evaluating as many accessions as possible. Some potato forms are able to withstand as harsh frosts as of -6^{o}C, drought, high air and soil temperatures, etc. Analysis of numerous studies allows conditional dividing the potato gene pool into forms that evolved through evolution and forms, the synthesis or selection of which was human need-oriented. The need for this approach is based on different criteria of natural and artificial selections. The natural evolution of a crop is directed towards improving the generative and vegetative parts of the plant, which would help preserve the potato diversity. Along with this, the natural evolution of the potato also resulted in the emergence of forms with higher expression of economically valuable features: from wild species to domestic ones and to endemic forms (as a separate evolutionary stage). Representatives of the potato gene pool are highly able to reproduce generatively and vegetatively; some of them are highly resistant to stressors (Podhaietskyi, 2002).

After the potato introduction to Europe in the second half of the 16th century, the evolution of mainly two domestic tetraploid species, *S. tuberosum* and *S. andigenum,* continued. These species evolved at the latest stages of phylogenetic development and had larger tubers and more yielding plants in comparison with diploid and other tetraploid species. However, both species significantly differed both in the environments, where they evolved, and in the plant morphology.

S. andigenum is a polymorphic and widespread species, which evolved in highlands under the influence of elevated ultraviolet radiation, drastic shifts of diurnal temperatures, and arid climate. The highland conditions affected the plant morphology: low bushes, small and mid-sized leaves, pigmented stems.

S. tuberosum – a typical species of lowland coastal plains with diffused sunlight and sufficient precipitation; it combined the features of a hygromorphic plant: broad, leafy bushes, large leaves, thick and succulent stems.

When *S. andigenum* was grown in a mixture with *S. tuberosum* (it was common in the early stages of the potato cultivation in Europe), spontaneous interspecies hybrids, which were heterozygous and highly plastic, appeared due to natural transpollination between these species. In addition, after several generations of sowing in a more humid and temperate climate, *S. andigenum* transformed into forms similar to *S. tuberosum*: leafy and latifoliate. Thus, under the influence of the new climate of Europe, a new subspecies of European potato, *ssp. Europeanum,* evolved within the species *S. tuberosum* (Kustaryov, 2002).

It should be noted that potato species have valuable adaptive genes, which allowed them to survive in nature for a long time. Therefore, it is now possible to trace the evolution of this crop on living material, despite the fact that some potato species have become extinct and are lost forever.

Materials and methods

Prevention of loss, preservation and rational use of their own genetic resources of plants are inviolable attributes of the state policy of sovereign states. In Ukraine, the National Bank of Plant Genetic Resources of Ukraine was started in 1992, and the National Center for Plant Genetic Resources of Ukraine

(NCPGRU) became its organizational core. After the proclamation of Ukraine's independence in 1991, since 1993, Ustymivka Experimental Station of Plant Production of the Plant Production Institute named after V.Ya. Yuriev of NAAS has been a basic scientific institution of the NCPGRU. Currently, the National Bank of Plant Genetic Resources of Ukraine has collected 151,300 specimens of domestic plants and their wild related species, including the potato gene pool, which comprises 3,719 samples belonging to 70 botanical species (2 domestic and 68 related wild species). At present, almost 90 gene banks of the world store 14,000 potato cultivars as well as significant numbers of locally cultured accessions and representatives of wild potato species. Potato research was started at Ustymivska Experimental Station of Plant Breeding in 1953 (Kharchenko et al., 2008; 2009). The study period was from 1954 to 2021. The study was conducted in accordance with conventional methods of potato growing (Cherepanova 1971; Bukasov et al., 1975; 1977; Kostina, 1976; 1983; 1992; Clark, Adams, 1977; Osipchuk et al., 1983; Zadina et al., 1984; Truskinov, 1987; Komarov et al., 1988; Podhaietskyi, 1991; 1993; Crowther, 1995; Podhaietskyi, Hrytsenko, 1996; Loebenstein et al., 2001; Kutsenko et al., 2002; Santala et al., 2010; King et al., 2012; Karasev, Gray, 2013; Lacomme, Jacquot, 2017).

Results and discussion

During the period of 1954–1974, 2,294 potato samples, mostly early and mid-early cultivars received from the N.I. Vavilov All-Union Research Institute of Plant Industry and other research institutions of the USSR, were evaluated (Olefir, 1975a). Due to a significant spread of the nematode (*Ditylenchus destructor* Thorne) at that time, scientists of the Experimental Station conducted surveys in 25 districts of the Poltavska, Kharkivska, Sumska, and Kirovohradska Oblasts. In the Kirovohradska Oblast, the nematode was detected in 100% of the surveyed farms, in the Poltavska Oblast – in 97.1%, in the Sumska and Kharkivska Oblasts – in 50.0%. This phenomenon was a serious problem in the potato growing as damage to tubers by stem nematodes in 1966–1970 in the Poltavska Oblast reached 28.9%. Losses of potato tubers during winter storage increased considerably, because nematodes continued to destroy tubers in storehouses (Olefir, 1971). In this regard, the Experimental Station paid special attention to the evaluation and selection of potato accessions that would be resistant to this parasite from the collection (Olefir, 1969). The work was carried out by V.V. Olefir under the guidance of Doctor of Agricultural Sciences A.Ya. Kameraz. During the period of 1967–1973, 160 accessions of tuber-reproduced wild species and 39 families of potato seedlings, which belong to 46 species of 20 series, were evaluated (Kameraz, Olefir, 1974). Four hundred and eighty-seven samples of *Solanum andigenum* Juz. et Buk. seedlings were assessed for resistance to stem nematodes in 1967–1970. During the period of 1970–1973, 232 tuber-propagated seedlings belonging to seven primitive domestic diploid species were studied, (Olefir, 1975b). In addition, 545 foreign and soviet cultivars as well as 313 clones of interspecies hybrids were evaluated for resistance to the nematode in 1966–1973 (Kameraz, Olefir, 1974).

The collection was studied under natural and artificial infestation of soil with stem nematodes. The purpose was to identify resistant forms that are valuable for breeding as starting material. Plants of wild species and *S. andigenum* forms were grown in pots (diameter 16–17 cm; volume 2,000 cm^3), as the biological specifics of the wild species (tuber formation in short photoperiod, long stolons) prevents their cultivation in the field. The pots were filled with humus-soil (1:2) mixture and placed in concrete greenhouses. The gaps between the pots were tightly filled with soil. Primitive domestic diploid species, cultivars and interspecies hybrids were tested in an area isolated from homesteads and the main crop rotation fields. The soil for the pot mixture was taken from the same area. Therefore, all accessions in the experiment were studied on the same soil. Plants were infested by joint planting of healthy and affected tubers in one box. Ella (Germany) and Priyekulskiy Ranniy (Russia) were taken as control cultivars. During harvest, the score of nematode-induced damage to tubers was visually determined. In addition, sections of three tubers from each bunch were microscopically monitored. Tubers that were not affected outwardly by the parasite were stored in a potato storehouse and thoroughly inspected again by the appropriate method before planting (Olefir, 1970).

The results of the 1966-1973 evaluation of the potato collection for resistance to stem nematodes showed that highly resistant forms could be distinguished within different wild potato species, which belong to several series: *S. bukasovii* Juz., *S. catarthrum* Juz., *S. sucrense* Hawk., *S. vernei* Bitt et Wittm., *S. simplicifolium* Bitt., *S. infundibuliforme* Phil., *S. acaule* Bitt., *S. chacoense* Bitt., *S. commersonii* Dun., *S. semidemissum* Juz., *S. stoloniferum* Schlechtd., *S. jamesii* Torr., etcl. (Olefir, 1971). As to the domestic potato species of the *Andigena* Buk. series, resistant accessions were found within the primitive diploid

species (*S. phureja* Juz. et Buk*., S. goniocalux* Juz. et Buk.) and others as well as within the polymorphic tetraploid species *S. andigenum.* Within the *Tuberosa* (Rydb.) Buk. series, Chilean domestic forms and numerous cultivars belonging to *S. tuberosum* L. were distributed according to the degree of susceptibility, but no highly resistant accessions were found (Olefir, 1975b). Provocative studies resulted in the identification of resistant and weakly susceptible to stem nematode interspecies potato hybrids of varying complexity. Most of them come from crossing wild species of the *Glabrescentia* Buk. or *Transaequatorialia* Buk. (or others) series with *S. tuberosum* cultivars (Kameraz, Olefir, 1974). Analysis of stem nematode-induced damage to complex interspecies hybrids proved a possibility of breeding cultivars that would be virtually resistant to this parasite from nematode-resistant forms of Latin American potato species.

In 1978, as continuation of previous studies, 60 wild tuber-reproduced potato accessions, which belonged to 16 species and 6 series, were evaluated. The accessions were provided by Moscow branch of the N.I. Vavilov All-Union Research Institute of Plant Industry and tested on artificial infestation. The test results indicated immunity to stem nematodes in lots of the accessions under investigation. These were the following species: *S. chacoense, S. leptostigma* Juz*., S. famatinae* Bitt. et Wittm*., S. spegazzinii* Bitt*., S. demissum* Lindl*.,* and *S. pinnatisectum* Dun. Plants of the above species developed well on the parasite-infested soil. It was noted that accessions of the *S. chacoense, S. vernei, S. jamesii, S. pinnatisectum,* and *S. kurtzianum* Bitt. et Wittm. species were less damaged by the Colorado potato beetle in the field. In addition, 267 clones grown from seeds of self-pollinated accessions, which were resistant or weakly affected by stem nematodes, were tested on the artificially infested soil. The test results showed that seedlings of the vast majority of accessions inherited high resistance to nematodes (Olefir, Turulyova, 1982). This gave reason to believe that in the future it was possible to use nematode-resistant wild potato accessions in breeding for resistance to this parasite.

However, despite the high harmfulness of the nematode, more significant damage to the potato in the Southern Forest-Steppe of Ukraine is caused by viral diseases. Scientists faced the tuber degeneration problems at the very beginning of the potato collection at the Experimental Station.

In the 1960s, in the context of insufficiently studied degeneration of potatoes in the South of the USSR, the work carried out by the N.I. Vavilov All-Union Research Institute of Plant Industry became of vital importance. Potato crops grown from locally reproduced tubers were surveyed in six sites of the USSR, which were located in the most typical environments of the country. Major forms of external signs of degeneration were described, and its spread under production conditions was assessed (Chesnokov, 1961). The most common for each area potato cultivars and new promising cultivars were studied. In order to study the environment effects on the potato degeneration, tubers of 10 cultivars from plants without external signs of degeneration, which were examined for latent viral infection, were sent to different sites from Pushkin (the area most favorable for growing potatoes, where physical and geographical conditions are similar to those in the historical homeland of the crop).

Such comprehensive work on previously studied potatoes allowed researchers within a relatively short time to get answers to questions which were essential to launch search for ways to protect this crop from degeneration. At Ustymivka Experimental Station of Plant Production, research was conducted by post-graduate student M.F. Muravyova under the guidance of Doctor of Biological Sciences P.G. Chesnokov (Muravyova, 1962; 1966). His well-known monograph "Potato Degeneration Diseases in the USSR" [9] contains data obtained in the area of activities of Ustymivka Experimental Station of Plant Production, which are the basis of the section "Potato Degeneration in the Forest-Steppe of the European Part of the USSR."

In 1989–1992, a researcher of the Ustymivka Experimental Station, A.V. Chygryn, under the guidance of Academician of the All-Union Academy of Agricultural Sciences K.Z. Budin continued studying the prevalence and harmfulness of the potato leaf roll virus (PLRV) in the Southern Forest-Steppe of Ukraine. Due to virological examinations of the potato collection on natural infection, 32 cultivars, 4 intraspecies and interspecies hybrids and 7 *S. andigenum* forms with field resistance to PLRV were identified. Serological tests showed that PLRV-resistant cultivars (Alpha, Bintje, Jaerla, Mansour, Sante, Tempora [Netherlands]; Turbella [Germany]) were also resistant to mosaic viruses X, Y and A. The selected cultivars can serve as starting material in potato breeding for group resistance to mosaic viruses (they penetrate the plant mainly through mechanical injuries; some of them can be transmitted by insects) and *Potato leaf roll virus* (virus-L) (transmitted by insects only). In Germany and the Netherlands, potato breeding must take into account the resistance of cultivars to PLRV. Having genealogically analyzed such cultivars, we found that all of them had virus-resistant parents in their pedigrees. Most of them are of

interspecies origin, for example, cultivar Jaerla derived from *S. tuberosum* forms and the *S. andigenum* and *S. demissum* species; cultivar Sante – from *S. andigenum, S. stoloniferum,* and *S. vernei*, cultivar Tempora – from *S. andigenum, S. acaule, S. demissum,* and *S. stoloniferum;* cultivar Turbella – from *S. andigenum, S. demissum,* and *S. chiquidenum* Ochoa.

In a three-year provocative study of 20 accessions of tetraploid domestic species *S. andigenum* and several accessions of diploid domestic species (*S. ajanhuiri* Juz. et Buk*., S. yabari* Hawk*., S. goniocalux* Juz. et Buk., and *S. rybinii* Juz. et Buk.), group resistance to PLRV and mosaic viruses was reported for 7 forms of *S. andigenun* (*v. tampumachay* (K-1793), *v. typicum* (K-1842), *v. catamarcense* (K-3153), *v. adpresse* (K-3156), *v. alcatarma* (K-3567), *v. yarochuacotto* (K-3570), and *v. catarthrisimle* (K-4005)).

The property of the tetraploid domestic polymorphic species *S. andigenum* to cross with other potato species, including cultivars of the tetraploid domestic species *S. tuberosum,* is widely used in breeding. This species served as starting material and is in the pedigrees of almost 800 potato cultivars, including 60 Ukrainian ones. It is readily used by breeders due to its inherent valuable features: resistance to nematodes, late blight, wart disease, some viral diseases (including PLRV), scab, bacterial rots and other diseases. *S. andigenum* is valuable material for the breeding of cultivars with high culinary and technological qualities required for industrial processing. Some of its accessions have increased contents of starch, protein, ascorbic acid, etc. Many forms give relatively high yields; their tubers have strong jackets and pulp. The following accessions were noticeable for the greatest tuber weights (g/bush) in the soil and climatic conditions of the Experimental Station (Southern Forest-Steppe of Ukraine): K-1793 (1,520 g), K-3567 (1,120 g), K-3570 (900 g). In virological evaluations, accessions with group resistance to PLRV and several other viruses were selected.

Scientists of Ustymivka Experimental Station of Plant Production evaluated 7 *S. andigenum* accessions, 7 cultivars, and 4 hybrids for resistance to the Colorado potato beetle. The study objective was to identify forms with complex resistance by improved methods of the All-Russian Institute of Plant Protection, which were tested and finalized at Ustymivka Experimental Station of Plant Production. Due to comparison of positions (ranks) occupied by each accession, this technique allows for determination of the resistance threshold of an accession. After data were statistically processed, the following findings were revealed: cultivar Sante showed antixenotic resistance (unattractiveness of plants for feeding and laying eggs); cultivar Alpha and *v. yarochyacotto* were distinguished by antibiotic resistance (adverse physiological effects of plants on the Colorado potato beetle feeding on them); *v. catamaracense* and two hybrids (181-1 and 43 (85) 5) combined different types of resistance.

In the late 1980s – early 1990s, very small numbers of potato cultivars were evaluated for resistance to the Colorado potato beetle. Therefore, the objective was set to evaluate the large collection of wild potato species, interspecies hybrids and unstudied accessions from the Department of Tuber Crops of the N.I. Vavilov All-Union Research Institute of Plant Industry. This work was supposed to localize resistance to the Colorado potato beetle and identify new foci.

In previous studies, V.V. Olefir (Kharchenko et al., 2008) found that resistant potato species were mainly from the Colorado potato beetle areal (North America). A.V. Chygryn evaluated 38 wild potato species (72 accessions) for resistance to the Colorado potato beetle and detected 6 highly resistant ones, including 2 species from North America (*S. pinnatisectum, S. lesteri* Hawk.) and 4 from South America. (*S. chiguidenum* Ochoa*., S. chomatophilum* Bitt*., S. multidissectum* Hawk*., S. multiinterruptum* Bitt.). These data confirmed the fact that Colorado potato beetle-resistant species are found both within the pest areal (North America) and outside it (South America, where more than 85% of all wild and domestic potato species are grown). This opens significant prospects for further research and use of these species in breeding.

Breeding material of the above species created at Ustymivka Experimental Station of Plant Production was sent to the Department of Tuber Crops of the N.I. Vavilov All-Union Research Institute of Plant Industry (to a senior researcher, L.M. Turulyova). Under the conditions of the High Pamirs (Ishkashim Base), L.M. Turulyova and D.O. Dzhongirov managed to obtain hybrid offspring from some of them. These and other hybrids were tested for resistance to the Colorado potato beetle. Their results indicated that *S. pinnatisectum* was not only a source but also a donor of resistance to the pest. Hybrids originated from the above species were resistant to the Colorado potato beetle, although the species that were crossed with *S. pinnatisectum* were not highly resistant at all.

Besides the resistance of a cultivar to biotic factors, adaptability to abiotic environmental factors is important. At the end of the 20th century, due to global climatic changes, a need arose to address the

problem of adaptive plant production. Mainstreams in plant breeding, in particular potato breeding, changed. The cultivar, as an open biological system, is subject to regulated and unregulated abiotic environmental factors in the field. Modern potato cultivars are characterized by high productivity potential, but are genetically insufficiently protected against adverse environmental conditions. In this regard, during the period of 1996-2021, the Experimental Station conducted special studies to establish the reaction norm of potato accessions to cultivation in the Southern Forest-Steppe of Ukraine. Two hundred and thirty-seven potato cultivars of different ripeness groups and 167 interspecies hybrids bred at the Institute of Potato Growing of UAAS were evaluated. Prospects of assessing backcrosses of complex remote multispecies hybrids for major economically valuable traits under specific soil and climatic conditions were outlined. It was revealed that the involvement of wild species (*S. chacoense, S. stoloniftrum*) and domestic species (*S. andigenum*) in breeding helped to breed more drought-resistant forms. Starting breeding material was differentiated by laboratory determining drought resistance as per L.S. Lytvynov's technique (Litvinov, 1988) and heat resistance as per F.F. Matskov's technique (Matskov, 1970). A possibility to use correlations for determination of heat resistance and especially water-replenishing and water-holding capacities of potato leaves by methods developed at the Institute of Potato Growing of UAAS (2002) was proven. Some hybrids in which the negative effect of drought was smaller in comparison with other factors were selected: 85.368s16, 90.676/83, 90.673/75, 85.568s9, 88.1450s2, 89.24s57, 90.691/38, 90.841s2, 91.497-92, 89.382s18, 96.977/3, 96.977/14, 91.561c3, 90.674/13, and others. The inclusion of selected parents in breeding programs of the Institute of Potato Growing of UAAS, Polissya Experimental Station named after O.M. Zasukhin, Sumy National Agrarian University became a practical result of this study.

The genetic potential of productivity and resistance of the potato to biotic and abiotic environmental factors is far from exhausted (Boland et al, 2004; Roos et al., 2011; Jones, 2014; Hameed et al, 2014; Torro et al., 2015; Lal et al., 2018). Various soil and climatic conditions positively affect the plant morphogenesis. Recently, under the leadership of the NCPGRU and with the participation of scientists of Ustymivka Experimental Station of Plant Production, N.I. Vavilov All-Russian Research Institute of Plant Industry and other foreign organizations, missions has been regularly conducted to collect landraces and wild related species in different soil/climatic zones of Ukraine: Steppe (Kirovohradska, Mykolaivska and Odeska Oblasts); Woodlands (Kyivska, Zhytomyrska, Volynska, and Rivnenska Oblasts); Forest-Steppe (Sumska, Kharkivska and Luhanska Oblasts); Central and Steppe regions of Ukraine (Poltavska, Dnipropetrovska, Zaporizhska, and Khersonska Oblasts).

As a result of enrichment of the collection with new samples and studies of introduced material, sources of economically valuable features are identified; they are transferred to breeding research institutions for further inclusion in breeding programs. Attention is paid to environmental assessments; the suitability of accessions for cultivation in different soil/climatic zones is established, which determines the further successful implementation of new cultivars into production.

As the potato spread, it was increasingly (and catholically used!); the potato breeding in each country acquired specific features and own traditions. The potato collection built up at Ustymivka Experimental Station of Plant Production comprises 665 cultivars, concentrating a significant number of breeding forms on a small area. As Kustaryov A.I. (2002) pointed out, each cultivar is a climatype, and the morphology of its plants more or less reflects the climatic conditions where it was created (Kustaryov, 2002). At the same time, these cultivars are always the end product of breeding for certain economic and biological characteristics.

In England, in the humid climate, cultivars were bred predominantly latifoliate and lost the ability to reproduce sexually. Such cultivars usually have low contents of dry matter and starch.

In the US, in the hot climate in major regions where potatoes are grown (Idaho, Maine, Washington), cultivars with dark leaves ensuring high heat resistance were bred. In Alaska, freeze-tolerant cultivars (Red Warb, Norland, Alaska, Frostless, Dazax) were bred; they had bushes and intensely pigmented stems. In general, American cultivars are characterized by large tubers and high marketability.

German breeders gave the mankind a number of high-starch cultivars (Voltman, Parnassia, Marker, Silesia) and a lot of high-yielding cultivars with excellent taste qualities (Emperor, Alma, Wagner, Paul, Foran, Carnea, Furstenkrone, Jubel, Delodara). German cultivars, with some exceptions, had abundant foliage (sympodial branching). These ecological and morphological features reflect the warm, humid climate of Southern Saxony, Bavaria and Hanover.

In the Netherlands, in the humid marine climate, rich-leafy cultivars with various timeframes of ripening were bred. The vast majority of Dutch cultivars boast good palatability, yellow pulp with a high

content of carotene and slender tubers. However, many of them are susceptible to viral diseases and drought.

The vast majority of cultivars turned out to be typical climatypes and did not go beyond the climatic regions where they were created. Only a very small number of cultivars (Lorhk, Nevskiy, Priekulskiy Ranniy, Gatchinskiy) became widespread in various climatic and soil conditions. These features, i.e. high environmental plasticity, were sure to be due to adaptations in their vegetative organs (Kustaryov, 2002).

Conclusions

The studies of the potato collection at Ustymivka Experimental Station of Plant Production under the soil/climatic conditions of the Southern Forest-Steppe of Ukraine provided an opportunity to select high-yielding accessions giving high-quality products, adapted and genetically protected from unfavorable biotic and abiotic environmental factors, as starting material for breeding and to determine some biological peculiarities of the growth and development of potato plants in this climatic zone. Due to multi-year research aimed at targeted involvement, effectively use and preservation of the authenticity of the valuable gene pool of potatoes as well as at optimization of the qualitative and quantitative composition of the National Bank of Plant Genetic Resources of Ukraine, a pedigree database on 301 accessions from 18 countries with certificates for 35 valuable unique accessions was formed and registered with the National Center for Plant Genetic Resources. We also built-up collections of 6 different types, specifically, a trait collection for yield including 46 accessions from 8 countries; a trait collection for starch content and technological scores (61 accessions from 5 countries); a working trait collection for large tubers (121 accessions from 16 countries); a working trait collection for resistance to viral diseases (31 accessions from 7 countries); and a trait collection for distinctness traits (568 accessions from 15 countries). The above collections and accessions of the potato gene pool are a concentrated reserve of valuable genes and material and an intellectual asset of the people of Ukraine; they play an important role in improving the welfare of the nation and the strength of the state, increase its scientific, intellectual and spiritual potentials and need preserving in a viable state and genetic integrity. Valuable accessions found or created during the studies have been included in breeding, scientific and educational programs and already found practical application at the Institute of Potato Growing of NAAS of Ukraine, Polissya Experimental Station named after O.M. Zasukhin, Sumy National Agrarian University, Taras Shevchenko Kyiv National University.

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Поліморфізм наявного генофонду картоплі за стійкістю до абіотичних та біотичних чинників середовища та його практичне використання

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У статті представлені дослідження стосовно вивчення поліморфізму картоплі впродовж 1954-2021 рр., як наслідку еволюції видів, та його практичне застосування. Розкрито питання адаптивності цінних генів картоплі, які сприяли її збереженню у природі впродовж тривалого часу, та простежено еволюцію картоплі на живому матеріалі. Відзначено, що окремі види даної культури припинили своє існування назавжди і, виходячи з цього, наголошено на важливості роботи генетичного банку рослин, як зберігача матеріального та інтелектуального надбання народу України. Проведені дослідження дозволили виділити цінні зразки картоплі, проаналізувати генеалогію окремих сортів, що надало можливість дослідити родинні взаємозв'язки сучасних сортів з їх батьківськими формами. Виділені або створені цінні зразки картоплі знайшли подальше практичне застосування шляхом їх включення до селекційних, наукових та навчальних програм. За результатами багаторічних досліджень з метою цілеспрямованого залучення, ефективного використання і збереження автентичності цінного генофонду картоплі, оптимізації якісного і кількісного складу Національного банку генетичних ресурсів рослин України було сформовано та зареєстровано у Національному центрі генетичних ресурсів рослин України з отриманням свідоцтв 35 цінних унікальних зразків, базу родоводів на 301 зразок з 18 країн та 6 різних типів колекцій, а саме: ознакова колекція за урожайністю, що включає 46 зразків з 8 країн; ознакова колекція за вмістом крохмалю та технологічними властивостями, що включає 61 зразок з 5 країн; робоча ознакова колекція за великобульбовістю, що включає 121 зразок з 16 країн; робоча ознакова колекція за стійкістю до вірусних

хвороб, що включає 31 зразок з 7 країн; ознакова колекція за ознаками відмінності, що включає 568 зразків з 15 країн. Вищевказані колекції та зразки генофонду картоплі є сконцентрованим резерватом цінних генів, матеріальним та інтелектуальним надбанням народу України, відіграють важливу роль у поліпшенні добробуту нації і міцності держави, зростанні її наукового, інтелектуального та духовного потенціалу і потребують збереження у високому життєздатному стані та генетичній цілісності.

Ключові слова: *картопля, поліморфізм, генофонд, генеалогія, сорти, селекція.*

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Marker-assisted selection and use of molecular markers in sunflower breeding for resistance to diseases and parasites

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Recently, the problem of phytosanitary condition of sunflower crops has been exacerbated, which is associated with violation of crop rotations and, as a consequence, spread of common diseases. Selection for resistance to biotic factors requires comprehensive research into the crop biology and pathogens. The use of starting material, which is resistant to major pathogens and environmental stressors, in selection is a prerequisite for the breeding of highly productive hybrids. Significant progress in the breeding of heterosis sunflower hybrids has been achieved primarily due to stable inbred lines. However, their creation is time-consuming, taking 8-12 years. Selection of desirable genotypes and initial forms for crossing is complicated by the fact that it is driven by a set of polygenic traits that are prone to significant modification variability. The use of molecular genetic markers is a way to accelerate breeding. Marker-assisted selection breeding (MAS) has been theoretically justified in numerous publications and implemented in most breeding institutions around the world. However, in domestic breeding programs, MAS has not become widespread compared to traditional methods. Nevertheless, this breeding trend opens new opportunities for studying genetic diversity and determining kinship at the intraspecies and genus levels. The review provides information on the status and prospects of implementation of MAS in traditional plant breeding and highlights the achievements of modern biotechnology in sunflower breeding for resistance to biotic factors owing to molecular genetic markers. The MAS principles are outlined and the advantages of this method are described. Specific examples of application of the molecular approach during the development of starting material of sunflower for breeding for resistance to common diseases and parasites are given. The main stages and components of PCR analysis are also described. Inbred sunflower lines – carriers of the gene for resistance to the downy mildew pathogen are characterized and genetic passports using STS markers to the Pl_6 locus have been formalized for 13 sunflower lines.

Key words: *DNA markers, Marker-Assisted Selection, sunflower, downy mildew, sunflower rust, broomrape.*

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Introduction

There are two main approaches in genetic studies - "direct" and "reverse" genetics. Since Gregor Mendel's discoveries of (1865), classical or "direct" genetics has been studying the inheritance of traits (phenotypes) in living organisms for several generations. Taking the phenotype as a starting point, "direct" genetics identifies genetic factors that affect the expression of any trait. Thus, "direct" genetics is

directed from phenotype to genotype. Production of bulky mutant populations for further search for phenotypic changes in them is a key stage in the experimental application of this approach (Sulima, Zhukov, 2015).

Since the 1980s, the knowledge gained over the past period (discovery of DNA as a carrier of genetic information, decoding of the genetic code, development of sequencing and genome-modifying methods) (Inge-Vechtomov, 2010) opened a new approach that involves analyses of DNA sequences and the effects that are exerted by changes in these sequences (mutations) rather than analyses of phenotypes and their genetic control. This concept is called "reverse" genetics (Struhl, 1983; Reski, 1998).

Modern genetics has entered the "post-genomic era", when information about the genome structure of a wide assortment of organisms has become available. Nowadays, it is especially important to identify biological functions of genes, the sequences of which are already known (Eisenberg et al., 2000; Griffiths, Stotz, 2006; Hsiao, Kuo, 2009).

The object of "reverse" genetics is usually a gene with an unknown function (which was detected by Expressed Sequence Tag (EST) sequencing), the entire genome, or a separate part of it, etc. The research strategy is in altering the gene structure or activity and subsequent analyzing associated changes in the phenotype. With the development of large-scale genomic sequencing technologies, "reverse" genetics has received significant support, taking a leading position both in fundamental science and in applied research (Alonso, Ecker, 2006; Barrangou et al., 2007; Small, 2007; Boutros, Ahringer, 2008; Hirochika, 2010; Bolle et al., 2011; Upadhyaya et al., 2011; Liu, Fan, 2014).

Traditional breeding focuses mainly on phenotypic selection and is unable to distinguish effects of the environment or other factors related to growing conditions from genetic characteristics. This problem can be solved by using molecular markers in direct selection (MAS). The trait of interest is marked by a molecular marker that is closely linked to the gene determining this trait or to the gene that affects the trait expression.

MAS allows selecting in accordance with the genotype, regardless of environmental effects. In addition, MAS is used as a tool that complements and significantly facilitates traditional breeding.

The European Technology Platform ‘Plants for the Future’ in its proposed strategic research agenda for plant genomics and biotechnology until 2025 regards a rise in the selection efficiency as a priority. The molecular approach, namely Marker-Assisted Breeding (MAB) or Marker-Assisted Selection (MAS) is a promising way to solve this problem (Kozhukhova, 2011).

Literature Review, Summarization of Basic Provisions. The term "Marker-Assisted Selection" was first used in the literature in 1986 to describe the possible use (Beckmann, Soller, 1983). The first ground-breaking article on MAS in plant breeding using DNA markers was devoted to resistance of soybean to nematodes (*Heterodera glycines* Ichinohe) (Concibido et al., 1996). According to the Glossary of Biotechnology for Food and Agriculture of the Food and Agriculture Organization of the United Nations (FAO), MAS is the use of DNA markers to increase the selection efficiency, basing on the detection of markers of breeding traits (Zaid et al., 2007).

The MAS principle is as follows: if the localization of a gene that affects the expression of an agronomically important trait is known, inheritance of the gene that controls it, or the presence of the required allele in breeding material (not the expression of this trait) is monitored (Kozhukhova, 2011).

Available molecular markers are a necessary prerequisite for any MAS project. A molecular marker can be any DNA fragment used to detect a polymorphism and having a close genetic association with the gene responsible for the trait under investigation (Kalendar, Glazko, 2002).

The rapid development of new methods of molecular biology, including automation and computerization of different processes, development of appropriate methods and software for statistical processing and creation of available databases needed to study DNA polymorphism contribute to the arsenal of molecular markers and their increasing use in various fields of fundamental and applied biology (Kozhukhova, 2011).

Methods of molecular genetics have been widely used in various biological branches: botany, entomology, phytopathology, genetics, etc. The idea of markers is not new, as it was formulated by AS Serebrovskiy as a method of signals in the 1930s. Genetic and breeding achievements in agricultural plants are often attributed to different marker systems. After all, to optimize and accelerate the breeding process, one has first to identify genes of the desired traits in staring and breeding materials. Traditional methods of their detection (hybridological analysis) are labor- and time-consuming.

MAS, a novel comprehensive combining traditional breeding and achievements of such new disciplines as genomics and bioinformatics, offers exciting possibilities for the creation of harmful organism-resistant plant varieties and hybrids (Sivolap et al., 2011).

Marker as a general concept in breeding is a trait that is easily recognizable and is closely associated with a gene of breeders' interest. That is, the presence of the marker is a signal that the important for breeding trait is present too. Markers make it possible to conduct the desired trait-oriented selection (for example, for resistance) and to add fundamentally new genes to the plant genome. Data on markers of resistance genes allow researchers to quickly find the desired resistance genes and their combinations in wild and domestic plants as well as to monitor the presence of this trait while creating resistant or tolerant varieties and hybrids. The method of biochemical markers was developed and investigated in the late 1970s. It was found that alleles of genes that determine the synthesis of storage proteins or isoenzymes are linked to genes of wheat resistance to fungal pathogens (Lisova, 1999). Due to these findings, individual proteins can be used as markers in breeding for resistance.

Markers for plant breeding began to gain popularity in the early 1980s, when isozyme markers were used to accelerate the introgression of monogenic traits from exotic germplasm into cultivated background (Tanksley, Rick, 1980; Tanksley, 1983). In the mid-1980s, the first use of restriction fragment length polymorphism (RFLP) markers in agricultural crop improvement was described, including theoretical issues related to marker-assisted backcrossing (MABC) to improve quality triats (Beckmann, Soller, 1983).

Marker systems can be categorized into three groups: morphological, biochemical (storage proteins and isoenzymes) and genetic (DNA markers). The first two groups of markers, morphological and biochemical ones (markers based on the polymorphism of storage proteins and some enzymes), were widely used in genetic and breeding studies of agricultural crops until the 1990s.

Lande and Thompson (1990) first conducted theoretical studies of MAS for quantitative traits, thus motivating other scientists to a number of modeling studies in the 1090s (Zhang, Smith, 1992, 1993; Gimelfarb, Lande, 1994a, 1994b, 1995; Hospital, Charcosset, 1997; Whittaker et al., 1997). In the early 2000s, additional theoretical discussions were held on the application of MAS and strategies for pyramiding the necessary alleles by recurrent crosses (Hospital et al., 2000; Frisch, Melchinger, 2001, 2005; Hospital, 2002; Servin et al., 2004; Bernardo et al., 2006). These studies have answered many key genetic questions about MAS systems, such as sample size, number and type of markers, population type, and genome size (Avise, 2004; Guimaraes et al., 2007).

In MAS, when one marker is used, the selection reliability increases from 95% to 99.5%, and when two flanking markers are used, it is 100% provided that this marker is located within the gene (Collard, Mackill, 2008). The markers to be used must be close to the target gene (<5 recombination units). This is mandatory to ensure that only a small percentage of specimens will be recombinant. Typically, two markers are used rather than one, to reduce the probability of an error caused by homologous combination. That is, the first step in MAS is to map the gene(s) or quantitative trait locus(loci) (QTL(s)) of interest by different techniques. The recombination frequency between the target locus and the first marker is approximately 5%. Thus, the recombination between the target locus and the marker can occur in approximately 5% of the offspring. The recombination probability between marker 1 and marker 2 (i.e. double crossingover) is much lower than for one marker (about 0.4%). Thus, the selection reliability is much higher when one use flanking markers (Kozhukhova, 2011).

MAS in breeding is used for almost all major crops in four broad directions, namely:

- Traits which are difficult to manage through traditional phenotypic selection because of significant resource costs or complex heredity;
- Traits, the expression of which depends on the specific environmental conditions or on stages of development, which affects the target phenotype;
- To accelerate backcross breeding and maintain recessive alleles in this breeding;
- To pyramide several QTLs for one target trait with complex heredity (drought resistance or other adaptive traits) or several monogenic traits (qualitative traits and resistance to diseases and pests) (Xu, Crouch, 2008).

If one combines simultaneous selections for large number of target traits in traditional breeding, it will lead to a general weakening of the resulting material and increase the number of selection cycles to obtain final accessions. On the other hand, MAS ensures fewer selections and fewer losses when breeders build up several target traits in the same genotype.

It should be noted that the improvement of complex traits, such as resistance to diseases and pests, is complicated by large numbers of additional genes with unpredictable epistatic effects, impacts of different environmental factors and weak heredity (Kozhukhova, 2011). Modern methods based on the achievements of DNA technologies enable searching for resistance genes in starting material more accurately and quickly. At present, in the breeding practice, it is DNA markers that are successfully used: short fragments of DNA that are closely linked with a gene that is responsible for a particular trait or directly characterizes the target gene. MAS, which is based on such markers, is suitable in the selection for different agronomic traits, including resistance to pathogens and pests. The main advantage of DNA markers is that it is possible to study almost any part of the DNA molecule, while there is no need for repeated reseeding of breeding material on infection backgrounds, which significantly shortens the time of creation of resistant varieties and hybrids.

Of molecular methods used in marker-assisted plant breeding, polymerase chain reaction (PCR), which is widely applied to identify most DNA markers, is the most effective tool of DNA analysis. PCR was invented by American biochemist Kary Mullis, and he won the 1993 Nobel Prize in chemistry for this invention (Korovaeva, Popova, 2015).

The PCR method is based on the identification of specific DNA (RNA) fragments in the test material, their selective synthesis to a concentration at which they are easy detected and subsequent determination of amplicons (amplification reaction products) (Fedorenko et al., 2007). Except for RNA viruses, DNA is a unique carrier of genetic information in all organisms on Earth (Glazko, 2003).

DNA has a unique property - the ability to double after unraveling the helix and the separation of DNA strands (replication). DNA replication is catalyzed by enzymes called DNA polymerases using the complementarity principle. To start replication, this enzyme needs an initial double-stranded DNA fragment. Such a fragment is formed when a short single-stranded DNA fragment (primer) interacts with a complementary region of the corresponding parental strand of DNA. Two strands of DNA are replicated, but they grow in opposite directions. As a result, two double-stranded molecules are synthesized from one double-stranded DNA molecule, each of which contains one strand from the parental molecule of DNA and the other daughter, newly synthesized strand (Oleshchuk et al., 2014).

Each DNA replication cycle includes three main stages:

- The DNA helix is unraveled and the double-stranded DNA template is separated into single strands (denaturation);
- Primers anneal, or bind, to the DNA template;
- A daughter strand of DNA is synthesized.

In the PCR machine, these processes are cycled *in vitro*. The transition from one stage of the reaction to another is achieved by changing the temperature of the incubated mixture (Lopukhov, Eldeinshtein, 2000; Fedorenko et al., 2007; Kutyrev et al., 2010). To perform PCR analysis, it is necessary to prepare a sample of biomaterial (DNA or RNA extraction), complete PCR (amplification) and to detect the PCR products (amplified nucleic acid) (Romanenko et al., 1998). The requirements for PCR laboratories are formulated and summarized in corresponding regulations and guidelines (Edwards et al., 2004; Kutyrev et al., 2010; Stehnii et al., 2010; Kalachniuk et al., 2012).

Polymerase chain reaction allows one to selectively synthesize certain (target) DNA sites of several hundred to thousands of nucleotide pairs (usually not longer than 2 kilobases (kb)), using any DNA sample as a template, including a sample of degraded DNA. Today, the PCR principles remain unchanged despite numerous modifications. They consist in DNA amplification via synthesis of complementary strands catalyzed by DNA polymerase. To start replication, this enzyme needs two artificially synthesized single-stranded oligonucleotide primers. Primers are normally between 18 and 30 nucleotides in length. The primers are oriented in opposite directions with their 3' ends pointing towards each other. so that the elongation reaction proceeds from 5′ to 3′ across the region between the two primers, i.e. the distance between the primers determines the length of DNA fragments amplified during PCR. As a result of amplification, new DNA fragments of a certain size are synthesized (they appear after cycle 2).

Polymerase chain reaction is a cyclic process and usually consists of 30-40 cycles. Starting from cycle 2, the newly synthesized DNA molecules serve as templates for further synthesis of the target DNA region. Therefore, PCR will lead to an exponential increase in the number of copies of the DNA region of interest, which was flanked on both sides by primers. The number of amplicons can be approximately

estimated by formula 2", where n is the number of cycles. Correspondingly, the target DNA region can be 2^{20}-fold amplified in 20 cycles (Patrushev, 2004).

Polymerase chain reaction has been widely used in medicine, veterinary science, biology, criminology, history, archeology and other branches of human activities. This method is supersensitive and specific in diagnostics of infections. Thanks to PCR, a lot of modern scientific challenges are successfully solved; organisms are genotyped; genetic diseases are diagnosed and liability to them is evaluated. PCR can accurately test family relations, identify individuals, analyze ancient remains and expose GMOs (Rybicki, 2005; Mahony, 2008).

To date, a lot of modifications of classical PCR analysis have been developed, among which the following types of PCR can be distinguished:

Real-time PCR. This approach is able to determine the number of DNA copies or mRNA in the sample under study. Today, real-time PCR is the most common method used in different sectors. This method is based on the quantitative determination of the PCR product content in the reaction mixture in each cycle of the reaction. Fluorescent-labeled oligonucleotides are used to quantify the PCR products. When performing real-time PCR, one should compare the obtained graphs of fluorescence accumulation between several samples, for example, between a standard sample and a test sample.

Real-time PCR is now widely used in medicine and plant production to determine viral load in living organisms, to elucidate transcript levels, to assess mononucleotide polymorphism and to quantitatively determine the content of a foreign DNA molecule in organisms and food (the presence of genetically modified sources).

Multiplex PCR. Several primers specific for different genetic loci are added to the reaction mixture simultaneously.

Polymerase chain reaction with reverse transcription (RT-PCR). cDNA is synthesized on the RNA template by reverse transcriptase, and the resulting DNA sequence is used for classical PCR. RT-PCR is used for the following purposes:

- To study the differential expression of genes at the transcription level;
- In DNA diagnostics of infectios.

PCR *in situ*. This technique is designed to amplify DNA or RNA sequences directly on fixed slides of tissues, cells or chromosomes.

Allele-specific PCR. It is used to detect mutations in genomic DNA with allele-specific primers, which are complementary to the mutant sequences, while the wild type (norm) is not amplified (Patrushev, 2004).

Different molecular methods are used to detect DNA markers of the trait under investigation. Gupta et al. (2008) grouped DNA markers according to the detection technique as follows:

1) DNA markers based on restriction fragment length polymorphism (RFLP). RFLP markers are detected after treatment of genomic DNA with restriction endonucleases.

2) DNA markers, which are detected by various types of PCR analysis. These include the following types of markers:

- RAPD (random amplified polymorphic DNA) - randomly amplified polymorphic DNA; RAPD markers include DNA sequences obtained through amplification with arbitrary primers (9-11 bp);
- ISSR (inter simple sequence repeats) – intermicrosatellite sequences; during amplification, a DNA fragment located between intermicrosatellite loci is replicated;
- AFLP (amplified fragment length polymorphism) – DNA regions are identified by treating the DNA with two restriction enzymes, ligating adapters to the ends of the target nucleic acid and further amplifying with primers that are complementary to the adapter nucleotides;
- SSR (simple sequence repeat) - simple repeating sequences or microsatellites; these are tandem repeats of 2 - 6 bp in length, for example, (A) n, (AT) n, (GA) n, where n varies between 10 and 80 bp;
- EST (expressed sequences tags) - expressed DNA sequences, anonymous sequences, or sequences of unknown function, obtained from sequencing cDNA libraries;
- SCAR (sequence characterized amplified region) - a sequence that characterizes the amplification region; first, the RAPD fragment is excised from the gel, cloned and sequenced, and then specific primers are designed for this site with a length of 14–20 bp;
- CAPS (cleaved amplified polymorphic sequence) - amplification polymorphic sequences that are cleaved; amplification products are treated with endonucleases;

- IRAP (inter-retransposon amplified polymorphism) - amplification polymorphism of interretrotransposon sequences; amplification occurs between primers that are complementary to the sequence of two adjacent LTR regions of the retrotransposon;

- STS (sequence tagged site) - a sequence that characterizes the locus. A fragment of genomic DNA obtained by amplification with primers that are specific to the primary structure of a known locus.

3) DNA markers, which are detected by sequencing and using DNA chips. Single nucleotide polymorphism (SNP). SNPs are sites in the genome at which more than one nucleotide is found in a population, and the frequency of the rare allele should be at least 1% (Malyshev, Kartel, 1997; Lesk, 2009).

As it has been mentioned above, MAS is applied to almost all major crops. Below, we describe some molecular markers of resistance genes to biotic factors exemplified by one of the most common oil crops in the world - sunflower.

Downy mildew (*Plasmopara helianthi* Novot.). Breeding for resistance to downy mildew is a difficult challenge due to the large number of pathogen races and their significant variability. To date, 20 genes (Pl_1 – Pl_{20}) are known to determine race-specific resistance to downy mildew. These genes were found in different accessions, and *Pl* alleles are dominant. Pl_1 and Pl_2 are the most common genes, which are present in almost all breeding specimens of sunflower. There are published data on marking some of them, which helps to significantly accelerate the selection of valuable genotypes. In search for markers of these genes, scientists extensively use 13 lines - differentiators, which are included in the international standard for the identification of the downy mildew pathogen (Table 1).

Table 1. Characteristics of lines – differentiators (Jocić et al., 2012)

Line	Genes of resistance	Downy mildew races, to which resistance is ensured
HA-288	–	susceptible
RHA-265	Pl_1	100
RHA-274	Pl_1, Pl_2	100, 300, 304, 310, 330, 334
DM-2	Pl_2, Pl_5	100, 300, 304, 700, 703
PM-I3	Pl_2, Pl_5	100, 300, 700, 703
PM-17	Pl_5	100, 300, 304, 310, 700, 710, 703, 714
803-I	Pl_8	100, 300, 304, 330, 334, 700, 710, 714, 730, 733, 734
HA-R4	Pl_2, Pl_{13}	100, 300, 304, 330, 334, 700, 710, 714, 730, 734, 770
HA-R5	Pl_2, Pl_{13}	100, 300, 304, 330, 334, 700, 710, 714, 730, 734, 770
QHP-1	Pl_8 , Pl_{13}	100, 300, 304, 330, 334, 700, 710, 714, 730, 734, 770
FT-226 (analogue of QHP-1)	Pl_8 , Pl_{13}	100, 300, 304, 330, 334, 700, 710, 714, 730, 734, 770
HA-335	Pl_1, Pl_2, Pl_6	100, 300, 330, 700, 710, 730, 733, 770
RHA-419	Pl_{ARG}	Universally resistant

To date, the following genes of resistance to downy mildew are marked: Pl_1, Pl_2, $Pl_{5\text{-}8}$, $Pl_{13\text{-}14}$, $Pl_{16\text{-}20}$, and Pl_{ARG}. Carriers of the Pl_{ARG} gene, which determines the universal resistance against all known races of *Plasmopara helianthi*, are the most valuable sources of resistance in further sunflower breeding for resistance to downy mildew (Jocić et al., 2010). The Pl_{ARG} gene was mapped with SSR markers in linkage group (LG) 1 of the sunflower genetic map (Duble et al., 2004), and it was shown to be closely linked with microsatellites ORS716, ORS509, ORS1128, and ORS543 (Wieckhorst et al., 2010).

Scientists from the Plant breeding and genetics institute - National center of seed and Cultivar Investigation (Solodenko et al., 2014) studied 16 microsatellites in LG 1 in order to identify markers of the Pl_{ARG} gene. Amplicons loci ORS543, ORS606, ORS710, ORS716, and ORS959 were obtained. Collection accessions of the wild species *Helianthus argophyllus* L. were compared with line - differentiator RHA-419 (the Pl_{ARG} gene carrier) and other lines - differentiators (carriers of the Pl_1, Pl_2, Pl_6, Pl_8, Pl_{13} genes). As a result, marker alleles of loci ORS509, ORS605, ORS610, ORS675, ORS1039, and ORS1182 that allow distinguishing the Pl_{ARG} carriers from the other studied genotypes, were identified. Thus, *H. argophyllus* carries the following marker alleles: 190 bp (ORS605), 220 bp (ORS1039), and 315 bp (OR716), while line RHA 419 carries 197 bp (ORS605), 130 bp (ORS610), and 190 bp (ORS1039).

Alleles 207 bp (locus ORS509), 165 bp. (ORS1182) and 220 bp (OR 675) can identify a fragment of LG1, which originates from *H. argophyllus* L. or line RHA-419.

Table 2. Characteristics of the *Pl*$_6$ locus (Bouzidi et al., 2002)

STS-marker	R/S*	Primer	Amplicon length,
Ha-NBS 1	S	HaP1	1,901
Ha-NBS 2	R	HaP1	1,484
Ha-NBS 3	S	HaP2	1,694
Ha-NBS 4	S	HaP2	1,979
Ha-NBS 5	R	HaP2	1,763
Ha-NBS 6	S	HaP2	1,700
Ha-NBS 7	R	HaP2	1,589
Ha-NBS 8	R	HaP2	1,414
Ha-NBS 9	R	HaP2	1,260
Ha-NBS 11	R	HaP3	1,811
Ha-NBS 12	S	HaP3	1,406
Ha-NBS 13	R	HaP3	1,119
Ha-NBS 14	R	HaP3	988

Note: R = resistance; S = susceptibility.

Table 3. Nucleotide sequences of the primers used to identify the *Pl*$_6$ locus

Primer	5′–3′ sequence
HaP1	F: GGTAATGGCTGTTGAATTTATGGAGC R: AGCATGATCCGGCTAGAGCCTTCTA
HaP2	F: GTCTACTACATGGTTTCCGTTTTC R: TGCTTCTTCCTTCTATCTCACTC
HaP3	F: GTTTGTGGATCATCTCTATGCG R: TGCTTCTTCCTTCTATCTCACTC

Table 4. Characteristics of the *Pl*$_5$ and *Pl*$_8$ loci (Bouzidi et al., 2002; Ramazanova, Antonova, 2018)

STS-marker	Locus	R/S*	Primer	Amplicon length, bp
Ha-NT8R 1	*Pl*$_8$	R	Ha-P1	1,569
Ha-NT8R 2	*Pl*$_8$	R	Ha-P1	2,119
Ha-NT8R 7	*Pl*$_8$	R	Ha-P1	2,237
Ha-NT8S 1	*Pl*$_8$	S	Ha-P1	1,153
Ha-NT8S 2	*Pl*$_8$	S	Ha-P1	1,610
Ha-NT5R 2	*Pl*$_5$	R	Ha-P1	2,021
Ha-NT5S 1	*Pl*$_5$	S	Ha-P1	1,303
Ha-NT5S 2	*Pl*$_5$	S	Ha-P1	1,424
Ha-NT5S 3	*Pl*$_5$	S	Ha-P2	387
Ha-NT8R 3	*Pl*$_8$	R	Ha-P3	1,584
Ha-NT5R 1	*Pl*$_5$	R	Ha-P3	1,584
Ha-NT8R 4	*Pl*$_8$	R	Ha-P4	1,840
Ha-NT8R 5	*Pl*$_8$	R	Ha-P5	2,419
Ha-NT8R 6	*Pl*$_8$	R	Ha-P6	2,437

Note: R = resistance; S = susceptibility.

Search for carriers of the *Pl*$_6$ resistance gene, which determines resistance to 11 races, including races 710, 730 and 330, is no less important in sunflower breeding for resistance to downy mildew (Ramazanova, Antonova, 2018). Table 2 summarizes the characteristics of 13 STS-markers within this locus and three primers that were developed by Bouzidi et al. (2002) and are used by researchers from different countries to identify the *Pl*$_6$ locus in sunflower genotypes. The nucleotide sequences of these primers are presented in Table 3.

The introgression of the *Pl*$_8$ locus into breeding accessions is also promising, since this locus is in the same LG with the *Pl*$_5$ locus, so one can use them in the crop breeding to create universally resistant sunflower lines and hybrids, i.e. with resistance to the most common races. To flank the *Pl*$_5$ and *Pl*$_8$ loci, researchers used six primers (Bouzidi et al., 2002), the characteristics of which are summarized in Table 4, and their nucleotide sequences are presented in Table 5.

To successfully breed sunflower for resistance to downy mildew, one should analyze not only plants, but also the pathogen. Hence nowadays, PCR methods are used to determine the molecular genetic variability of *P. helianthi*. This approach allows elucidating the structure of the pathogen population and the rate of its variability, which significantly accelerates the breeding work to create resistant starting material (Radwan et al., 2008).

Table 5. Nucleotide sequences of the primers used to identify the *Pl*$_5$ and *Pl*$_8$ loci

Primer	5′–3′ sequence	Locus
Ha-P1	F: GCCCAAAATTGAAAGAAAGGTGTG R: GGCGAAATTGGTTCCCGTGAGTCG	*Pl*$_5$, *Pl*$_8$
Ha-P2	F: AATCTTGAGTCATTACCCGAGC R: CAGCGTCTCTGGTAGATCGTTCACC	*Pl*$_5$, *Pl*$_8$
Ha-P3	F: GCTGTTACTGCCCTCTTCAAAGTC R: TTTGAAAGATAAGTTCGCCTCTCG	*Pl*$_5$, *Pl*$_8$
Ha-P4	F: GCTGTTACTGCCCTCTTCAAAGTC R: CCCAACTCGACATATCTTCAAACC	*Pl*$_8$
Ha-P5	F: TAGTTAACATGGCTGAAACCGCTG R: CCCCATATTGACAAAGAGTTGAGG	*Pl*$_8$
Ha-P6	F: TAGTTAACCATGGCTGAAACCGCTG R: CGTCTCTGGTAGATCGTTCACCTT	*Pl*$_8$

Sunflower rust (*Puccinia helianthi* Schw.). The introgression of resistance to rust from wild species, mainly from *H. argophyllus*, in modern sunflower varieties and hybrids has led to the discovery of 13 resistance genes (*R*$_{1\text{-}5}$, *R*$_{10\text{-}12}$, *R*$_{13a}$, *R*$_{13b}$, *R14*, *R*$_{adv}$ and *P*$_{u6}$) (Bachlava et al., 2011). Molecular mapping of 11 genes showed they were located in several LG of the genetic map of sunflower. Thus, five *R* genes are located in LG 2 (*R*$_5$), LG 8 (*R*$_1$), LG 11 (*R*$_{12}$ and *R*$_{14}$) and LG 14 (*R*$_2$), while the remaining six genes (*R*$_4$, *R*$_{11}$, *R*$_{13a}$, *R*$_{13b}$, *R*$_{adv}$ and *P*$_{u6}$) are located in LG 13, which is divided into two subclusters, subcluster 1 (*R*$_{11}$, *R*$_{adv}$ and *P*$_{u6}$) and subcluster 2 (*R*$_4$, *R*$_{13a}$ and *R*$_{13b}$) (Lawson et al., 1996; Yu et al., 2003; Qi et al., 2011, 2012a, 2012b, 2015b; Gong et al., 2013a, 2013b; Bulos et al., 2013).

The fact that sunflower rust resistance genes quickly lose their effectiveness due to the emergence of new virulent races in a short period of time after the introduction of resistant varieties and hybrids into production poses a serious problem. Therefore, it is important to search for new genes of rust resistance and for molecular markers that can identify these genes as well as to pyramide several resistance genes in one genotype.

The first molecular studies were conducted to detect markers of the *R*$_1$ and *R*$_{adv}$ genes using RAPD- and SCAR-markers (Bulos et al., 2013; Lawson et al., 1998). Subsequent molecular studies of the *R* genes were designed to identify molecular markers linked to the *R*$_1$, *R*$_{adv}$, *P*$_{u6}$, *R*$_{11}$, *R*$_{13a}$, and *R*$_{13b}$ genes, which are located in LG 13. Qi et al. (2015b) identified two markers flanking the *R*$_4$ gene (ORS581 and ZVG61), which were later also used to mark the rust resistance genes *R*$_{13a}$ and *R*$_{13b}$ (Bulos et al., 2013; Qi et al., 2015a, 2015b; Talukder et al., 2014, Solodenko, Fait, 2016) (Table 6). Bulos et al. (2014) mapped the *P*$_{u6}$ gene and identified closely linked SSR-markers (ORS316, ORS224) in LG 13.

Today, *R*$_5$ is the only sunflower rust resistance gene in LG 2. Qi et al. (2012b, 2015a) identified two SSR- and two SNP-markers that flank the gene (SFW03654, ORS653$_a$, NSA_000267, ORS1197-2).

Table 6. Markers of the sunflower genes of resistance to *Puccinia helianthi*

Linkage group	Resistance gene	Accession used to map the gene	Marker	Reference
LG 2	R_5	Ha-R_2	SFW03654 ORS653a NSA_000267 ORS1197-2	Qi et all., 2012a Qi et all., 2015a
LG 8	R_1	RHA 279	$SCT06_{950}$	Lawson et all., 1998
LG 11	R_{12}	RHA 464	NSA_003426/NSA_004 55/ NSA_000064/NSA_008884/ NSA_003320. CRT275 ORS1227 ZVG53	Talukder et al., 2014
	R_{14}	PH3	NSA_000064 ORS1227 ORS542 ZVG53	Zhang et al., 2016
LG 13	R_4	HA-R3	SFW05240/SFW05630/SFW06095/ SFW08283 SFW01497/SFW05630/SFW08875 ORS581 ZVG61	Qi et al., 2011 Qi et al., 2015a
	R_{HAR6}	HA-R6	ZVG61 ORS581	Bulos et al., 2013
	R_{13a}	HA-R6	SFW05743 RGC15/16 ORS316/ZVG61 ZVG61 SUN14	Gong et al., 2013a Qi et al., 2015a
	R_{13b}	RHA 397	SUN14 SFW00757 RGC15/16 ZVG61, ORS316 SFW04275/SFW04317/SFW05743 ZVG61, ORS316	Gong et al., 2013a Qi et al., 2015a
	R_{adv}	Advance RHA 340	SCX20600 RGC260 ORS316	Lawson et al., 1998 Bachlava et al., 2011
	P_{u6}	P386	ORS316 ORS224	Bulos et al., 2014
	R_{11}	Rf ANN-1742	ORS728 ORS728 ORS45	Qi et al., 2012b
LG14	R_2	MC 29 (USDA)	SFW01272 NSA_002316 SFW00211	Qi et al., 2015b

LG 11 contains two rust resistance genes: R_{12} and R_{14}. The two genes were aligned with markers ORS1227 and ZVG53. Talukder et al. (2014) used five SNP-markers (NSA_000064, NSA_004155, NSA_003426, NSA_008884, NSA_003320) to identify the R_{12} gene, but only two of these markers (NSA_003426 and NSA004155) were effective in identifying the R_{12} gene.

Qi et al. (2012b) also used previously developed SSR- and SNP-markers to identify homozygous multi-race-resistant genotypes in a population of F_2 hybrids derived from crossing BC3F2 accession - carrier of the R_5 gene and HA-R6 accession - carrier of the R_{13a} gene. The offspring obtained from plants selected from this hybrid population were more resistant to races 336 and 777 compared to lines with only one resistance gene. The researchers also pyramidized the R_5 and R_{13a} genes in confectionery sunflower using SSR- and SNP-markers (Qi et al. 2015a). They revealed that pyramidation of the *R* genes could ensure long-term resistance to the causative agent of sunflower rust. Thus, the creation of sunflower genotypes combining several rust resistance genes is a very important objective in breeding, and the breeding process can be significantly facilitated and accelerated by using the molecular markers described above.

Broomrape (*Orobanche cumana* Wallr.). Broomrape (a plant - parasite)-caused damage significantly reduces sunflower yields. The most reliable way to control this parasite is to create resistant varieties and hybrids with prior studies of the inheritance of resistance to broomrape. The genetics of this trait is studied by Ukrainian and foreign scientists. Sunflower has been bred for resistance to broomrape for almost a century (Shindrova, 2006). Currently, 8 broomrape races with different virulence are known. They are denoted by the Latin letters as A, L, B, C, D, E, F, G, H (Melero-Vara et al., 1989; Alonso *et al.,* 1996; Akhtouch *et al*., 2002; Shindrova, 2006; Pacureanu et al., 2009; Antonova *et al*., 2011). Until recently, the first five physiological races of broomrape were spread in all regions of sunflower cultivation; resistance to them is determined by individual *Or* genes (Sukno, 1999). Studies have confirmed that resistance to races A to E is determined by the genes Or_1 to Or_5, which are allelic or strongly linked, and resistance to race E is controlled by a single dominant gene, Or_5 (Lu et al., 2000; Fernández-Martínez et al., 2000; Fernández-Martínez et al., 2008).

Most molecular analyses were performed to study and create different types of molecular markers to identify the Or_5 gene, which determines resistance to races E and below (Tang et al., 2003; Fernández-Martínez et al., 2004; Guchetl et al., 2012). Foreign scientists (Tang et al., 2003) identified some DNA markers in the same linkage group with Or_5. The nearest SCAR-marker is mapped at a distance of 5.6 cM from the distal end of Or_5 (Table 7).

Table 7. Markers of the sunflower broomrape resistance genes

Linkage group	Gene of resistance	Accession used to map the gene	Marker	Genetic distance, cM	Reference
LG 3	Or_5	RPG01	CRT214	1.1	Lu et all., 2000
		PHD	RTS05	5.6	Tang et all., 2003
			CRT392	6.2	
			ORS1036	7.5	
LG 13	$Or_{ab\text{-}vl\text{-}8}$	AB-VL-8	ORS683	1.5	Imerovski et all., 2016
			ORS657	4.7	

Tang et al. (2003) identified SSR-markers closely linked to Or_5 and mapped this locus in the upper part of LG 3 in the genetic map of SSR loci. The nearest SSR-markers are at distances of 6.2 cM (CRT392) and 7.5 cM (ORS1036) from the Or_5 locus.

Search for donors of resistance to highly virulent broomrape race F is urgent today. This race originated at the end of the last century in Spain. Imerovski I. et al. (Imerovski et al., 2016) used line AB-VL-8, resistant to this broomrape race, to map markers of a resistance gene, which was named $Or_{ab\text{-}vl\text{-}8}$ by the researchers. $Or_{ab\text{-}vl\text{-}8}$ was established to be in LG 13. In their molecular studies, the researchers found the nearest SSR-markers, which are at distances of 1.5 cM (ORS683) and 4.7 cM (ORS652) from the locus.

For traits with weak heredity, typical breeding programs involve the cultivation of millions of individual plants in thousands of populations to achieve greater homozygosity in lines, which occurs in approximately F_5-F_6 generations. This process requires significant resource costs and a significant time of 5-12 years. Due to the rapid development of agriculture and life in general, breeding programs with their scale, complexity of selection, numbers and sizes of populations, etc., require the latest approaches, which certainly include MAS.

Conclusions

Thus, MAS has been theoretically justified in numerous scientific publications and is implemented in most breeding institutions around the world. This trend in breeding opens new opportunities for studying genetic diversity and relations at the species and genus levels. Molecular marker-based selection is necessary in modern plant breeding, including in sunflower breeding for resistance to biotic factors.

The achievements of scientists in marking genes of resistance to the pathogen of downy mildew and sunflower rust as well as to the plant - parasite, broomrape, opens opportunities of identifying reliable sources of resistance and involving them in breeding programs in order to create valuable starting material. The emergence of new virulent races of these diseases and broomrape forces researchers to carry out deeper studies in finding new resistance genes and identifying molecular markers for these genes.

The effectiveness of molecular markers in sunflower breeding for resistance to diseases and parasites is confirmed by the results of scientific studies. Today, the search for donors of resistance to highly virulent races, which will significantly accelerate the selection of valuable genotypes in breeding for resistance, is urgent.

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Маркери генів стійкості соняшнику до основних хвороб та паразитів

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Останніми роками відмічається загострення проблеми фітосанітарного стану посівів соняшнику, що пов'язано із порушенням сівозмін і як наслідок – поширенням основних хвороб. Селекція на стійкість до біотичних чинників потребує всебічного вивчення біології культури та патогенів. Необхідною умовою для створення високопродуктивних гібридів є використання в селекційному процесі вихідного матеріалу, стійкого до основних патогенів та стресових умов середовища. Значних успіхів в селекції гетерозисних гібридів соняшнику досягнуто, насамперед, шляхом використання стійких інбредних ліній. Проте нині процесс їх створення є досить тривалим та займає 8–12 років. Процес добору потрібних генотипів та вихідних форм для схрещування ускладнюється тим, що іде за комплексом полігенних ознак, які зазнають значної модифікаційної мінливості. Одним з шляхів прискорення селекційного процесу є використання молекулярно-генетичних маркерів. Маркер-асоційована селекція (MAS) отримала теоретичне обґрунтування в численних публікаціях та впроваджена у більшості селекційних установ різних країн світу. Але у вітчизняних селекційних програмах маркер-асоційована селекція порівняно з традиційними методами не набула широкого розповсюдження. Проте даний напрям в селекції відкриває нові можливості вивчення генетичного різноманіття, визначення спорідненості на внутрішньовидовому і родовому рівнях. В огляді наведено інформацію щодо стану та перспектив впровадження в традиційну селекцію рослин так званого добору за допомогою генетичних маркерів Marker-Assisted Selection (MAS), висвітлено досягнення сучасної біотехнології в селекції соняшнику на стійкість до біотичних чинників з використанням молекулярних маркерів. Представлено принципи MAS, охарактеризовано переваги даного методу. Наведено приклади конкретного використання молекулярного підходу при створенні вихідного матеріалу соняшнику для селекції на стійкість до основних хвороб та паразитів. Також описано основні етапи та компоненти для проведення ПЛР-аналізу. Надано характеристику

інбредних ліній соняшнику з геном стійкості до збудника несправжньої борошнистої роси та сформовано генетичні паспорти 13 ліній соняшнику за STS-маркерами до локусу Pl_6.

Ключові слова: *ДНК-маркери, маркер-асоційована селекція, соняшник, несправжня борошниста роса, іржа соняшнику, вовчок соняшниковий.*

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Inheritance of traits in F_1 hybrids of diploid einkorn wheat of the spring crop

Hao Fu, L.O. Atramentova

Diploid einkorn wheat (2n = 14) is an ancient crop that people cultivate for 10 thousand years. The grain of this wheat is a valuable product for a healthy diet, which determines the increasing interest in einkorn wheat by scientists and agricultural producers. Meanwhile, the wide use of this crop is hindered by several shortcomings that complicate the usage of modern technologies: low yield, ear fragility, a tendency to lodging, and difficult grain threshing. Nevertheless, there are some preconditions for improving the agronomic properties of this crop. We carried out crosses in seven combinations with the use of three wheat species (*T. boeoticum*, *T. monococcum*, *T. sinskajae*) to improve the diploid einkorn wheat in terms of productivity and threshing. In total, the hybrid seed set in the crosses varies from 6.3 % to 79.7 %. In the combination of cultivated wheat *T. sinskajae* with wild *T. boeoticum*, differences in the results of reciprocal crosses are observed specifically in the hybrid seed set (in the forward cross it equals 6.3 %; in the reverse one, 48.9 %). Hybrids from reciprocal crosses of *T. monococcum* var. *sofianum* UA0300649 and *T. sinskajae* f. *aristata* were equivalent in seed set (72 and 82 %) and inheritance patterns and had similar quantitative traits. In other combinations, the seed set varied from 12.5 to 45.6 %. Hybrid depression was the most frequent (22 cases out of 49) inheritance type of the F_1 quantitative traits in einkorns; dominance of the parent form with a large trait manifestation was registered in 11 cases, and heterosis in four cases. In hybrids, the inheritance of spike length is correlated with the inheritance type of the ear number (r = 0.92) and the grain number (r = 0.78) per spike. The dominance degrees after these two traits are also highly correlated (r = 0.89). The combination UA0300400 *T. boeoticum* var. *thaoudar* ARM / UA0300224 *T. sinskajae* var. *sinskajae* RUS, which manifested heterosis for kernel number per spike (*Hp* = 1.2), the weight of spike (*Hp* = 11.8) and weight of kernels per spike (*Hp* = 5.4) is of particular interest. The combination UA0300222 *T. monococcum* var. *hohensteinii* / UA0300224 *T. sinskajae* var. *sinskajae* is promising for creating easily threshed material.

Key words: *einkorn wheat, productivity elements, hybrids, degree of dominance*.

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Introduction

Diploid einkorn wheat (2n = 14) is an ancient crop that mankind has been cultivating for 10 thousand years (Heun et al., 1997). The grain of this wheat is a valuable product for a healthy diet (Hidalgo, Brandolini, 2014). It may also be beneficial for people with wheat gluten sensitivity (Di Stasio et al., 2020). All this determines the increasing interest in einkorn wheat of scientists and agricultural producers. Meanwhile, the wide use of this crop is hindered by a number of shortcomings that make it difficult to use modern technologies: low yield, tendency to lodging, fragility of ear, difficult grain threshing. Nevertheless, there are preconditions for improving the technological qualities of this crop. For example, threshing can be facilitated by using for hybridization a species *Triticum sinskajae* A. Filat. et Kurk. with easy threshing although not highly productive (Filatenko, Kurkiev, 1975). It is possible to predict the effectiveness of hybridization for the genetic improvement of einkorn wheat by manifestation of traits in F_1 hybrids (Kostylev, Nekrasova, 2015), which is also important for the development of commercial heterotic hybrids.

The purpose of the research is to find out the trait inheritance modes in F_1 hybrids of einkorn wheat accessions and to identify promising hybrid combinations for a prospective increase of subsequent generations' productivity.

Materials and methods

Seven hybrid combinations involving representatives of three einkorn wheat species were used as

the material for the study. Among them, there were two reciprocal combinations (1/2 and 5/6, Table 1). The studies were carried out in the experimental field of the Plant Production Institute named after V.Ya. Yuryev of the National Academy of Agrarian Sciences of Ukraine (eastern forest-steppe).

Table 1. Crossability of einkorn wheat

№	Maternal form ♀	Paternal form ♂	C %	CI
1	UA0300649*, *T. monococcum* var. *sofianum***, BLR***	*T. sinskajae* f. *aristata***, UKR***	81.9	73.5–88.1
2	*T. sinskajae* f. *aristata***, UKR***	UA0300649*, *T. monococcum* var. *sofianum***, BLR***	72.2	65.6–78.0
3	UA0300113*, *T. monococcum* var. *macedonicum***, SYR***	UA0300224*, *T. sinskajae* var. *sinskajae***, RUS***	19.7	12.3–30.0
4	UA0300222*, *T. monococcum* var. *hohensteinii*** GEO***	UA0300224*, *T. sinskajae* var. *sinskajae***, RUS***	45.6	37.9–53.6
5	UA0300224*, *T. sinskajae* var. *sinskajae***, RUS***	UA0300400*, *T. boeoticum* var. *thaoudar***, ARM***	6.3	3.4–11.1
6	UA0300400*, *T. boeoticum* var. *thaoudar***, ARM***	UA0300224*, *T. sinskajae* var. *sinskajae***, RUS***	48.9	39.1–58.9
7	UA0300401*, *T. boeoticum* var. *kurbagalensense***, UKR***	UA0300221*, *T. monococcm* var. *monococcum***, AZE***	12.5	3.5–36.0

*Note: * – number of the National Catalog of Ukraine, ** – species and variety, *** – country of origin, C – Crossability, CI – Confidence interval at the probability level of 95 %.*

The soil of the experimental field is chernozem (black soil). During the growing season in 2021 from March to July, the average temperature was +14℃ and the total precipitation was 188 mm whereas the long-term temperature is of +13℃ and the total precipitation is 219 mm. Thus, the year is characterized as dry.

The "single cross" method was used to create the hybrids. At least 10 spikes were pollinated in each combination, at least 200 flowers in total. The F_1 hybrid seeds were sown manually in blocks according to the scheme: mother–hybrid–father. The row length was 1 m, and the row spacing was 15 cm. The number of rows depended on the number of available seeds in each hybrid combination. The evaluation of hybrids by biological and economic characteristics was carried out according to the guidelines (Merezhko et al., 1999).

Hullness (*H*) is calculated by the formula:

$$H = \frac{WS - WKS}{WS} \times 100\% \quad (1)$$

where *WS* – weight of spike, *WKS* – weight of kernels per spike.

Crossability (*C*) is determined by the formula:

$$C = \frac{PFN - SS}{PFN} \times 100\% \quad (2)$$

where *PFN* – pollinated florets number, *SS* – seed setting.

The degree of dominance (*Hp*) of the trait in F_1 hybrids was determined by the Griffing formula (Griffing, 1956):

$$Hp = \frac{F_1 - Mp}{P_{max} - Mp} \quad (3)$$

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Where F_1 – arithmetic mean value of a trait in a hybrid F_1, M_p – arithmetic mean value of a trait in a parental form, P_{max} – the best parental form for the trait under study. At $Hp > 1$, the expressiveness of the trait is designated as heterosis i.e. overdominance of the parent with a higher value of the trait. *Hp* value < –1 is designated as hybrid depression or overdominance of the parent with a lower value of the trait. The value $–1 < Hp < 1$ indicated intermediate dominance, i.e., incomplete dominance. The value $Hp = 1$ indicates the complete dominance of the parent with a higher value of the trait, $Hp = –1$ indicates the complete dominance of the parent with a lower value of the trait.

Results

Crossability. In plant breeding, crossability serves as a guideline for the selection of parental pairs. This indicator in hybrid combinations of einkorns varies from 3 to 88 % (Table 1). When *T. sinskajae* is taken as the maternal form and wild wheat *T. boeoticum* is taken as the paternal form (combination 5), the lowest crossability is obtained – an average of 6 %. In the reciprocal cross (combination 6), the setting percentage was 49 %. Both reciprocal combinations *T. monococcum* var. *sofianum* UA0300649 / *T. sinskajae* f. *aristata* showed high seed setting (72–82%) (1 and 2 in Table 1). In other combinations, the crossability was at the level of 13–46 %, which is a good result for the dry conditions of spring 2020.

Table 2. Degree of dominance (*Hp*) of the traits in F_1 hybrids

№	Spike length	Spikelet number in spike	Kernel number per spike	1000 kernel weight	Spike weight	Kernel weight per spike	Proportion of husks
1	–0.07	–0.6	–5.2	–3.0	–1.8	–12.6	0.8
2	–0.03	–1.0	–5.0	–1.4	–4.8	–6.6	0.6
3	–1.9	–2.0	–1.3	–8.9	–3.2	–3.4	52.9
4	–0.2	0.5	0.4	–0.2	0.5	0.7	–143.4
5	–0.4	1.0	0.4	–0.5	0.0	–0.01	–0.04
6	0.8	0.8	1.2	–0.3	11.8	5.4	0.4
7	–4.3	–4.5	–13.8	–1.5	–2.1	–1.7	–5.7

Note: No – the cross number in the Table 1.

Degree of dominance. Of the 49 indicators (Table 2), in four cases there is a overdomination of the parent with higher trait index: in the combination 3 it is shown for proportion of husks, in the combination 6 – for grain number per spike, for weight of spike, for grain weight per spike. In 22 cases there is overdomination of the parent with lower trait index, in five – no dominance (*Hp* is close to zero) is observed, in 11 – dominance of the parent with higher trait index, in seven – dominated the parent with lower trait index. In the combination 7, overdomination of the parent with lower trait index is observed for all traits.

Table 3. Correlation coefficients between the trait dominance degrees in F_1 einkorns hybrids

Traits	a	b	c	d	e	f
Spikelet number in spike (b)	0.92**					
Kernel number per spike (c)	0.78*	0.89**				
Thousand kernel weight (d)	0.31	0.38	0.02			
Spike weight (e)	0.45	0.50	0.47	0.40		
Kernel weight per spike (f)	0.05	0.27	0.40	0.35	0.71	
Proportion of husks (g)	–0.21	–0.33	–0.19	–0.58	–0.11	–0.25

*Note: a – Spike length, b – Spikelet number in spike, c – Kernel number in spike, d – thousand kernel weight, e – Spike weight, f – Kernel weight per spike, g – Proportion of husks, * $p \le 0.05$, ** $p \le 0.01$.*

It should be noted that in reciprocal combinations 1 and 2, the inheritance patterns of all traits are similar. In contrast, in reciprocal combinations 5 and 6, the inheritance patterns for five of the seven traits are not the same, and only two – the spikelet number in spike and the mass of 1000 grains – are near. This result is consistent with crossability in these combinations and points to the role of crossing direction.

Among all the combinations, No. 6 (UA0300400 *T. boeoticum* var. *thaoudar* ARM / UA0300224 *T. sinskajae* var. *sinskajae* RUS) stood out, in which heterosis was manifested by the kernel number per spike (*Hp* = 1.2), for weight of spike (*Hp* = 11.8) and for kernel weight per spike (*Hp* = 5.4).

The analysis shows a correlation between the dominance degrees of the traits. It is established (Table 3) that dominance for the spike length is near to the dominance for spikelet number (r = 0.92) and for kernel number per spike (r = 0.78). The degrees of dominance in the latter two traits are also closely correlated (r = 0.89).

Discussion

The inheritance of quantitative traits in bread and durum wheat including their hybrids is well studied whereas for einkorns such studies are rare (Kuspira et al.,1989). In F_1 hybrids of spring polyploid wheat, overdomination of the best parent is often observed for productivity elements, (Ljubičić et al., 2014; Valekzhanin, Korobeynikov, 2016; Mukhordova, 2018). In our experiments with diploid wheat, this type of inheritance was observed in only one combination. This phenomenon requires more thorough analysis in special experiments.

From a practical point of view, combination 6 is of interest because it is more likely than other combinations to select relatively more productive forms in subsequent generations.

Conclusions

1. In the combination of cultivated *T. sinskajae* / wild *T. boeoticum*, differences in hybrid grain setting are observed: in direct cross it equals 6 %, in reciprocal cross it equals 49 %. In other combinations, the index varied from 13 % to 46 %. Both reciprocal crosses between *T. monococcum* var. *sofianum* UA0300649 and *T. sinskajae* f. *aristata* showed high seed setting (72–82 %) (1 and 2 in Table 1).

2. In F_1 hybrids of einkorn wheat, the inheritance pattern of the spike length is similar to the inheritance patterns of the spikelet number per spike and kernel number per spike (the correlation coefficients are 0.92 and 0.78, respectively). Also closely correlated are the dominance degrees in the last two traits (r = 0.89).

3. The combination UA0300400 *T. boeoticum* var. *thaoudar* ARM / UA0300224 *T. sinskajae* var. *sinskajae* RUS is of interest for breeding as showing heterosis for kernel number per spike (*Hp* = 1.2), for spike weight (*Hp* = 11.8) and for kernel weight per spike (*Hp* = 5.4); the combination UA0300222 *T. monococcum* / UA0300224 *T. sinskajae* is valuable for developing easily threshing material.

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Успадкування ознак у гібридів F_1 диплоїдних пшениць однозернянок у ярій культурі

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Диплоїдна однозернянка (2n = 14) — давня культура, яку людство вирощує 10 тисяч років. Зерно цієї пшениці є цінним продуктом для здорового і профілактичного харчування. Це зумовлює зростання інтересу науковців і виробничників до однозерної пшениці. Між тим, широкому використанню цієї культури перешкоджає низка недоліків, які ускладнюють використання сучасних технологій: низька врожайність, схильність до вилягання, ламкість колосу, важкий вимолот зерна. Тим не менш, є передумови для покращення технологічних якостей цієї культури. Для поліпшення диплоїдної пшениці однозернянки (2n = 14) за продуктивністю та вимолочуваністю проведено схрещування у семи комбінаціях за участі трьох видів цих пшениць (*T. boeoticum*, *T. monococcum*, *T. sinskajae*). Загалом схрещуваність у комбінаціях гібридів однозернянки коливається від 6,3 % до 79,7 %. У комбінації культурної пшениці *T. sinskajae* з дикою *T. boeoticum* спостерігаються реципрокні відмінності щодо зав'язування гібридних зерновок (у прямій комбінації 6,3 %; у зворотній 48,9 %). Реципрокні комбінації *T. monococcum* var. *sofianum* UA0300649 та *T. sinskajae* f. *aristata* були подібні за схрещуваністю (72 and 82 %) та характером спадкування і близькі за кількісними показниками. У решті комбінацій зав'язуваність становила 12,5–45,6 %. Найчастішим (22 випадки з 49) типом успадкування кількісних ознак F_1 однозернянок була гібридна депресія, домінування батьківської форми з більшим проявом ознаки відзначено в 11 варіантах, гетерозис у чотирьох. У гібридів успадкування довжини колоса корелює з типом успадкування кількості колосків у колосі (r = 0,92) та кількістю зерен у колосі (r = 0,78). Ступені домінування за цими двома ознаками також пов'язані між собою (r = 0,89). Селекційний інтерес представляє комбінація UA0300400 *T. boeoticum* var. *thaoudar* ARM / UA0300224 *T. sinskajae* var. *sinskajae* RUS, яка проявила гетерозис за кількістю зерен у колосі (Hp = 1,2), масою колосу (Hp = 11,8) та масою зерен з колосу (Hp = 5,4). Для створення матеріалу, що легко вимолочується, перспективною є комбінація UA0300222 *T. monococcum* var. *hohensteinii* / UA0300224 *T. sinskajae* var. *sinskajae*.

Ключові слова: *пшениця однозернянка, елементи продуктивності, гібриди, ступінь домінування.*

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Ecological and faunistic analyses of the trematodes of the Little Grebe (*Tachybaptus ruficollis* (Pallas, 1764)) in Azerbaijan

Y.A. Mahmudova

The research was conducted from 1998 to 2019 at the nine water bodies of Azerbaijan. During the study, 94 individuals of the Little Grebes (*Tachybaptus ruficollis* (Pallas, 1764)) were examined by the method of complete helminthological dissection. As a result, 12 trematode species belonging to one order, seven families, and eight genera were found: *Patagifer bilobus, Petasiger megacantha, P. skrjabini, Echinochasmus coaxatus, E. dietzevi, E. mordax, Mesorchis pseudoechinatus, Cryptocotyle concavum, Metorchis intermedius, Eucotyle cohni, Strigea falconis, Hysteromorpha triloba.* Of these, three species (*Petasiger megacantha, Echinochasmus coaxatus* and *E. dietzevi*) are specific grebe parasites, while the others infect various waterfowl. Except for *Strigea falconis*, whose cercariae penetrate actively into the bird's body and transform into metacercarie, all the trematode found are ingested by the birds and mature in their intestine. The grebe, as a fish-eating bird, is infected with six trematode species (*Patagifer bilobus, Petasiger megacantha, Mesorchis pseudoechinatus, Cryptocotyle concavum, Metorchis intermedius, Hysteromorpha triloba*) that parasite in fish at the stage of metacercaria. Other species use aquatic invertebrates as second intermediate hosts. We established that species diversity of the grebe trematodes depended on the reservoir size and the richness of its hydrofauna, increasing with the increase of both factors. The differences between the faunas of grebe trematodes in various water bodies depended on the distance between them and the similarity of their living conditions. Seven grebe trematode species (*Petasiger megacantha, P. skrjabini, Echinochasmus coaxatus, E. dietzevi, Cryptocotyle concavum, Metorchis intermedius, Eucotyle cohni*) belong to the northern group of helminthes, while the five species (*Patagifer bilobus, Echinochasmus mordax, Mesorchis pseudoechinatus, Strigea falconis, Hysteromorpha triloba*) are ubiquitous. Southern trematode species were absent from the examined birds. Presumably, this can be explained by the dominance of grebes from northern populations wintering on the water bodies of Azerbaijan.

Key words: *Azerbaijan, waterfowls, parasites, helminthes, Trematodes.*

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Introduction

The Little Grebe (*Tachybaptus ruficollis* (Pallas, 1764)) is a widespread bird in the internal water bodies of Azerbaijan. It lives on the freshwater bodies and on the coast of the Caspian Sea. Most individuals are migratory; only an insignificant part of the birds spend winter in this region. The grebe feeds on little fish, amphibians, crustaceans, shell-fish and water plants. The helminth fauna of the little grebe in Azerbaijan was studied by S.M. Vaidova (1978), who recorded 11 trematode species throughout the country. Although the study of bird parasites, including grebe trematodes, has theoretical and practical importance, not a single work has been published since that time.

Material and method

The material was collected from 1998 to 2019 at nine water research stations in various regions of Azerbaijan: the Devechi Firth, the Small Gizilagach Bay, the coast of the Absheron Peninsula of the Caspian Sea, the Middle Kura River, the Lower Kura River, the Kura delta, the Mingechevir Reservoir, the Varvara Reservoir, and the Lower Araz River (Fig. 1). The birds and their helminth were studied all year round, but most observations were made in April-June and September-November. As a result, 97 specimens of the little grebe were examined by complete helminthological dissection (the method is described in Dubinina,

1971; Pronina, Pronin, 2007; Musselius et al., 2008; Dorovskikh, Stepanov, 2009). Only adult birds were subjected to autopsies; shooting of birds with scientific purposes was carried out with the permission of the Ministry of Ecology and Natural Resources of the Republic of Azerbaijan. The birds were used in a complex study by ornithologists and parasitologists. Some specimens were obtained from amateur hunters; birds that died from natural causes were also studied. In most cases, the birds were dissected on the spot; some individuals were frozen and delivered to the laboratory for complete helminthological dissection. All the trematodes found were collected and fixed in 70° alcohol, and then they were treated with carmine dye and identified.

Figure 1. Map of the sampling localities. (1 – Devechi Firth, 2 – Small Gizilagach Bay, 3 – Absheron Peninsula coasts of the Caspian Sea, 4 – Middle Kura, 5 – Lower Kura, 6 – Kura River Delta, 7 – Mingechevir Reservoir, 8 – Varvara Reservoir, 9 – Lower Araz)

The collected trematodes were identified using classical monographs and key guides McDonald, 1961; Bykhovskaya-Pavlovskaya, 1962; Gaevskaya et al. 1975; Smogorjevskaya, 1976; Ginetsinskaya T.A., Dobrovolsky, 1978 etc.). Faunistic similarity of the little grebe trematodes of various water bodies was assessed by the Czekanowski–Soerensen index (Czekanowski, 1913; Soerensen, 1948).

Results

During the study, 12 trematode species were discovered in the little grebe. In systematic list, each species is provided with the following data: collecting localities, extensity (% of infected birds) and intensity (individuals per bird) of invasion, localization in the host body, and a brief information on species biology.

Class TREMATODA RUDOLPHI, 1808
Order FASCIOLIDA SKRJABIN ET GUSCHANSKAJA, 1962
Family ECHINOSTOMATIDAE Dietz, 1900
Patagifer bilobus (Rudolphi, 1819)

Localities: Devechi Firth (28.67%; 2-4 specimens), Small Gizilagach Bay (33.3%; 4-16 specimens), Middle Kura (25.0% 1 specimen), Lower Kura (22.2%; 1-6 specimens), Kura River delta (15.4%; 1-4 specimens)

Localization: gut.

A parasite of fish-eating wetland birds; sometimes occurs in cranes and gallinules (Ginetsinskaya T.A., Kulik, 1952; Faltýnková et al., 2008).

Petasiger megacantha (Kotlan, 1922)

Localities: Mingechevir (10.0%; 1 specimen) and Varvara (6.7%; 2 specimens) reservoirs.

Localization: gut.

A parasite of grebes. Freshwater mollusks and fish are its intermediate hosts (Selbach et al., 2014).

Petasiger skrjabini Baschkirova, 1941

Localities: Mingechevir (18.2%; 1-2 specimens) and Varvara (13.3%; 1-3 specimens) reservoirs.

Localization: gut.

A parasite of Anserinae and grebes (Bykhovskaya-Pavlovskaya, 1962).

Family ECHINOCHASMIDAE Odhner, 1910

Echinochasmus coaxatus Dietz, 1909

Localities: Devechi Firth (35.7%; 3-5 specimens), Small Gizilagach Bay (25.0%; 1-3 specimens), Kura River delta (7.7%; 3 specimens).

Localization: gut.

Mainly parasitizes grebes, sometimes occurs in other water birds; first intermediate hosts are gastropods (Chikhlyayev et al., 2012).

Echinochasmus dietzevi Issaitschikoff, 1927

Localities: Lower Kura (11.1%; 2 specimens).

Localization: gut.

A parasite of grebes (Bykhovskaya-Pavlovskaya, 1962).

Echinochasmus mordax Looss, 1899

Localities: Mingechevir reservoir (10.0%; 2 specimens).

Localization: gut.

A parasite of grebes and Pelecaniformes, sometimes occurs in other water birds (Bykhovskaya-Pavlovskaya, 1962).

Mesorchis pseudoechinatus (Olsson, 1876)

Localities: Devechi Firth (21.4%; 1-3 specimens), Small Gizilagach Bay (33.3%; 2-7 specimens), Absheron Peninsula coasts (9.1%; 3 specimens), Kura River delta (7.7%; 2 specimens).

Localization: gut.

It parasitizes mainly gulls, rarely Anserinae, toadstools and Pelecaniformes; the first intermediate hosts are gastropods; the second are fish (Urazbaev, Kurbanova, 2006; Shakarboev et al., 2012).

Family HETEROPHYIDAE Odhner, 1914

Cryptocotyle concavum (Creplin, 1825)

Localities: Devechi Firth (14.3%; 2-6 specimens), Absheron Peninsula coasts (9.1%; 2 specimens), Kura River delta (7.7%; 1 specimen).

Localization: gut.

A parasite of fish-eating birds; the second intermediate hosts are various fishes (Wootton, 1957).

Family OPISTHORCHIDAE Braun, 1901

Metorchis intermedius Heinemann, 1937

Localities: Small Gizilagach Bay (25.5%; 2-9 specimens), Absheron Peninsula coasts (18.2%; 1-3 specimens), Lower Araz (20.0%; 2 specimens).

Localization: gut, bile ducts of liver.

A parasite of various fish-eating birds; the first intermediate hosts are water-shells; the second intermediate hosts are various fishes (Razmashkin, 1978; Linnik, 1984; Isakova, 2008).

Family EUCOTYLIDAE Skrjabin, 1924

Eucotyle cohni Skrjabin, 1924

Localities: Mingechevir reservoir (21.4%; 1-2 specimens), Varvara reservoir (20.0%; 2-3 specimens).

Localization: kidneys.

Mainly is parasitic on divers (Gavia) and grebes; sometimes occurs in other waterfowls (Turemuratov, 1964; Aliyev, 2006).

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Family STRIGEIDAE Railliet, 1919

Strigea falconis Szidat, 1928

Localities: Devechi Firth (28.6%; 3-9 specimens), Small Gizilagach Bay (41.7%; 1-3 specimens), Lower Kura (22.2%; 2-3 specimens), Kura River Delta (23.1%; 1-2 specimens).

Localization: subcutaneous fat tissue and connective tissue, esophagus and trachea, fascia of the neck and head muscles.

Definitive hosts are birds of the Falconiformes order; mesocercaria localizes in the tissues of amphibians, metacercariae – in different tissues of the waterfowls (Lunaschi, Drago, 2009).

Family DIPLOSTOMATIDAE Poirier, 1886

Hysteromorpha triloba (Rudolphi, 1819)

Localities: Middle Kura (12.5%; 2-5 specimens), Lower Kura (33.3%; 2-5 specimens).

Localization: gut.

A parasite of cormorants and grebes; the first intermediate hosts are water-shells; the second intermediate hosts are different fishes (Iskova, 1983).

The 12 trematode species found in the examined grebes belong to one order, seven families and eight genera. Of these only three species – *Petasiger megacantha, Echinochasmus coaxatus,* and *E. dietzevi*, are specific grebe parasites, the rest infect various water birds. Nine trematode species of our collection occurred only in the gut, one species was found in the liver bile-duct and in the gut, one species in kidneys, and the one in subcutaneous fat and connective tissues, esophagus, trachea, neck and head muscles' fascia. Out of all the trematodes found, only *Strigea falconis* uses water birds as intermediate hosts. Its cercaria penetrates actively into the bird's body and transforms into metacercaria. The definitive hosts are predator birds of the Falconiformes order. For the other trematode species, water birds are definitive hosts; they are infected via ingestion of fishes, frogs and/or aquatic invertebrates

As the grebe is fish-eating bird, 6 species (*Patagifer bilobus, Petasiger megacantha, Mesorchis pseudoechinatus, Cryptocotyle concavum, Metorchis intermedius, Hysteromorpha triloba*) of 12 species trematodes that found in it, namely the half, parasites in the fish in metacercarial stage and infect birds when they feed on a fish.

Species richness of the trematode fauna depends of environmental conditions of the water body and diversity of its flora and fauna, which created favorable conditions for all members of the parasite life cycle. The three water bodies studied, the Devechi Firthhe, Small Gizilagach Bay and the Kura River delta are the richest in mollusk, fish and bird fauna. Each of them hosted five trematode species. Four species were recorded form the Lower Kura and the Mingechevir Reservoir, three species from the Absheron Peninsula and the Varvara Reservoir, two species from the Middle Kura, and one species from the Lower Araz (Table 1).

Table 1. Distribution of the little grebe trematodes in the study area. (Abbreviations: Dev – Devechi Firth, SQ – Small Gizilagach Bay, Ab – Absheron Peninsula coast of the Caspian Sea, MK – Middle Kura, LK – Lower Kura, KD – Kura River Delta, Min – Mingechevir Reservoir, Var – Varvara Reservoir, LA – Lower Araz)

Species	Localities								
	Dev	SQ	Ab	MK	LK	KD	Min	Var	LA
Patagifer bilobus	+	+		+	+	+			
Petasiger megacantha							+	+	
P. skrjabini							+	+	
Echinochasmus coaxatus	+	+				+			
E. dietzevi					+				
E. mordax							+		
Mesorchis pseudoechinatus	+	+	+			+			
Cryptocotyle concavum	+		+						
Metorchis intermedius		+	+			+			+
Eucotyle cohni							+	+	
Strigea falconis	+	+			+	+			
Hysteromorpha triloba				+	+				
Total	5	5	3	2	4	5	4	3	1

Comparison of the little grebe trematode faunas of the studied water bodies showed that it includes the same species in the Devechi Firth and the Kura River delta (K_{cz-s}=100%). Similarity indices were also high in the pairs Small Gizilagach Bay / Devechi Firth, Small Gizilagach Bay / Kura River Delta – 66.7% each; Lower Kura River / Small Gizilagach Bay – 51.1%, and Middle Kura River / Lower Kura River – 50.0%.

Table 2. Species similarity of the little grebe trematode faunas of the studied water bodies. (Czekanowski-Soerensen index, %). For abbreviations, see Table 1.

	LA	Var	Min	KD	LK	MK	Ab	SQ
Dev	0	0	0	100.0	28.6	16.7	33.3	66.7
SQ	20.0	0	0	66.7	51.1	16.7	33.3	
Ab	33.3	0	0	33.3	0	0		
MK	0	0	0	16.7	50.0			
LK	0	0	0	28.6				
KD	0	0	0					
Min	0	75.5						
Var	0							

The closely located Mingechevir and Varvara reservoirs are inhabited be one population of the little grebe. It is isolated from the populations of other water bodies and has no trematode parasites in common with them. On the contrary, similarity index of the trematode species composition in the little grebes of the two reservoirs amounts for 75.5%. Therefore, faunistic similarity of the trematodes depends on the distance between the water bodies and the similarity of their living conditions.

In the previous article (Mahmudova, Ibrahimov, 2020), we divided all the waterfowl trematode species of Azerbaijan into three groups: northern, southern and ubiquitous, in accordance with the division proposed by V.A. Dogel (1949), M.M. Belopol'skaya (1966), and A.A. Smogorzhevskaya (1976). The first group can infect birds only in the northern part of their range during the nesting period, the second group infects them only in the south during wintering, and the third one is able to infect birds throughout their range. Of the trematodes recorded in our study, seven species (*Petasiger megacantha, P. skrjabini, Echinochasmus coaxatus, E. dietzevi, Cryptocotyle concavum, Metorchis intermedius, Eucotyle cohni*) belong to the northern group, while five species (*Patagifer bilobus, Echinochasmus mordax, Mesorchis pseudoechinatus, Strigea falconis, Hysteromorpha triloba*) are ubiquitous. The southern species were absent. The noticeable predominance of the members of the northern trematode group and the absence of southern ones, apparently, should be explained by the prevailing of the birds of northern grebe population in Azerbaijan, which do not fly into wintering areas of southern populations and therefore they are not available for the southern species of trematodes.

Of the trematodes found in the little grebe, only *Strigea falconis* is the causative agent of bird diseases. Its metacercariae are localized in various tissues and infect wild and domestic birds. That results in birds´ weakening and a significant decrease in the quality of their meat (Krone, Streich, 2008; Drago et al., 2014; Olinda et al.2015).

Conclusion

Parasitological studies of the 94 little grebe individuals at nine water bodies of Azerbaijan revealed 12 trematode species. The research was conducted in 1998-2019. Only three species are specific grebe parasites. Differences of the grebe trematode faunas of various water bodies depended on the distance between them and similarity of their living conditions. Seven species in the studied trematode fauna belong to the northern group of helminths, while the five ones are ubiquitous.

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Екологічний аналіз трематод малої поганки – *Tachybaptus ruficollis* (Pallas, 1764) в Азербайджані

Є.А. Махмудова

У 1998-2019 рр. на 9 водоймищах Азербайджану методом повного гельмінтологічного розтину було досліджено 94 особин малої поганки – *Tachybaptus ruficollis* (Pallas, 1764), виявлено наступні 12 видів трематод, що належать до одного ряду, 7 родин та 8 видів: *Patagifer bilobus, Patagifer bilobus, Echinochamus coaxatus, E. dietzevi, E. mordax, Mesorchis pseudoechinatus, Cryptocotyle concavum, Metorchis intermedius, Eucotyle cohni, Strigea falconis, Hysteromorpha triloba*. З них 3 види (*Petasiger megacanth, Echinochasmus coaxatus* та *E. diezevi*) є специфічними паразитами лише поганок, інші паразитують і в інших водоплавних птахів. За винятком *Strigea falconis*, церкарії якого активно потрапляють в організм водоплавних птахів і перетворюються там на метацеркарії, всі знайдені види досягають статевої зрілості в організмі водоплавних птахів. Мала поганка, як рибоїдний птах, заражена 6 видами трематод (*Patagifer bilobus, Petasiger megacantha, Mesorchis pseudoechinatus, Cryptocotyle concavum, Metorchis intermediateus, Hysteromorpha triloba*), які паразитують у риб на стадії метацеркарія. Дослідження показали, що видова різноманітність трематод поганки залежить від розмірів водойми та ступеня багатства його гідрофауни. Більше видів трематод було виявлено у водоймах з більшими розмірами та більш багатою гідрофауною. Відмінності фауни трематод поганки у різних водоймах залежать від відстані між ними та подібності умов проживання. Серед трематод поганки 7 видів (*Petasiger megacantha, P. skrjabini, Echinochasmus coaxatus, E. dietzevi, Cryptocotyle concavum, Metorchis intermediateus, Eucotyle cohni*) належать до північної групи гельмінтів, а 5 видів (*Patagifer bilochasmus, Echinochasmus mordax, Mesorchis pseudoechinatus, Strigea falconis, Hysteromorpha triloba*) є убіквістами. Південні форми були відсутні у фауні трематод цього птаха. Це, мабуть, можна пояснити тим, що в Азербайджані серед особин малої поганки значно переважають представники північних популяцій, які зимують у цьому регіоні.

Ключові слова: *Азербайджан, водоплавні птахи, паразити, гельмінти, трематоди*.

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The red blood cells cytometric characteristics of young fresh-water fish of various families

T.S. Sharamok, N.B. Yesipova, V.O. Kurchenko

The morphometric indices of red blood cells of young fish of various species inhabiting coastal zones of the water bodies were studied. The subjects of the research were the fish of four families: Carp (*Alburnus alburnus*, *Carassius gibelio*, *Rhodeus amarus*, *Abramis brama*, *Rutilus rutilus*), Needles (*Syngnathus abaster nigro lineatus*), Centrarchidae (*Lepomis gibbosus*), Loaches (*Cobitis taenia taenia*), Gobies (*Neogobius fluviatilis*). The hydroecological conditions were characterized by an intense oxygen regime, high content of phosphates, and heavy metals (zinc). The fish peripheral blood was taken from the tail vein; smears were made according to the classical Romanowsky-Giemsa method. Our research showed that the red blood cells of young fish belonging to the ecological group of inactive and unpretentious species (*Neogobius fluviatilis*, *Carassius gibelio*) had the largest cross-sectional area and a high index of nuclear-cytoplasmic ratio. The indicators of erythrocyte eccentricity were the highest in the active fish with high energy costs (*Alburnus alburnus*, *Lepomis gibbosus*). The largest number of erythrocytes with pathological events (cytolysis, karyolysis, pyknosis, poikilocytosis) was observed in the young *Alburnus alburnus* (14%), and the smallest one in *Rhodeus amarus* and *Lepomis gibbosus* (2–4%). In the individuals of *Syngnathus abaster nigro lineatus* affected by parasitic nematodes of the genus *Ascaris*, the number of erythrocytes with pathologies increased to 81%. Under the toxic load, destabilization of the fish circulatory system begins with the appearance of the young forms of erythrocytes as compensation for depleted mature erythrocytes and ends with the mass destruction of mature erythrocytes. Given these patterns, as well as the relatively low number of young ballast forms of erythrocytes and mature erythrocytes with pathological features, we can assume that the state of the red blood cells in the studied young fishes meets the conditional norm, with the exception of the blood of *Syngnathus abaster nigro lineatus* infected with parasites. In our opinion, the main characteristics of the fish red blood that reflect the fishes' adaptive capacities are as follows: eccentricity ratio of erythrocytes, the nuclear-cytoplasmic ratio, ratio of the young ballast forms of erythrocytes, and the relative number of erythrocytes with pathology.

Key words: *erythrocytes, cytometric indices, pathological changes, Zaporizhzhia Reservoir.*

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Introduction

In modern aquatic ecosystems, significant changes occur under the impact of natural (climate change) and human (economic activity) factors. As a result, the structure of populations and the physiological status of fish are changing (Gamito et al., 2015; Zeng et al., 2017; Năstase, Oţel, 2017; Fedonenko et al., 2018). The younger age groups of fish are hypersensitive to environmental changes; firstly, they have not formed the mechanism of physiological adaptation yet, and secondly, most of the young fish live in the reservoirs' coastal zones, where the influence of external factors is the strongest. Therefore, the studies on young fish's adaptogenic potential are very important for forecasting the further development of industrial fish populations and assessing their strategic stocks, as well as for the environmental introductions and the artificial formation of ichthyofauna in inland water bodies.

The self-regulating blood system plays a major role in adapting to changing conditions. Blood, as an internal part of an organism, responds quickly to environmental changes and always reflects accurately the physiological state of an organism. Therefore, in the ecological monitoring system, hematological indicators

are often used as a benchmark for the adaptive capacity of fish to anthropogenic impact (Ahmed et al., 2020; Fazio, 2019; Fazio et al., 2020; Yilmaz, 2015; Belokon et al., 2013; Fedonenko et al., 2019).

When analyzing formed blood elements, special attention is paid to the cells of the erythroid series. Many authors indicate the expediency of using erythrocytes as indicators of hypoxic conditions. Thus, during the research of *Neogobius melanostomus* (Pallas, 1814) adult specimens' blood that was kept for 10 days under experimental hypoxia conditions (1.7–1.8 mg O_2 / l), a probable increase in the volume of the mature erythrocyte was detected as a result of their swelling, the ellipsoidal form of the erythrocytes changed to a circular, and there was a nearly two-fold increase in the number of the immature erythroid cells (Parfenova, Soldatov, 2011). After six-hour experimental hypoxia (2 mg O_2 / l), the number of red blood cells with chromatolysis, karyolysis, and poikilocytosis in the three-year-old pikes and breams increased significantly. In the silver carp, 1.8 times increased the number of erythrocytes with amitosis (Moroz, Yesipova, 2011).

In recent years, more and more researchers have been using the parameters of erythrocytes to assess the status of fish populations living in areas with anthropogenic pollution. Thus, 22 erythrocyte pathologies were detected in the adult roach, bream, perch, and gobies from the Saratov Reservoir (Russia) under conditions of the high content of phenols, petroleum products, copper, and sulphates in the water. The most widespread was the deformation of the cell and nucleus, carriolysis, chromatolysis, agentric location of the nucleus, and amitozes (Mineev, 2013). In the roach from the Zaporizhzhia Reservoir (Ukraine), inhabiting areas with heavy metal and organic contamination, there were detected a decrease in the relative proportion of mature erythrocytes and an increase in young blast forms of erythrocytes, changes in the shape of erythrocyte nuclei, anisocytosis, hypochromia, cytolysis, cell membrane destruction, karyolysis and karyopyknosis (Sharamok et al., 2016).

Despite the popularity of ichthyological research on formed blood elements, information on the red blood cell cytometric indices is limited. In particular, in the early stages of ontogenesis. A small number of researches in this direction suggest that the morphological features of erythrocytes and their functional properties in young fish have certain differences from that in adult fish. Thus, comparing the morphology of fingerlings and adult erythrocytes of roach and crucian carp, it was found that with age in fish, the ratio of the nucleus area to the erythrocytes' area is likely to decrease, and the number of mature forms of erythrocytes increases by almost 20% (Fedonenko et al., 2016). It is known that mature erythrocytes of fish, compared to young cells, have a higher concentration of hemoglobin protein, the main carrier of the blood respiratory function (Soldatov, 2005). Therefore, the determination of the ratio of various forms of erythrocytes could indirectly characterize the hematopoietic function of the blood activity. It was also established that in conditions of environmental pollution, the number of erythrocytes with various pathologies (vacuolation of the cytoplasm, nucleus hemolysis, carioerexis, amitosis) in the young bream *Abramis brama* (L., 1758) reaches 44.4% of the total number of red blood cells in an organism (Konkova, Fedorova, 2016). Thus, the existing data prove the expediency of using erythropoiesis indicators in young fish to assess the impact of the aquatic environment on ichthyofauna and to understand the mechanism of fish adaptation to changing hydroecological conditions.

Our research aimed to study the cytomorphological indices of young fish red blood cells from the Zaporizhzhian reservoir coastal populations.

Materials and methods

The selection of fish for hematological research was carried out in the summer of 2016–2017 in the coastal zone of the central part of the Zaporizhzhia (Dnipro) Reservoir (Figure 1). The water reservoir was created on the Dnipro River in 1932; it has an area of 420 km^2. It is located in the southeast of Ukraine, in the territory of agrarian-industrial zones under heavy anthropogenic influence.

The young fish were taken in a small 10 m long frying net. The young fish were divided by species and counted. Nine fish species of different families were researched. The carp family was represented by four species: *Alburnus alburnus* (Linnaeus, 1758), *Carassius gibelio* (Bloch, 1782), *Rhodeus amarus* (Bloch, 1782), *Abramis brama* (Linnaeus, 1758), *Rutilus rutilus* (Linnaeus, 1758); Family of Needles – a *Syngnathus abaster nigro lineatus* (Eichwald, 1831); Family Centrarchidae (Sunfish) – *Lepomis gibbosus* (Linnaeus, 1758); Family Cobitidae (Loaches) – *Cobitis taenia taenia* (Linnaeus, 1758); Family Gobiidae (Gobies) – *Neogobius fluviatilis* (Pallas,1814). All experimental fish, except the *Syngnathus abaster nigro lineatus*, were the fingerlilngs (0+); *Syngnathus abaster nigro lineatus* were represented by two-year-olds (1+).

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Blood from the fish was taken from the tail vein. The smears were made using the generally accepted method and stained by the Romanowsky-Giemsa method. The smears were studied with a 40^X objective using a microscope with the Sciencelab T500 5.17 M digital camera. Atlases of blood cells were used to identify cells of the erythroid population (Ivanova, 1983; Sharon, Zilberg, 2012). The preparations were looked at 100 fields of vision. The following parameters were determined: large longitudinal (D) and small transverse (d) diameters of mature erythrocytes, the ratio of small and large erythrocytes diameters (d / D), erythrocyte separation area (S), area of erythrocyte nucleus (s), nuclear-cytoplasmic ratio (s / S), the ratio of mature and young forms of erythrocytes. The eccentricity coefficient (E), which characterizes the degree of the cell deviation from the round shape, was determined as the square root of the square difference ($1 – d / D^2$). Measurements of cytometric erythrocytes indices were performed using the Science Lab View 7 program.

Fig. 1. Study area on the scheme of the Zaporizhzhia Reservoir

The data were analyzed as Mean ± S.E.M. at reliability of 95% and significant level of $P < 0.05$. To test the significance of the test, the *t*-test method was used.

Ethical Statements

Bioethical standards were not violated during the research. It was performed under the Regulations on the Ethics Committee (Bioethics) in Ukraine (Regulations on the Ethics Committee (Bioethics), 2019).

Results

The central part of the Zaporizhzhia reservoir, where the selection of fish was carried out, is characterized by weak flow and a large shallow water area. The coastal populations of young fish are concentrated. The hydroecological regime of the area is affected by the household and domestic water of Dnipro city and agrarian enterprises.

According to long-term monitoring, the average annual mineralization of water in the site is 334 mg / l, hardness – 3.2 mg equ / l, pH 7.5–8.6. The content of ammoniacal nitrogen in water during the experimental period varied from 0.22 to 0.97 mg / dm^3 at different points (average 0.54 mg / dm^3 in the reservoir); nitrites – from 0.01 to 0.14 mg / dm^3 (0.019 mg / dm^3), nitrates – 0.11–2.8 mg / dm^3 (0.47 mg / dm^3), phosphates – from 0.25 to 0.62 mg / dm^3 (0.39 mg / dm^3). The content of dissolved oxygen in summer varies between 2,2–6,8 mg O_2 / dm^3 (Fedonenko et al., 2018).

The most of the Zaporizhzhia reservoir toxicants are heavy metals. The content of toxic metals in the water of the reservoir's central part corresponded to the existing Ukrainian norms for the water of fish-water reservoirs. There was a high concentration of zinc, which was 2.7 MPCs (0.027 ± 0.007 mg / l) and copper – 12 MPCs (0.012 ± 0.006 mg / l). Persistent contamination with copper may be a result of sewage emissions from enterprises of the chemical, metallurgical industry, mine waters, and aldehyde reagents

used for the destruction of algae. Zinc enters the natural waters as a result of natural processes of destruction and dissolution of rocks and minerals. A considerable amount of zinc gets into water bodies with anthropogenic pollution, namely the wastewater from ore dressing factories and galvanic shops, parchment paper production, and mineral plants. The most important external source of zinc in the Zaporizhzhia reservoir is a runoff that changes zinc distribution in the littoral.

The obtained data made it possible to calculate the chemical index of water quality, where the index of quality according to the trophic and saprobiological indicators was 4.4 and for indicators of specific toxic effect was 3.8. Thus, the weighted chemical index was 4.1, which characterizes the water quality of the Zaporizhzhia reservoir as "satisfactory" and "poorly polluted".

In the research of species composition of fish coastal populations, the domination of the short-cycle species as the *R. amarus* was identified. Its number was 40–45% of the total from the fish in catches. In the second place was the *R. rutilus* – 24–28%, followed by *A. alburnus* and *N. fluviatilis* – 6–10%. The number of other fish species varied from 1.5% to 4%. The prevalence of a small population of low-value species with a short growing cycle has been observed in the reservoir during the last 10 years and it indicates the degradation changes in the ichthyotsenoses of the Zaporizhzhia reservoir. The rapid increase in the number of invasive species of the self-settler Sunfish was another negative feature of the coastal population structure (Fedonenko et al., 2018).

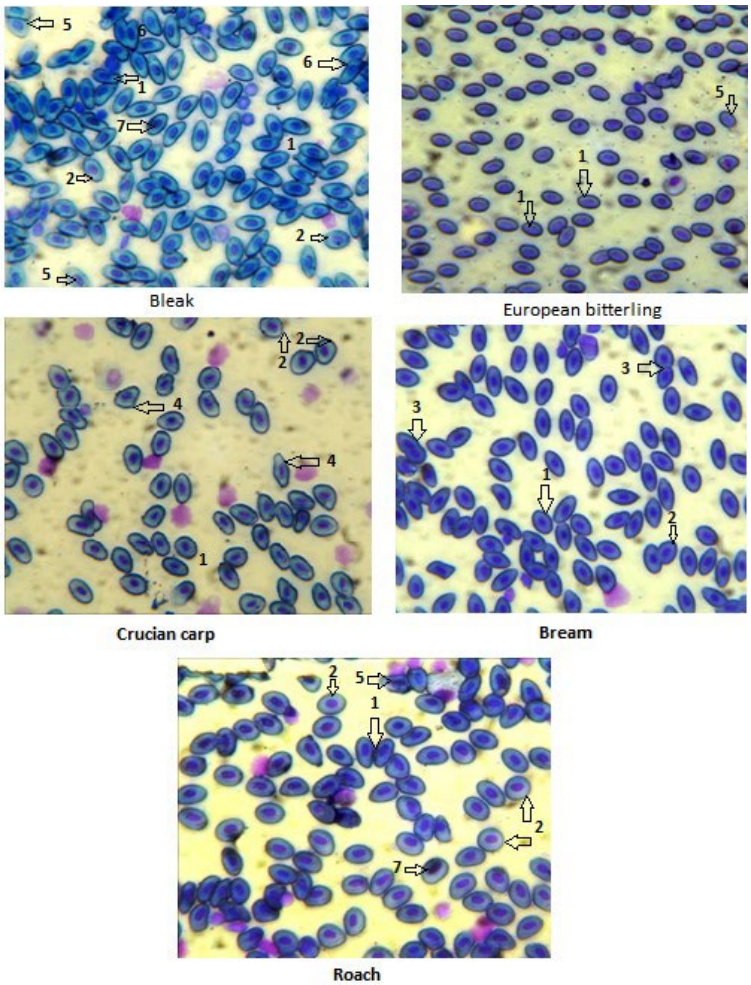

Fig. 2. The picture of the Carp family young fish peripheral blood:
1 – mature erythrocytes; 2 – young red blood cells; 3 – amitosis; 4 – poikilocytosis; 5 – karyolysis; 6 – adhesion of red blood cells; 7 – pyknosis (40^x)

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The picture of the carp fish fingerlings' peripheral blood is presented in Figure 2. In all the fish, mature erythrocytes had a clear ellipsoidal form. The nuclei are well-expressed, dark purple, and have a central location. The cytoplasm is transparent and homogeneous.

The erythrocytes with pathological events were found in all the carp fish. The disturbances were in the form of cytolysis, karyolysis, pyknosis, poikilocytosis, and amitosis. The most common cells with variable forms (poikilocytosis) are pear-shaped and crescent-shaped. The relative number of such cells ranged from 26 to 68% of the total number of cells with pathologies. Cytolysis was detected in 15–32% of erythrocytes in the form of partial lysis of cells. Nuclear pathologies were found in the form of karyopyknosis (shrinkage of the nucleus) and karyolysis (partial or complete dissolution of the nucleus) and occurred in 28–54% of variable erythrocytes. Erythrocytes in the state of amitotic division were much less common – in 8–14% of the total number of cells with pathologies. The largest indicator of erythrocyte pathologies is noted in *A. alburnus* – 14% of the red blood cells total number, then in *A. brama* – 12%, the *R. rutilus* – 9%, *C. gibelio* – 7%, and the smallest number of red blood cells with pathologies was in the *R. amarus* – 2%.

The relative number of mature erythrocytes in all the carp fishes significantly exceeded the number of young red blood cells and ranged from 93% to 98%. The young forms of erythrocytes were represented mainly by blast forms – early erythrocytes (EE) and i polychromatophilic erythroblasts (PE). They had a well-defined perinuclear zone, a basophilic cytoplasm, and a large nucleus with prominent chromatin granules. PE differed in the absence of the perinuclear zone and in the presence of oxyphilic properties in the cytoplasm.

In our research of the ratio PE : EE of the carp fish was as follows: *Abramis brama* and *A. alburnus* – 0.8–1 : 1, *R. rutilus* – 2.5 : 1, *C. gibelio* – 3 : 1, *R. amarus* – 4 : 1. Thus, among the carp fish, the highest percentage of functionally active erythrocytes forms in blood was in *R. amarus* and *C. gibelio* fingerlings.

The morphological signs of erythrocytes in the young fish of other families had their peculiarities. The red blood cells of *L. gibbosus* and *C. taenia taenia* had a classic ellipsoid form, whereas, in *N. fluviatilis* and *S. abaster nigro lineatus*, mature erythrocytes had a more rounded shape. The nuclei and shells of erythroid cells were clearly expressed in the majority, the cytoplasm was homogeneously colored (Figure 3).

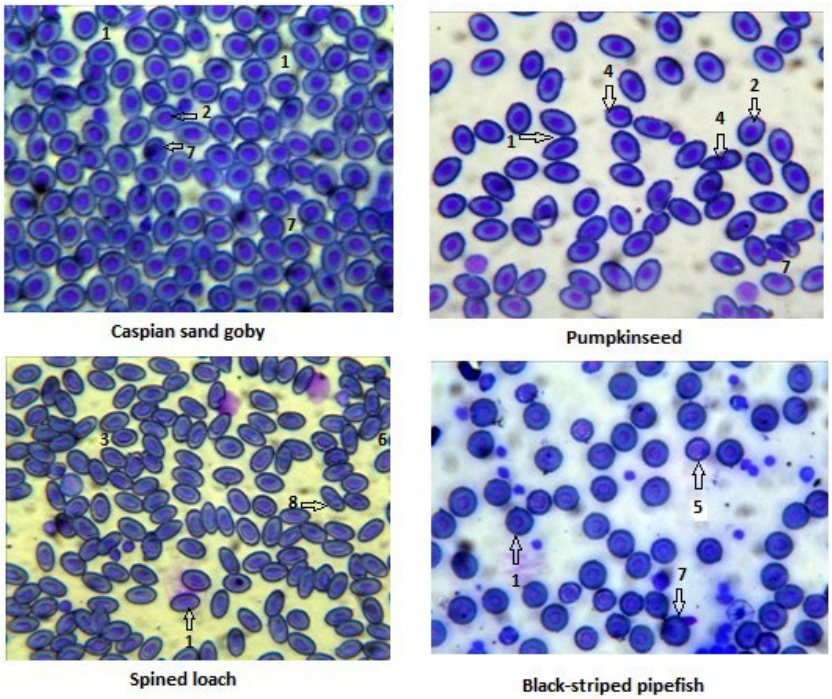

Fig. 3. Picture of the peripheral blood of different families of young fish:
1 – mature erythrocytes; 2 – young red blood cells; 3 – amitosis; 4 – poikilocytosis; 5 – karyolysis; 6 – adhesion of red blood cells; 7 – pyknosis; 8 – micronuclei (40^x)

The relative number of mature erythrocytes in *L. gibbosus* and *C. taenia taenia* was 98 and 97% respectively, *N. fluviatilis* – 72%, *S. abaster nigro lineatus* – 68%. The ratio of young forms of erythrocytes (PE : EE) in the first two fish species was 1 : 1, in *N. fluviatilis*, polychromatophilic erythroblasts (1 : 2) predominated, but in *S. abaster nigro lineatus*, on the contrary, it was early erythrocytes (1 : 1,5).

The smallest number of erythrocytes with pathological features was observed in the *L. gibbosus* – from 2 to 4%, mostly these were cells with poikilocytosis, and nuclei were more common in a state of piknosis (Fig. 3). In *N. fluviatilis*, the relative number of erythrocytes with pathology ranged from 4 to 7%, with more frequent lysis and nucleus picnosis. In *C. taenia taenia*, the number of erythrocytes with pathology was 10% on average. The pathological phenomena appeared in infractions associated with cell division (amitosis), the appearance of several nuclei (micronuclei) in cells, and the adhesion of red blood cells. The largest number of erythrocytes with pathological changes was detected in blood smears of the *S. abaster nigro lineatus* – from 16 to 27%. There were the erythrocytes with picnosis and asymmetry of nuclei.

In the course of small-scale fishing, we found specimens of the *S. abaster nigro lineatus*, damaged by *Ascaris (Contracoecum)* larvae. The one parasite localized in the abdominal cavity of the fish. The infection incidence of *S. abaster nigro lineatus* population was 20%. It was interesting to see how the picture of the fish's blood changes in the presence of parasitic invasion. In the Figure 4, we could see that pathological changes affect almost all erythroid cells. The total number of erythrocytes with pathology reached 77–81%. Most often, there were erythrocytes with a displacement of the nucleus, karyolysis, and karyopiknosis. In many cells, the membrane had a festooned edge, or it was completely disturbed. There were poikilocytosis and cytolysis.

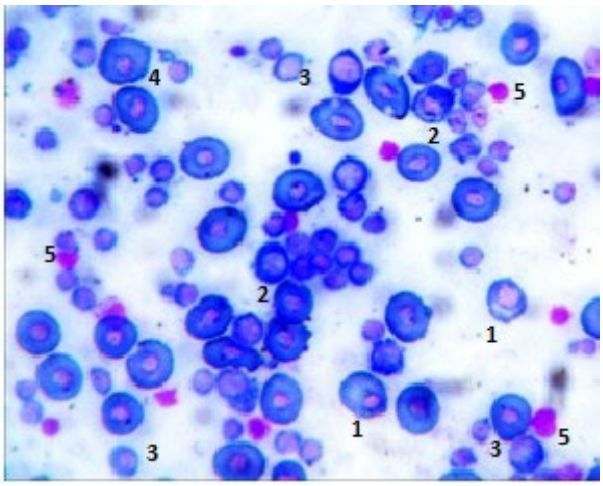

Fig. 4. The picture of the female Black-striped pipefish peripheral blood, damaged by parasitic larvae of the genus *Ascaris (Contracoecum)*:
1 – displacement and lysis of the nucleus, vacuolation of the cytoplasm, infraction of the cell membrane; 2 – karyorrhexis; 3 – cytolysis; 4 – piknosis, poikilocytosis; lymphocytes (40^x)

The large number of lymphocytes that are concentrated near the affected red blood cells, which indicates the active course of phagocytic processes is drawing attention.

The research on erythrocytes cytometric indices in different species of the fish allowed to reveal the following features. In the carp fish, longitudinal diameters of erythrocytes (D) ranged from 10,27 ± 0,08 μm, in the *R. amarus* to 13,4 ± 0,1 μm, in *A. brama* (Table 1). The difference between the indicators reached 23% and was significant ($p \leq 0.05$).

The largest cross-sectional diameters were in red blood cells of *C. gibelio*, *A. brama,* and *R. rutilus* (8.1–8.7 μm). In *R. amarus* and *A. alburnus*, they were on average less for 20% (6.2–6.6 μm).

In other fish families, longitudinal diameter indices ranged from 11.7 ± 0.06 μm in the *S. abaster nigro lineatus*, to 13.5 ± 0.08 μm in *C. taenia taenia*. The difference between the indicators did not exceed 6–10% ($p \geq 0.05$). The values of the cells' transversal diameter, on the contrary, had significant differences. The largest cross-sectional diameter was in *S. abaster nigro lineatus* erythrocytes (9.7 ± 0.04 μm), and the

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smallest – *L. gibbosus* erythrocytes (6.7 ± 0.02 μm). The difference was probable ($p \leq 0.05$) and reached 30%.

The largest area of erythrocytes (S) among the carp fish was in *C. gibelio* and *A. brama* and was 88.9 ± 0.47 and 89.04 ± 0.58 mkm^2, respectively. The area of erythrocytes of *R. rutilus* was less by 16%, *A. alburnus* by 22%, and *R. amarus* by 45%. A similar dependence was observed in the indices of the area of erythrocyte nucleus division (s). In *C. gibelio,* it was 17.4 ± 0.16 μm^2, in *A. brama* was less by 9%, *R. rutilus* by 26%, *A. alburnus* by 37%, and *R. amarus* by 43%.

In the case of other families of fish, the highest rates of S and s had the erythrocytes of the young *N. fluviatilis* – respectively 98.9 ± 0.9 and 21.7 ± 0.3 μm^2. Compared to carp fish (*A. brama*), they were higher to S by 10%, and to s – by 27%. In *S. abaster nigro lineatus*, the area of erythrocytes and their nuclei occupied an intermediate position among the indicators of other species of fish. The *C. taenia taenia* had rather large area of the cores of erythrocytes – 17.7 ± 0.33 μm^2, which exceeded the value of this index in carp fish by 10–44%. In *L. gibbosus*, S and s did not differ much from *A. alburnus*.

Table 1. Mature erythrocytes cytometric indices of fish fingerlings of different families

Species of fish	Diameter of erythrocyte, μm		Eccentricity (E)	Area of erythrocyte division, (S, μm^2)	Area of erythrocyte nucleus division (s, μm^2)	s / S
	longitudinal (D)	transversal (d)				
Alburnus alburnus	13.03 ± 0.09	6.6 ± 0.07	0.86	69.6 ± 0.62	10.9 ± 0.13	0.16
Rhodeus amarus	10.27 ± 0.08	6.2 ± 0.05	0.80	49.3 ± 0.29	9.9 ± 0.12	0.20
Carassius gibelio	12.8 ± 0.08	8.7 ± 0.07	0.73	88.9 ± 0.47	17.4 ± 0.16	0.20
Abramis brama	13.4 ± 0.08	8.3 ± 0.06	0.79	89.04 ± 0.58	15.9 ± 0.15	0.18
Rutilus rutilus	11.9 ± 0.06	8.1 ± 0.07	0.73	75.4 ± 0.57	12.8 ± 0.15	0.17
Neogobius fluviatilis	12.7 ± 0.07	9.6 ± 0.08	0.65	98.9 ± 0.9	21.7 ± 0.3	0.22
Lepomis gibbosus	12.4 ± 0.06	6.7 ± 0.02	0.84	66.8 ± 0.5	10.8 ± 0.12	0.16
Cobitis taenia taenia	13.5 ± 0.08	7.7 ± 0.06	0.82	80.4 ± 0.65	17.7 ± 0.33	0.22
Syngnathus abaster nigro lineatus	11.7 ± 0.06	9.1 ± 0.04	0.62	89.9 ± 0.38	14.7 ± 0.18	0.16

To evaluate the functional state of erythrocytes, the nuclear-cytoplasmic ratio (s / S) is often used. According to our calculations among carp fish, the highest s / S was in *R. amarus* and *C. gibelio* – 0.20; the lowest – was in *A. alburnus* – 0.16. Among the fish of other families, the maximum values were in *N. fluviatilis* and *C. taenia taenia* – 0.22; the least – in *L. gibbosus* – 0.16. As the s / S value decreases, the researched fish were constructed in the following series: *N. fluviatilis = C. taenia taenia > R. amarus = C. gibelio > A. brama > C. gibelio > A. alburnus = L. gibbosus = S. abaster nigro lineatus*.

Discussion

In the conditions of the Zaporizhzhia reservoir, several features concerning the morphology and ratio of red blood cells, nuclear-cytoplasmic ratio, and pathological abnormalities in erythrocytes were detected in the young fish blood of different families.

According to our research, the mature erythrocytes of the young fish of all the researched fish species had an ellipsoidal shape, but the coefficient of eccentricity (ellipsoidity) of the cells differed significantly.

The highest elevation of erythrocytes (E) among the carp fish was in *A. alburnus* – 0.86. In other carp fish, it was less than 7–15%. Compared to other families, the highest values of E were at *L. gibbosus* –

0.84, and the smallest – in *S. abaster nigro lineatus*. – 0.62. In species of the Black-striped pipefish was noted a decline in E index to 0.56 in infection by parasitic nematodes. The dynamic reduction of E by the species of fish is as follows:

A. alburnus > *L. gibbosus* > *C. taenia taenia* > *R. amarus* > *A. brama* > *C. gibelio* = *R. rutilus* > *N. fluviatilis* > *S. abaster nigro lineatus*.

The coefficient of eccentricity (ellipsoidity) of erythrocytes characterizes not only morphological but also functional features of cells. It is known that in warm-blooded – for example, humans and higher animals (mammals) common erythrocytes have a round shape. The appearance of ellipsoid cells with an eccentricity index E > 0.62 is regarded as a pathological symptom and is observed in hemolytic anemias. On the contrary, in cold-blooded animals – fish, and frogs, the ellipsoid form of erythrocytes is normal, and an increase in the number of red blood cells with a round form is regarded as a deviation from the norm. Thus, in the round goby in the conditions of experimental hypoxia, red blood cells became larger, acquired a rounded form, and increased the volume of their nuclei (Parfenova, Soldatov, 2011). In the research of erythropoiesis in the frog *Rana ridibunda* L., three groups of red blood cells were noted according to morphological features: normal ellipsoid cells (eliptocytes) with E = 0.74 ± 0.004; strong ellipsoid cells (magnulocytes) – E = 0.81 ± 0.004; rounded cells (teretiocytes) – E = 0.63 ± 0.012 (Zelentsova, Skorkina, 2004).

If we classify erythrocytes of fish by the E index in analogy with red blood cells, then in the group of fish with normal ellipsoid erythrocytes (E = 0.73 – 0.80) there are *A. brama*, *C. gibelio*, *R. rutilus*, *R. amarus*; with strongly ellipsoid eritrocytes (E = 0.81–0.86) – *A. alburnus*, *L. gibbosus*, *C. taenia taenia*; with rounded erythrocytes (E = 0.62 – 0.72) – *N. fluviatilis*, *S. abaster nigro lineatus*.

Thus, the eccentricity coefficients of erythrocytes of the different species of young fish within the same family had high variability and depended not so much on the systematic affiliation of the fish but the biological and physiological features of the species. So, the active, mobile fish (*A. alburnus*, *L. gibbosus*) with high energy costs had erythrocytes with a high ellipsoid index, and the sedentary fish (*N. fluviatilis*, *S. abaster nigro lineatus*) had erythrocytes with a more rounded excentric form. Besides, the infection of the *S. abaster nigro lineatus* by parasitic nematodes (*Contracoecum*) resulted in a 10% decrease in this index and an increase in the number of red blood cells with a rounded form. A similar phenomenon was observed in *N. melanostomus* under conditions of experimental hypoxia (Parfenova, Soldatov, 2011), as well as in *Siganus rivulatus* under conditions of lead and cadmium intoxication (Ezzat et al., 2013).

The research on the ratio of erythrocytes in different forms showed that in all fish species the majority of red blood cells were mature erythrocytes – 93–98%. This fact is emphasized by many authors on different fish species (Parfenova, Soldatov, 2011; Mineev, 2013; Kurchenko, Sharamok, 2020) and because mature erythrocytes have more hemoglobin and are able to more actively bind oxygen compared to young erythrocyte species (Speckner et al., 1989).

Concerning the ratio of young erythroid cells, different species of fish were distinguished by their peculiarities. The following regularities could be observed: in active, mobile fish species, blood was more actively rejuvenated by young ballast forms of erythrocytes – early erythrocytes (EE) and therefore in relation to more mature ballast forms – polychromatophilic erythroblasts (0.8 – 1 : 1). We noticed this pattern in the *A. brama*, *A. alburnus*, *L. gibbosus*, *C. taenia taenia*, *R. rutilus*. On the contrary, in the blood of the sedentary species – *N. fluviatilis*, *C. gibelio*, and *S. abaster nigro lineatus* early erythrocytes were much smaller, and the ratio of PE: EE was as follows – 2.5–4 : 1.

We also noted the fact that in *S. abaster nigro lineatus*, when infected with parasitic nematodes, the number of early erythrocytes increased several times, exceeding the number of polychromatophilic erythroblasts (PE: EE = 1: 1.5). Apparently, the appearance of young ballast forms of erythrocytes was a protective reaction of the body to parasite intoxication.

According to the cytometric data, the largest area of erythrocyte segregation was in the Caspian sand goby, *S. abaster nigro lineatus*, and *A. brama,* and the *C. gibelio*, and the smallest in the *R. amarus*, the *L. gibbosus,* and the *A. alburnus*. It is thought that in smaller cells, a shorter diffusion path and faster oxygen transfer occur. Our data, in general, coincide with this conclusion – namely, in the bleak, the perch, and the bitter, which were fast-moving and therefore actively needed oxygen for respiratory processes, the erythrocytes' area was the smallest among other types of fish. While in the slow-moving fish (*N. fluviatilis*, *C. gibelio*), which are unpretentious to oxygen conditions, the area of erythrocytes was 30–50% higher.

The indicators of the nuclear-cytoplasmic ratio differed in fish with varying agility to environmental conditions. The highest were in the *N. fluviatilis*, the *C. taenia taenia*, and the *C. gibelio* (0.20–0.22) – that

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is fish that was resistant to oxygen conditions and pollution. In more sensitive fish (*A. alburnus*) they were 20–25% lower. High indices of the nuclear-cytoplasmic ratio can indicate the ability of erythrocytes to rapidly accumulate nuclear mass and switch to an amyotrophic division, which may be one of the adaptive reactions of fish to adverse conditions.

The erythrocytes with pathological abnormalities were found in all the fish species studied, but their relative numbers differed. Among the carp fish family, the largest number of erythrocytes with pathology (cytolysis, karyolysis, piknosis, poikilocytosis, amitosis) was in the *S. abaster nigro lineatus* (14%), the lowest was in the *R. amarus* (2%); then in descending order followed by the *A. brama*, *Rutilus rutilus*, *Carassius gibelio*. In the fish of other families, the proportion of damaged erythrocytes ranged from 2% (*L. gibbosus*, *N. fluviatilis*) to 10% (*C. taenia taenia*). In the *S. abaster nigro lineatus* only, the number of erythrocytes with pathological features reached 27%. In the *S. abaster nigro lineatus* individuals infected with parasitic nematodes, the number of erythrocytes with pathological features (nucleus displacement, karyolysis, karyopiknosis, poikilocytosis, and cytolysis) increased to 81%.

Unfortunately, we did not have the opportunity to compare the data obtained with the literature data for all the fish species researched. In the literature available to us, we did not find information on the status of red blood cells in the *R. amarus*, *A. alburnus*, *L. gibbosus*, *C. taenia taenia*, and *S. abaster nigro lineatus*. That is, we can assume that these researches were conducted for the first time and need to be continued. The most researched erythrocyte hematopathies in the *A. brama*, *R. rutilus*, and *C. gibelio*, which are common and common species in reservoirs. Thus, according to Russian scientists in the contaminated areas of the Kuibyshev reservoir in the *A. brama* and the *R. rutilus* the number of red blood cells with pathological signs increased from 26.4% to 40.6%. In this case, the most common cell pathologies were: vacuolation of the cytoplasm, acentric location of the nucleus, cytolysis, karyolysis, and karyopiknosis (Mineev, 2016). In the young *A. brama*, contaminated sites of the Volga delta with kernel displacement, chromatinolysis, anisakidosis, and other pathologies reached 32–42% (Konkova, Fedorova, 2016). We saw a similar pattern in the *R. rutilus* from the Zaporizhzhian reservoir. The number of damaged erythrocytes in the fish from the contaminated area of the reservoir increased from 8% to 33% compared to the conditionally clear area (Sharamok et al., 2016).

It is known that in conditions of toxic loads on fish, destabilization of the circulatory system begins with the appearance in the blood of young forms of erythrocytes as compensation for depleted mature erythrocytes, and ends with the mass destruction of mature erythrocytes.

Given these patterns, as well as the relatively low number of young ballast forms of erythrocytes and mature erythrocytes with pathological features, we can assume that the state of red blood cells in the young fish species researched by us meets the conditional norm, except for the picture of the parasite-infected *S. abaster nigro lineatus*.

Conclusion

The hematological research of the fish was carried out at the central section of the Zaporizhzhia (Dnipro) reservoir, which is characterized as “satisfactory” and “poorly contaminated” by toxicity indicators. The morphometric indices of erythroid cells were used to assess the adaptive capacity of the young fish populations. According to the results of research, the young fish which belong to the ecological group of sedentary and oxygen-less fish species (*Neogobius fluviatilis*, *Carassius gibelio*) had the largest erythrocytes area and a high nuclear-cytoplasmic ratio. Indicators of eccentricity (ellipticity) of erythrocytes had the highest values in the mobile fish (*Alburnus alburnus*, *Lepomis gibbosus*) with high energy costs. The mobile fish also had a high content of young ballast forms of early erythrocytes, indicating active rejuvenation of red blood cells. The relative number of erythrocytes with pathological features (nucleus displacement, karyolysis, karyopiknosis, poikilocytosis, and cytolysis) was not high in all fish species (2–10%). Only in the *Syngnathus abaster nigro lineatus*, the percentage of erythrocytes with pathology ranged from 16–27%, and in individuals affected by *Ascaris* parasitic nematodes, it reached 81%.

Thus, the main indicators of the red and blood of fish, which, in our opinion, reflect the adaptive capacity of fish, are the eccentricity ratio of erythrocytes, the ratio of young ballast forms of erythrocytes, nuclear-cytoplasmic ratio, and the relative number of erythrocytes with pathology.

In the less mobile fish, the physiological adaptation of red blood cells to the environmental conditions was more pronounced than in the actively moving fish.

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Цитометрична характеристика еритроцитів молоді прісноводних риб різних родин

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Досліджено морфометричні показники еритроцитів молоді різних видів риб, що мешкають у прибережних зонах водойм. Об'єктом дослідження були риби чотирьох родин: коропові (верховодка, карась сріблястий, гірчак звичайний, лящ, плітка), іглицеві (голка пухлощока чорноморська), центрархові (сонячний окунь), в'юнові (щиповка звичайна), бички (бичок пісочник). Гідроекологічні умови характеризувались напруженим кисневим режимом, високим вмістом фосфатів і важких металів (цинку). Периферичну кров риби відбирали з хвостової вени; мазки виготовляли за класичною методикою та фарбували за методом Романовського-Гімзи. За результатами досліджень було виявлено, що у молоді риб, які належать до екологічної групи малоактивних і невибагливих видів (бичок пісочник, карась сріблястий), еритроцити мають найбільшу площу поперечного перерізу та високий показник ядерно-цитоплазматичного співвідношення. Показники ексцентричності еритроцитів були найвищими у активних риб з високими енергетичними витратами (верховодка, сонячний окунь). Найбільшу кількість еритроцитів з патологічними явищами (цитоліз, каріолізис, пікноз, пойкілоцитоз) спостерігали у молоді верховодки (14%), а найменшу – у гірчака звичайного та сонячного окуня (2–4%). У особин голки пухлощокої чорноморської, уражених паразитичними нематодами р. *Ascaris*, кількість еритроцитів з патологією зросла до 81%. В умовах токсичного навантаження дестабілізація кровоносної системи у риб починається з появи в крові молодих форм еритроцитів як компенсація руйнування зрілих еритроцитів, а закінчується масовим руйнуванням зрілих еритроцитів. Враховуючи ці закономірності, а також відносно низьку кількість молодих форм еритроцитів і зрілих еритроцитів з патологічними ознаками, можна вважати, що стан еритроцитів у досліджених нами видів молоді риб відповідає умовній нормі, за винятком крові зараженої паразитами голки пухлощокої чорноморської. Таким чином, основними показниками червоної крові риб, які, на нашу думку, відображають адаптаційні можливості риб, є: коефіцієнт ексцентричності еритроцитів, співвідношення молодих баластних форм еритроцитів, ядерно-цитоплазматичне співвідношення та відносна кількість еритроцитів з патологією.

Ключові слова: *еритроцити, цитометричні індекси, патологічні зміни, Запорізьке (Дніпровське) водосховище.*

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Регуляція селективним світлом мітотичної активності кореневих меристем та ростових процесів проростків сої культурної з контрастною фотоперіодичною реакцією

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У роботі представлені результати дослідження впливу опромінення селективним світлом різного спектру – червоного (ЧС), 660 нм, зеленого (ЗС), 530 нм та синього (СС), 450 нм на проліферативну активність клітин кореневої меристеми, накопичення біомаси і ростові процеси в надземній та підземній частинах етіольованих проростків сої культурної. Як рослинний матеріал в роботі використовували контрастні за фотоперіодичною реакцією проростки сої культурної (*Glycine max* (L.) Merr.) двох сортів – короткоденного сорту Хаджибей та фотоперіодично-нейтрального сорту Ятрань. Стерилізоване насіння сої пророщували у чашках Петрі протягом 3-х діб в темряві за температури 22±1°С, після чого проводили активацію фоторецепторних систем опроміненням монохроматичним світлом різного спектру: червоним, зеленим та синім світлом щодня по 30 хвилин протягом 5-ти діб за допомогою LED матриць Коробова. Контрольні рослини культивували за тих же умов, але без опромінення селективним світлом. Відбір проб для аналізу проліферативної активності проводили у динаміці на – 6-ту, 7-ту та 8-ту добу експерименту, ростову реакцію проростків аналізували в кінці досліду – на 11-ту добу. Результати експериментів показали, що за опромінення селективним світлом різного спектру осьові органи проростків сої культурної реагують неоднаково: лінійні розміри надземної частини проростка більшою мірою залежать від дії ЧС, тоді як коренева система активніше реагує на дію СС. Надземна частина проростків за активації фітохромної системи шляхом опромінення ЧС змінює морфогенетичну програму розвитку зі скотоморфогенезу на фотоморфогенез. Водночас було показано, що опромінення усіма застосованими спектрами впливало на накопичення біомаси етіольованих проростків короткоденного сорту сої Хаджибей, тоді як у фотоперіодично нейтральної сої сорту Ятрань – лише опромінення ЧС. На кореневу меристему етіольованих проростків сої культурної сорту Хаджибей мало вплив опромінення ЧС та СС, тоді як проліферативна активність меристем коренів проростків сорту Ятрань більшою мірою залежала від дії СС та ЗС. Висловлюється припущення стосовно різного складу та активності фоторецепторних систем у проростків сої культурної з контрастною фотоперіодичною чутливістю, що проявляється у регуляції проліферативної активності меристем та ростових і морфогенетичних процесів.

Ключові слова: *Glycine max (L.) Merr., фотоперіодична реакція, селективне світло ЧС (660 нм), ЗС (530 нм), СС (450 нм), фоторецептори, мітотичний індекс, біомаса, ростові процеси*.

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Вступ

Рослини, як організми, що ведуть прикріплений спосіб життя, змушені узгоджувати свій ріст та розвиток з умовами навколишнього середовища, які постійно змінюються. Серед екологічних факторів, що діють на рослинний організм, найважливішим є світло, яке виступає не тільки основним джерелом для фотосинтезу, але й бере участь у регуляції процесів росту та розвитку рослин, також

виконує функцію координації морфогенетичних процесів (Franklin et al., 2003). Світло є багатогранним фактором, що характеризується якісними (широким діапазоном довжини хвилі) та кількісними параметрами (інтенсивністю, інтегральною добовою радіацією, фотоперіодом) (Zhong et al., 2012). Фоторецепторна система вищих рослин включає п'ять основних найбільш поширених типів ідентифікованих фотосенсорів. Серед них виділяють три фоторецептори СС/УФ А – криптохроми CRY1, CRY2 та CRY3 (або CRY DASH) (Mishra, Khurana, 2017), фоторецептори СС – фототропіни PHOT1 та PHOT2 (Briggs Christie, 2002), фоторецептори сімейства ZTL/FKF1/LKP2 (Schultz, 2006), фоторецептори ЧС/ДЧС – фітохроми PHY A-E (Wang, Haiyang, 2015), а також фоторецептор УФ В – UVR8 (Tilbrook et al., 2013).

За літературними даними, фітохром практично повністю контролює хід індивідуального розвитку рослин – починаючи від проростання насіння, закінчуючи цвітінням та плодоносінням (Kami et al., 2010; Quail, 2010; Wang, Haiyang, 2015). З фотоіндукованим утворенням активної форми фітохрому пов'язані різноманітні зміни у метаболізмі, диференціації клітин, активності ферментів, тропізмах, процесах росту та розвитку рослин. Під регуляторною дією фітохромів знаходяться такі процеси, як деетіляція (Zhong et al., 2012), проростання насіння деяких видів рослин (Quail, 2010), «синдром уникнення тіні» (Franklin, Whitelam, 2005), закладка листових примордіїв і розвиток листка, закладка квіткових бруньок і цвітіння тощо (Franklin, Quail, 2010). Фототропіни беруть участь у регуляції таких відповідей рослин на синє світло, як фототропізм, відкривання/закривання продихів, а також регуляції рухів хлоропластів в умовах слабкої освітленості або високої інтенсивності світла (Christie, 2007). Загалом вважається, що фототропіни беруть участь у процесах, пов'язаних з оптимізацією фотосинтезу та зменшенням ризику фотоушкоджень (Briggs, Christie, 2002). Криптохром виконує функцію основного активатора цвітіння у *Arabidopsis thaliana* (Christie et al., 2015). Також криптохроми активують експресію всіх генів фотосинтетичного апарату, білки яких кодуються в ядрі, а дія СС CRY1 викликає фотоморфогенез пластид клітин кореня (позеленіння коренів) (Mishra, Khurana, 2017). Як сенсори співвідношення СС/ЗС, при зниженні освітленості у синій області спектру криптохроми можуть викликати реакцію “уникнення тіні”, подібну до реакції, контрольованої фітохромами (Golovatskaya, Karnachuk, 2015). Таким чином, під контролем криптохромів перебувають циркадні ритми, індукція цвітіння, СС-залежний фотоморфогенез та інші функції рослин. Передбачається, що сімейство білків ZTL/FKF1/LKP2 (або ZEITLUPE/FLAVINBINDING, KELCH, FBOX1/LOV KELCH PROTEIN2) бере участь у регуляції добових ритмів та фотоперіодичності цвітіння (Weller, Ortega, 2015), контролюючи залежну від синього світла деградацію білків (Schultz, 2006). За літературними даними, ZTL контролює добові циркадні ритми, FKF1 контролює перехід до цвітіння, а LKP1 необхідний для обох процесів (Schultz, 2006). За деякими літературними даними, ЗС високої інтенсивності не лімітує процеси фотосинтезу, але активно регулює ростові процеси (Golovatskaya, Karnachuk, 2015). Зелене світло, як і червоне, регулює багато процесів життєдіяльності рослин: від проростання насіння до цвітіння (Golovatskaya, 2005). Крім того, ЗС, нарівні з СС, є одним із факторів, що контролюють реакції рослин при «синдромі уникнення тіні». Природа рецептора ЗС на даний момент залишається невивченою, проте передбачається одночасна участь у регуляторній ролі ЗС декількох фоторецепторів (CRY1–2, PHOT1–2, ZTL/FKF1/LKP2, PHYA–B та ін.), які неоднаково активовані цим світлом та взаємодіють між собою. Зокрема, показано залежну від світла взаємодію фоторецепторів CRY1 та PHYB (Golovatskaya, Karnachuk, 2015). Також було зроблено припущення про існування рецептора ЗС зеаксантинового типу (Hoang et al., 2014).

Для рослинного організму характерні видоспецифічні особливості в процесах росту та розвитку. З цього можна зробити висновок, що у рослин різних видів або різних екологічних груп можуть виявлятися специфічні особливості у сприйнятті, трансдукції та проявах ефектів активації фоторецепторних систем. Фотоперіод є одним із головних факторів зовнішнього середовища, який визначає тривалість вегетаційного періоду рослин і пов'язану з нею поширеність по зонах вирощування, продуктивність та якість урожаю (Zhmurko, 2009). В залежності від фотоперіодичної реакції (ФПР) виділяють наступні групи рослин: короткоденні рослини (КДР), в яких ФПР індукується, коли фотоперіод коротший, ніж критична довжина дня, довгоденні рослини (ДДР), в яких ФПР індукується, коли фотоперіод перевищує критичну довжину дня; нейтральноденні рослини (ФПН), що переходять до цвітіння одночасно за різної тривалості фотоперіоду. Рослини, що відрізняються за фотоперіодичною чутливістю по-різному реагують на активацію фітохромної системи червоним

світлом (Avksentieva, Batuieva, 2020), тому можна зробити припущення, що їхня реакція на активацію інших фоторецепторних систем селективним світлом також буде відрізнятися.

В основі активації процесів росту рослини на органному рівні лежить стимуляція процесів клітинного росту, складовою якої є проліферативна активність меристеми. Саме мітотична активність визначає інтенсивність росту рослинного організму. Меристематичні тканини є найбільш чутливими і активно реагують на зовнішні впливи (Hopkins et al., 2002). Вважається, що будь-який неспецифічний вплив навколишнього середовища, зокрема вплив світла різного спектрального складу, може призвести до деяких змін життєдіяльності клітин, які відображає такий показник, як мітотичний індекс (Reichler et al., 2001). Передбачається, що індукція проліферації здійснюється за допомогою різних сигнальних систем, до яких можуть входити і фоторецепторні сигнальні системи.

Переважна більшість досліджень ефектів активації фоторецепторів була проведена з модельним об'єктом *Arabidopsis thaliana* (Franklin et al., 2003; Franklin, Quail, 2010; Zhong et al., 2012) та іншими рослинами, без урахування їх фотоперіодичної реакції. Це не дозволяє зробити висновки про роль фотоперіодичної реакції рослин в ефектах впливу селективного світла на процеси фотоморфогенезу.

Відповідно до цього, метою нашої роботи було дослідити вплив опромінення селективним світлом на проліферативну активність клітин кореневої меристеми та ростові процеси в надземній та підземній частинах проростків сої культурної в залежності від їх фотоперіодичної реакції.

Матеріали та методи

Рослинний матеріал. У якості рослинного матеріалу в роботі використовувались контрастні за фотоперіодичною реакцією рослини сої культурної (*Glycine max* (L.) Merr.) короткоденного сорту (КДР) Хаджибей та фотоперіодично нейтрального сорту (ФПН) Ятрань.

Дизайн дослідження. Насіння дослідних рослин стерилізували в 15%-ому розчині гіпохлориту натрію і 70%-ному етанолі та пророщували у чашках Петрі на зволоженому фільтрувальному папері у термостаті за температури 26°С у темряві протягом 3-х діб. Після чого проводили активацію фоторецепторних систем досліджуваних проростків опроміненням монохроматичним світлом різного спектру: червоним світлом (ЧС), 660 нм, зеленим (ЗС), 530 нм, та синім (СС), 450 нм, щодня по 30 хвилин протягом 5 днів за допомогою LED матриці. Контрольні рослини культивували за тих же умов без активації фоторецепторних систем селективним світлом. Відбір проб для аналізу проліферативної активності проводили на 6-ту, 7-ту та 8-ту добу експерименту. На 11-ту добу експерименту проводили аналіз ростової реакції проростків.

Проліферативна активність кореневих меристем. Інтенсивність поділу клітин кореневої меристеми визначали, аналізуючи мітотичний індекс (МІ) за стандартною методикою. Фіксацію рослинного матеріалу – головного кореня проростків проводили у фіксаторі Кларка (96% етиловий спирт:крижана оцтова кислота (3 : 1)) протягом 24 годин за температури 0–3°С. Надалі проводили забарвлення ацетокарміном методом гарячого гідролізу і готували давлені тимчасові мікропрепарати за стандартною методикою. Препарати аналізували за допомогою світлового мікроскопа Мікромед XS-2610 при збільшенні ×400, у кожному препараті проглядали не менш 5 полів зору у двох діагоналях, для кожного варіанту було проаналізовано не менше 1000 клітин. Мітотичний індекс (МІ) розраховували як відношення клітин, які перебували у мета-, ана- і телофазі мітозу, до загальної кількості клітин у полі зору, що виражене у відсотках (Graña, 2018).

Аналіз ростової реакції. Ростову реакцію визначали за показниками лінійного росту, вимірюючи загальну довжину проростка, довжину надземної і підземної частин, та за накопиченням біомаси осьовими органами проростка, що виступає показником ростових і біосинтетичних процесів. Вимірювання проводили, аналізуючи в кожному варіанті по 15–20 проростків.

Статистичний аналіз. Було проведено 3 біологічні серії експериментів, статистичний аналіз отриманих даних проводили за допомогою пакету програми Statistica 5.0. Істотність відмінностей між контрольними та дослідними варіантами визначали з використанням *t*-критерію Стьюдента за $p \leq 0{,}05$ з урахуванням поправки Бонферроні (Atramentova, Utevskaya, 2008). В таблицях та на графіках наведені середні значення та їх стандартні похибки.

Результати та обговорення

Проліферативна активність. В апікальних меристемах головного кореня сої культурної сортів Хаджибей та Ятрань у віці 6–8 діб, як у контрольних, так і у дослідних проростках, МІ був

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невисоким: 0,97–2,83%, що може свідчити про низьку проліферативну активність, що виражається невеликою кількістю клітин, які перебували у мета-, ана- та телофазах мітотичного циклу (рис. 1).

За результатами дослідження у короткоденного сорту Хаджибей протягом експерименту спостерігається зниження MI з 2,59% на 6-ту добу до 1,49% на 8-му добу. Це свідчить про поступове зниження проліферативної активності меристематичних клітин кореня. Опромінення ЧС та ЗС не призводило до суттєвих змін у динаміці MI, лише на 7-му добу експерименту у рослинах, опромінених ЧС, спостерігалося різке збільшення MI, порівняно з контролем. Це може бути пояснено періодичними змінами проліферативної активності клітин зони поділу головного кореня, зумовленими генотиповими особливостями сорту. Різка зміна MI на 7-му добу експерименту при опроміненні ЧС КДР проростків сої спостерігалась і у наших попередніх експериментах (Avksentieva, Batueva, 2020). Водночас, при опроміненні СС спостерігалося значне зменшення MI, порівняно з контролем, вже на 6-ту добу, та подальше зниження MI з 1,84% на 6-ту добу експерименту до 1,09% на 8-му. Це свідчить про наявність деякої чутливості меристематичних клітин до синього спектру світла.

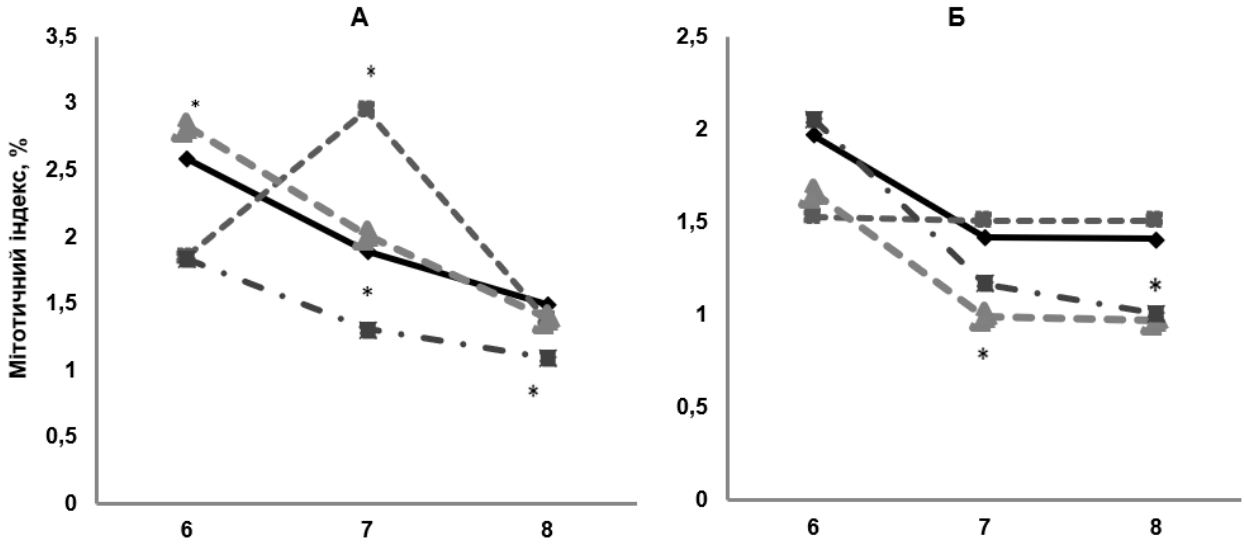

Рис. 1. Вплив опромінення селективним світлом на інтенсивність поділу клітин у апікальних меристемах коренів сої культурної КДР сорту Хаджибей (А) та ФПН сорту Ятрань (Б)
Контроль (б/о) ——— , ЧС (660 нм) - - - - - , ЗС (530 нм) — — — , СС (450 нм) — · — ·
Примітка: * різниця з контролем істотна при $p \leq 0{,}05$

Fig. 1. The influence of selective light irradiation on the cell division rate in the apical meristems of the roots of SDP of Khadzhibey variety (A) and PPN of Yatran variety (B)
Control (b/o) ——— , RL (660 nm) - - - - - , GL (530 nm) — — — , BL (450 nm) — · — ·
Note: * the difference with the control is significant at $p \leq 0.05$

При дослідженні фотоперіодично нейтрального сорту Ятрань протягом досліджуваного онтогенетичного періоду 6-8 діб спостерігалось незначне гальмування проліферативної активності меристематичних клітин апексу коренів у контрольних проростків. Опромінення ЧС на 6-ту добу призводило до зниження MI, порівняно з контролем, але не призводило до подальшого зниження мітотичної активності протягом зазначеного онтогенетичного періоду. Це може свідчити не тільки про невисоку чутливість кореневих меристем фотоперіодично нейтральної сої до ЧС, але й про наявність деяких перешкод на шляху трансдукції сигналу з надземної частини проростку до кореневої частини. Опромінення СС призводило до суттєвого зниження MI порівняно з контролем, майже у два рази, хоча на 6-ту добу різниця з контролем була неістотною. Ефект ЗС у меристематичних тканинах кореневої системи був схожий з ефектом СС. Це може бути пов'язано з роботою криптохромної системи, яка приймає участь у рецепції ЗС (Golovatskaya, 2005).

Таким чином, за результатами наших досліджень показано, що стимуляція проліферативної активності кореневих меристем відбувається за дії ЧС, опромінення яким призводить до

фотоактивації саме фітохромної системи – головної фоторецепторної системи рослинного організму. Стимулювання проліферації меристематичних клітин більшою мірою відбувається саме у фотоперіодично чутливих рослин – короткоденного сорту Хаджибей та за нашими попередніми дослідженнями (Avksentieva, Batueva, 2020) – довгоденного гороху посівного сорту Меценат. Фотоперіодично нейтральні рослини майже не реагують на дію ЧС. Опромінення СС світлом незалежно від фотоперіодичної чутливості дослідних рослин, навпаки, інгібує процеси проліферації клітин кореневих меристем, що також було показано іншими дослідниками (Hopkins et al., 2002).

Ростові показники. За результатами дослідження впливу селективного світла на ростові показники етіольованих проростків сорту Хаджибей, активація фоторецепторних систем суттєво впливала на масу надземної частини проростків (рис. 2А). Найбільший ефект мало опромінення ЗС, рецепція якого, можливо, відбувається за участі фітохромної та криптохромної систем. Порівняно до контролю спостерігалось збільшення маси надземної частини проростків також і при опроміненні синім світлом. Однак збільшення маси за опромінення СС було меншим, ніж те, яке відбувалося за опромінення ЗС. Це засвідчує, що, у етіольованих проростках сої сорту Хаджибей присутні у великій кількості криптохроми, фототропіни та, можливо, фітохроми, які сприймають хвилі застосованих спектрів.

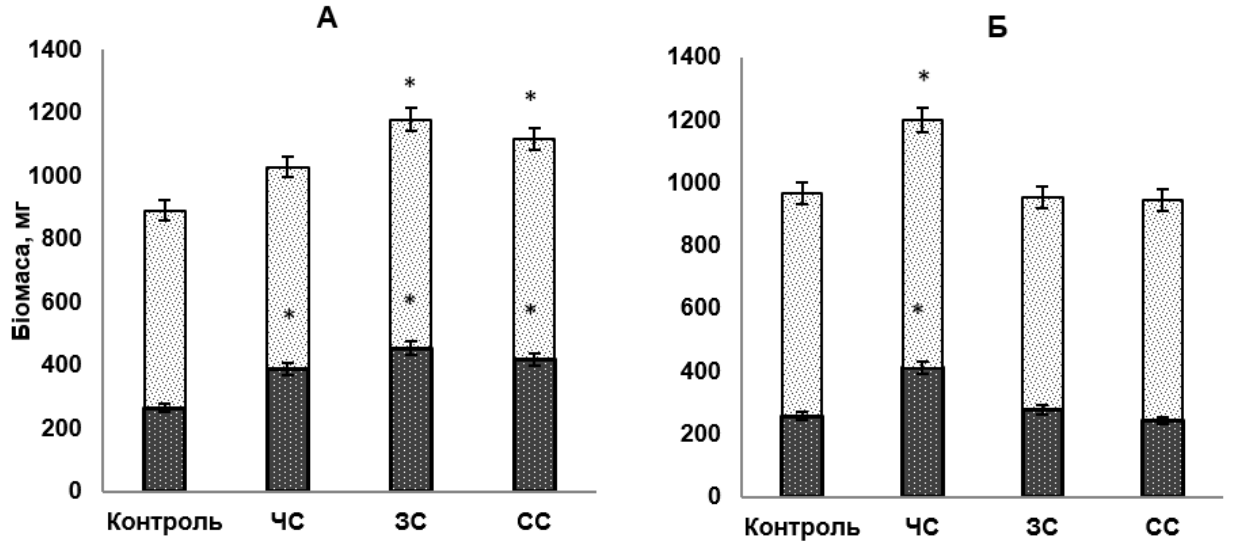

Рис. 2. Вплив опромінення селективним світлом на біомасу осьових органів проростків сої культурної КДР сорту Хаджибей (А) та ФПН сорту Ятрань (Б). Надземна частина, підземна частина

Примітка: * різниця з контролем істотна при $p \leq 0{,}05$.

Fig. 2. The influence of selective light irradiation on the biomass of the axial organs of soybean seedlings of SDP of Khadzhibei variety (A) and PPN of Yatran variety (B). Above-ground part, underground part

Note: * the difference with the control is significant at $p \leq 0.05$.

Ефекти опромінення селективним світлом на кореневу систему були іншими. Значне збільшення маси кореневої системи проростків спостерігалось при опроміненні світла усіх застосованих спектрів у дослідженні, але за рівнем прояву ефектів на біомасу коренів дію спектрів світла можна ранжувати наступним чином: ЗС>СС>ЧС. При цьому спостерігався більший вплив селективного світла на підземну частину, порівняно з надземною, ще дає підставу припустити, що надземна і підземна частина досліджуваних проростків відрізняються за вмістом фоторецепторів використаних спектрів та їх активністю, що має підтвердження у літературних даних (Kami et al., 2010).

Як на фотоперіодично нейтральний сорт, на сорт Ятрань селективне світло впливало менше, ніж на короткоденний сорт Хаджибей. За результатами дослідження дії селективного світла на

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біосинтетичні процеси етіольованих проростків ФПН сорту Ятрань (рис. 2 Б) опромінення ЧС та ЗС призводило до зміни біомаси надземної частини, але у протилежних напрямках. Опромінення ЧС призводило до збільшення біомаси, порівняно з контролем, тоді як опромінення ЗС – до гальмування ростових процесів. Це дає підстави зробити припущення, що у надземній частині етіольованих проростків сорту Ятрань міститься велика кількість фітохромів, які й приймають участь у рецепції ЧС та ЗС.

Біомаса підземної частини проростків ФПН сорту Ятрань в основному залежала від ЧС, опромінення яким призводило до збільшення біомаси у 1,6 разів. Також до збільшення маси призводило опромінення ЗС, але у меншій мірі, а СС призводило до зменшення маси кореневої частини проростків.

Відомо, що саме фітохроми та криптохроми, фоторецептори ЧС та СС, приймають участь у запуску реакцій фотоморфогенезу. За літературними даними, у темряві реалізація фотоморфогенетичної програми блокується у тому числі і репресором фотоморфогенезу COP1. Активація фоторецепторів на світлі призводить до інгібування COP1 та його переміщення до цитоплазми, в результаті чого активатори фотоморфогенезу стають стабільними, накопичуються та починають регулювати транскрипцію світлочутливих генів (Smirnova et al., 2012).

На лінійні розміри надземної частини досліджуваних проростків сорту Хаджибей (рис. 3А) найбільший вплив мало опромінення ЧС, яке призводило до зменшення довжини пагону у півтора рази. Опромінення ЗС та СС призводило до незначного зменшення лінійних розмірів надземної частини. Це свідчить про наявність фітохромів, імовірно, саме фітохрому А (Kami et al., 2010), у етіольованих проростках, які запускають реакцію фотоморфогенезу: зупинку зростання гіпокотилю, розгортання сім'ядолів, позеленіння проростків і завершення розвитку фотосинтетичного апарату пластид.

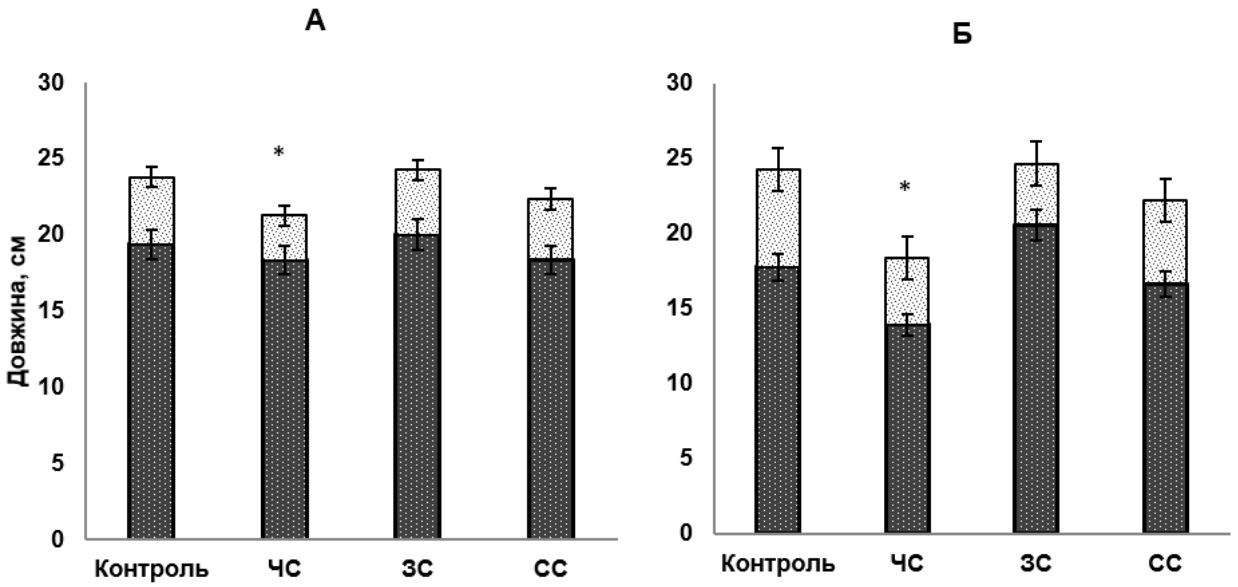

Рис. 3. Вплив опромінення селективним світлом на лінійні розміри осьових органів проростків сої культурної КДР сорту Хаджибей (А) та ФПН сорту Ятрань (Б). Надземна частина ⁙, підземна частина ▩

Примітка: * різниця з контролем істотна при $p \leq 0{,}05$.

Fig. 3. The influence of selective light irradiation on the linear measures of the axial organs of soybean seedlings of SDP of Khadzhibei variety (A) and PPN of Yatran variety (B). Above-ground part ⁙, underground part ▩

Note: * the difference with the control is significant at $p \leq 0.05$.

Опромінення ЧС та СС призводило також до деякого зменшення довжини і підземної частини проростків, тоді як опромінення ЗС призводило до незначного збільшення довжини, що свідчить про інший склад фоторецепторів у кореневій системі досліджуваних проростків.

Реакція етіольованих проростків сорту Ятрань на опромінення селективним світлом була аналогічною (рис. 3Б). Надземна частина зазнавала значного гальмування ростових процесів за впливу червоного та зеленого світла. СС також призводило до зменшення довжини надземної частини, але у меншій мірі. Коренева система реагувала дещо інакше. Активація фітохромної системи ЧС призводила до суттєвого зменшення довжини кореневої системи, тоді як ЗС впливало у протилежному напрямку: спостерігалося збільшення лінійних розмірів підземної частини на 16%. Тобто, як і при дослідженні проростків сорту Хаджибей, на лінійний ріст проростків сорту Ятрань суттєвий вплив мала активація фітохромної системи, але реакція надземної та підземної частин відрізнялася, що свідчить про різний склад фоторецепторних систем у асиміляційному апараті та коренях сої. Подібність реакцій на селективне світло проростків сортів Ятрань та Хаджибей може бути пояснена генотиповими особливостями виду.

Таким чином, за опромінення селективним світлом різного спектру – ЧС, ЗС та СС – було встановлено, що осьові органи проростків сої культурної реагують різним чином: лінійні розміри надземної частини проростка залежали від дії ЧС, тоді як коренева система реагувала ще й на СС. Надземна частина за активації фітохромної системи шляхом опромінення ЧС змінює програму розвитку із скотоморфогенезу на фотоморфогенез. Водночас було встановлено, що на накопичення біомаси етіольованих проростків КДР сої сорту Хаджибей мало вплив опромінення усіма застосованими спектрами, у той час коли на біомасу проростків ФПН сої сорту Ятрань значний вплив мало лише ЧС. Ростові процеси тісно пов'язані з проліферативною активністю меристематичних клітин. На кореневу меристему етіольованих проростків сої культурної сорту Хаджибей вплив мало опромінення ЧС та СС, тоді як активність меристем коренів проростків сорту Ятрань залежала від СС та ЗС.

Висновки

• На проліферативну активність кореневої системи проростків короткоденного сорту Хаджибей найбільший вплив мали ЧС та СС. Активність меристематичних клітин кореневої системи проростків фотоперіодично нейтрального сорту Ятрань залежить головним чином від СС та ЗС.

• Під впливом ЧС, ЗС та СС відбувалося стимулювання біосинтетичних процесів у етіольованих проростків короткоденного сорту Хаджибей, у етіольованих проростків ФПН сорту Ятрань збільшення біомаси відбувається тільки за дії опромінення ЧС.

• На лінійний ріст проростків сої культурної значний вплив має ЧС, запускаючи реакції фотоморфогенезу, що призводить до зменшення довжини проростків КДР сорту Хаджибей та ФПН сорту Ятрань.

• Зроблено припущення, що різна реакція надземної та підземної частин досліджуваних проростків обох сортів на опромінення селективним світлом може бути пов'язаною з різним складом та активністю фоторецепторів у цих органах.

• Показано, що етіольовані проростки короткоденного сорту сої Хаджибей є більш чутливими до дії різних спектрів опромінення в порівнянні з реакцією фотоперіодично нейтрального сорту Ятрань, що призводить до змін проліферативної активності кореневих меристем, ростових та біосинтетичних процесів.

Роботу виконано в рамках проекту фундаментального дослідження Міністерства освіти і науки України «Методологія дослідження біологічної природи фотоперіодичної чутливості рослин за використання комплексної системи генетичних, фізіологічних та біохімічних показників», номер держреєстрації 0118U002041.

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Regulation of the mitotic activity of root meristems and growth processes of soybean seedlings with a contrasting photoperiodic response by selective light

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The present paper concerns the influence of selective light irradiation of various spectrums – red (RL, 660 nm), green (GL, 530 nm), and blue (BL, 450 nm) on the proliferative activity of root meristem cells, biomass accumulation, and growth processes in the above-ground and underground parts of etiolated soybean seedlings. Seedlings of the soybean (*Glycine max* (L.) Merr.) of two varieties, contrasting in photoperiodic reaction, the short-day variety Khadzhibei and the photoperiodic-neutral variety Yatran, were used as plant material. Sterilized soybean seeds were germinated in Petri dishes for three days in darkness at 22±1°C. After that, their photoreceptor system was activated by irradiation with monochromatic light of red, green, and blue spectrums for five days, 30 minutes daily, with the use of Korobov LED matrices. Control plants were cultivated under the same conditions but without selective light exposure. Samples for the proliferative activity analysis were taken in dynamics on the 6th, 7th, and 8th days of the experiment. The seedlings' growth reaction was analyzed at the end of the experiment, on the 11th day. The experiments showed that axial organs of soybean seedlings react in different ways when exposed to selective light of various spectrums: linear measures of the seedlings' above-ground part largely depend on the RL impact, while the root system reacts more actively to the BL impact. Under activation of the phytochrome system with RL, the above-ground part of seedlings changes its morphogenetic development program from scotomorphogenesis to photomorphogenesis. At the same time, biomass accumulation in the etiolated seedlings of the short-day Khadzhibey variety was influenced by irradiation with all the applied spectra; the biomass of photoperiodic-neutral soybean seedlings of the Yatran variety was affected only by RL. The root meristem of etiolated soybean seedlings of the Khadzhibey variety was sensitive to both RL and BL irradiation, while that of the seedlings of the Yatran variety reacted to a greater extent to the BL and GL exposure. Based on the results obtained, we suppose that soybean seedlings with contrasting photoperiodic sensitivity have different compositions and activity of photoreceptor systems. It is manifested in regulation of the meristem proliferative activity, growth, and morphogenetic processes.

Key words: *Glycine max (L.) Merr., photoperiodic reaction, selective light, RL (660 nm), GL (530 nm), BL (450 nm), photoreceptors, mitotic index, biomass, growth processes*.

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List of abbreviations: photoperiodic response (PPR), short-day plants (SDP), photoperiodically neutral plants (PPN), red light (RL), green light (GL), blue light (BL), mitotic index (MI).

ПРАВИЛА ДЛЯ АВТОРІВ
журналу «Вісник Харківського національного університету імені В. Н. Каразіна. Серія «Біологія»

У журналі публікуються результати досліджень за всіма напрямками біологічних наук. До публікації приймаються:
– закінчені оригінальні роботи, що досі ніде не видавалися;
– описи оригінальних методів та приладів;
– теоретичні та оглядові статті;
– матеріали та повідомлення про події наукового життя;
– рецензії на книги.

Статті друкуються українською та англійською мовами.

Текст експериментальної статті має складатися з наступних розділів: «Вступ», «Методика» («Об'єкти та методи дослідження»), «Результати», «Обговорення» (можливий об'єднаний розділ «Результати та обговорення»), «Перелік посилань». Тексти статей повинні бути виконані у редакторі Ms Word з використанням шрифту Arial – 10 pt; абзац – 1 см; міжрядковий інтервал – одинарний; поля: верхнє та нижнє – 3,5 см; ліве – 2,5 см, праве – 2 см. Текст статті починається з індексу УДК, далі зазначається, мовою оригіналу, назва статті (Arial – 12 pt), ініціали та прізвища авторів (Arial – 10 pt), анотація (Arial – 9 pt), список ключових слів (Arial – 9 pt). Далі наводиться англійською мовою (якщо стаття написана українською): назва статті (Arial – 12 pt), прізвища та ініціали авторів (Arial – 10 pt), анотація (Arial – 9 pt), список ключових слів (Arial – 9 pt). Обсяг кожної анотації – не менш ніж 1800 фонетичних символів. Таблиці і рисунки розміщуються у тексті. Назви таблиць і рисунків та примітки до них подаються українською та англійською мовами. Посилання на літературу у тексті подаються у круглих дужках із вказуванням прізвища автора та року видання. Список використаних джерел оформлюється за алфавітом (спочатку – джерела кирилицею, потім – латиницею), без нумерації.

Бібліографічний опис джерел та посилань у тексті виконується відповідно до вимог МОН України, зокрема – ДСТУ 8302:2015, але у варіанті, наближеному до норм стилю APA (American Psychological Association). При описі друкованого джерела обов'язково слід зазначати місце видання (місто), видавництво, рік видання, загальну кількість сторінок (у періодичних виданнях – сторінки статті). Бібліографічний опис джерел англійською мовою (References) оформлюється відповідно до норм стилю APA (American Psychological Association). Джерела після слова «References» розташовуються за англійським алфавітом, без нумерації. До посилань обов'язково треба додавати DOI, якщо він присвоєний.

Електронні версії статей надсилаються до редакції електронною поштою. Разом з електронною версією до редакції надсилається друкована копія, підписана авторами. На окремій сторінці вказують прізвища та ініціали усіх авторів, повні назви наукових установ та поштові адреси установ, адреси електронної пошти авторів та посилання на їх профілі у мережі ORCID. Ця інформація наводиться українською та англійською мовами.

Стаття, яка надходить до редакції, реєструється та передається рецензентам, які рекомендують статтю до публікації або відхиляють її. При наявності зауважень статтю повертають авторам для доопрацювання.

Наукове видання

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