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Jalal A. Aliyev (1928–2016): A Great Scientist, A Great Teacher and A Great Human Being

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Jalal A. Aliyev was a distinguished and respected plant biologist of our time, a great teacher, and great human being. He was a pioneer of photosynthesis research in Azerbaijan. Almost up to the end of his life, he was deeply engaged in research. He left us on February 1, 2016, but many around the world remember him as he was engaged in international dialog on solving global issues, and in supporting international conferences on ‘‘Photosynthesis Research for Sustainability’’ in 2011 and 2013.

Jalal Alirza Aliyev was born on June 30, 1928, in Nakhchivan, a city with a 5000-year old history. After receiving primary school education, he graduated, in 1946, from the Faculty of Natural Sciences of Nakhchivan Pedagogical College. In June 1946, he went by train to Baku, the capital city of Azerbaijan for further studies. There, he attended the Azerbaijan (now Baku) State University; as a student he worked as a laboratory assistant in the Department of Plant Physiology. In 1951, he graduated from the Biological Faculty of Azerbaijan State University, receiving a diploma with honors.

During 1951–1954, Jalal Aliyev was a graduate student in the Plant Physiology Laboratory of the Azerbaijan Academy of Sciences, working under the supervision of academician Muzaffar Abutalubov, who had many graduate students and technical personnel. Jalal Aliyev’s PhD thesis, in 1955, was on ‘‘The effects of microelements on the growth and productivity of wheat’’.

After graduation Jalal Aliyev worked as a junior researcher, head of a group, and then head of a laboratory at the Research Institute of Crop Husbandry, Ministry of Agriculture.

Young Jalal entered the world of photosynthesis research and, in 1971, received a ‘‘Doctor of Biological Sciences’’ degree with a thesis on ‘‘Photosynthetic activity, mineral nutrition and productivity of plants’’.

In the 1970s, Jalal Aliyev was a group leader in the Institute of Botany. In 1974, his creative group became the Laboratory of Molecular Basis of Bioenergetics, under the broader umbrella of Physicochemical Biology. In 1983, a Laboratory of Molecular-Genetic Basis of Production Processes was created, which at the end of 1988 became a full-fledged department by the same name. In 2010, this department became the Department of Fundamental Problems of Biological Productivity. Currently, fifteen laboratories are housed in the new Institute of Molecular Biology and Biotechnology, ANAS, created, in 2015, on the basis of the earlier department. In addition, Professor Jalal Aliyev served as the Head of the Department of Plant Physiology and Biotechnology at the Research Institute of Crop Husbandry as well as the Head of the Department of Fundamental Problems of Biological Productivity in the Institute of Botany.

Jalal Aliyev was much loved and respected by students and staff throughout Azerbaijan. He had been a long-time member of the Azerbaijan Academy of Sciences, had served as Academician-Secretary in the Department of Biological Sciences, and had been a member of the Presidium of the National Academy of Sciences of Azerbaijan.

Professor Jalal Aliyev was deeply involved in dynamic research on several aspects of photosynthesis for 65 years; he focused mainly on an important crop wheat; this research was

integrative in its approach, involving physiological, biophysical, biochemical, and molecular-genetic basis of plant productivity. Further, it dealt with production processes, and structural-functional organization of plants – from the molecular level to the whole plant. Under Jalal's leadership, the main principles determining productivity and high-yield capacity of an ''ideal'' type of wheat was made available to all of Azerbaijan and neigh-boring countries (see Aliyev, 1974).

Delving deeper into CO_2 exchange, carbon metabolism, transport and distribution of assimilates in the leaves and ear elements of contrasting genotypes, (through the use of $^{14}CO_2$), he revealed the role of separate organs in grain filling (Aliyev et al., 1996). Jalal's team also continued their detailed studies on structural and functional organization of carbonic anhydrase; this enzyme was isolated and crystallized from *Cicer arietinum* leaves for the first time and a molecular model of its quaternary structure was proposed (see Aliyev et al., 1985).

Jalal Aliyev's team went further into illuminating the structural molecular organization of the two photosystems and presented a model for the topography of pigment-protein complexes in thylakoid membranes (Asadov et al., 1987). These studies included biosynthesis, molecular biology and genetics of the system. His team elucidated the general mechanisms of drought response and their application in drought-resistance improvement in wheat genotypes (Huseynova et al., 2007).

Under his leadership, the distribution of ^{90}Sr and ^{137}Cs in a soil-vegetative cover was studied (Aliyev et al., 1991); in addition, the group promoted research to obtain a data-base of plant promoters, development of computer pro-grams for the prediction of RNA polymerase II promoters, as well as potential regulatory elements of transcription. Jalal's research group revealed some peculiarities of organelle-to-nucleus DNA transfers and of the organization and expression of genes in nuclear genomes of rice and Arabidopsis (Shahmuradov et al., 2003).

A major point that emerged from his research team was that, under field conditions, photorespiration is one of the vital metabolic processes in plants, and attempts to reduce this process by various ways with the purpose of increasing the crop productivity is not viable (see Aliyev, 2012).

Professor Jalal Aliyev instigated the development of new directions of research, namely molecular biology, molecular genetics, gene and cellular biotechnology, mathematical and computational biology, and bioinformatics as applied to crop productivity in Azerbaijan. Under his initiative, about 300 doctoral students were trained; further, under his personal supervision, 85 Ph.D. and 12 Doctor of Science (Dr. Sci) students successfully defended their theses. At present, these former students are leading scientists in research centers and universities of Azerbaijan and many others around the world (e.g., Australia, Canada, France, Israel, Japan, South Korea, Russia, the UK, and the USA).

As chairman of the Problem Council on Biological Sciences of the Scientific Research Management and Coordination Council of the Azerbaijan Republic and member of many scientific and academic councils, Jalal promoted development of photosynthesis research in Azerbaijan and elsewhere.

Jalal Aliyev served as an editor-in-chief of the ''Proceedings of Azerbaijan National Academy of Sciences (Biological and Medical Sciences), a member of the Editorial Board of ''Reports of Azerbaijan National Academy of Sciences'', and a scientific editor of a number of books and proceedings. He was elected an honorary member of the editorial board of ''Bioinformatics and Comparative Genomics'', ''Plant Biochemistry and Physiology'', ''Computational Biology and Bioinformatics'', and ''The Infectious and Non-infectious Diseases''.

A major contribution of Jalal Aliyev was in the development of high-yielding wheat *Triticum durum* Desf. (durum) and *Triticum aestivum* L. (bread) with a productivity of 7–8 tons per hectare. These wheat lines have excellent grain quality, and are being cultivated widely in Azerbaijan. In acknowledgment of his success in the field of fun-damental and agricultural sciences, Professor Jalal Aliyev was elected as a foreign member of the Russian Academy of Agricultural Sciences (1995), the Ukrainian Academy of Agrarian Sciences (1996) and the Academy of Agrarian Sciences of Belarus (1996).

As a research scientist, Jalal Aliyev was highly prolific. He authored or coauthored more than 800 scientific publications including 25 monographs and books, and 10 book chapters.

The National Plant Genetic Resource Program was created, a strategy on preservation and rational use of biodiversity was developed, and the Plant Gene Pool of Azerbaijan was created under his leadership.

Bioethical problems of research in modern biology, agriculture and medicine were also of great interest to him, and ways of tackling these issues were established under his leadership.

Professor Jalal Aliyev received many distinguished honors in Azerbaijan: Order of Independence of Azerbaijan (1998); the Order of

Glory of the Republic of Georgia (2003); the Honorary diploma from the President of the Republic of Azerbaijan (2008); the Order of Glory of the Republic of Azerbaijan (2013), and, together with Irada Huseynova, Hasan Bey Zardabi Prize of the Azerbaijan National Academy of Sciences (2014). In addition, he was elected as a Member of Parliament of the Republic of Azerbaijan four times (1995, 2000, 2005, and 2010), a rare honor, especially for a scientist.

In 2011 and 2013, the meetings on ‘‘Photosynthesis Research for Sustainability’’, held in Baku, Azerbaijan, were highly successful, attracting outstanding scientists from around the world. The 2013 meeting celebrated his 85th birthday and honored him for his outstanding contributions to Plant Science in general and Photosynthesis in particular, with a significant impact on improving crop productivity. His leadership support and, at the same time, his outstanding contributions were highly appreciated by the International Photosynthesis Community (see Allakhverdiev et al., 2013).

Professor Jalal Aliyev left us on February 1, 2016, but many around the world remember him as he was engaged in international dialog on solving global issues, and in supporting international conferences on ‘‘Photosynthesis Research for Sustainability’’ in 2011 and 2013.

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Natural Occurrence of Tomato Viruses in Azerbaijan

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During growing season of 2015-2017 (June-September), phytopathological surveys were conducted in the southern part of Azerbaijan. A total 263 suspicious tomato (*Solanum lycopersicum* L.) samples of various cultivars exhibiting symptoms of leaf curl, dwarfing, leaf narrowing, necrosis, mosaics or yellowing with reduced fruit yield and quality were collected in three regions (Jalilabad, Masally, Lankaran) of Azerbaijan. Collected samples were first tested serologically (rapid one-step assay AgriStrip, DAS-ELISA) depending on virus symptoms and then molecular tests (RT-PCR, PCR) were performed to confirm the virus presence. According to the result of serological tests, 179 samples (68%) positively reacted with the virus antibodies. DNA and RNA genome virus infections were confirmed by polymerase chain reaction (PCR) and reverse - polymerase chain reaction (RT-PCR). Results showed the presence of *Tomato mosaic virus* (T_0MV), *Tobacco mosaic virus* (TMV), *Tomato etech virus* (TEV), *Tomato spotted wilt virus* (TSWV) and *Cucumber mosaic virus* (CMV) in tomato samples. CMV was the most common virus, infecting about 25% of the tomato samples. TMV and ToMV, infected tomato samples by about 21% and 13%, respectively, followed CMV in frequency. This study reports the natural incidence and prevalence of tomato viruses in the southern part of Azerbaijan.

***Keywords*:** *Solanum lycopersicum* L., virus diseases, *tomato mosaic virus*, *tobacco mosaic virus*, *tomato etech virus, tomato spotted wilt virus, cucumber mosaic virus*

INTRODUCTION

Agriculture still stands in the first place for most Azerbaijani people, despite the fact that the share of industry is constantly growing. It should also be noted that vegetable growing occupies an economically important place in Azerbaijan. The total volume of vegetable production in Azerbaijan for a year is about 1.72 million tons. Vegetable crops are frequently affected by a wide range of diseases showing varying degree and kind of symptoms. Most of the causal agents of the diseases are biotic, not ruling out the involvement of abiotic factors too. Among the biotic factors, virus diseases constitute a bulk of the diseases observed in all plant types, with variable symptoms including leaf curling and distortion, green or yellow foliar mosaic, stunting of plants, and reduced yields (Rakhshandehroo et al., 2011). Plant diseases caused by viruses can be devastating on crops leading the yield reduction. At present, one of the leading vegetables in the country is tomato wihch is mainly produced in the southern regions of Azerbaijan, such as Masally, Jalilabad and Lankaran. Tomato has a particular importance to meet the demand of the population for food. According to the State Statistics Committee, 27,400 hectares of tomatoes are grown in our country and productivity of tomato is 463,200 tons. Several viruses, such as, *Cucumber mosaic virus* (CMV), *Tomato yellow leaf curl virus* (TYLCV), *Pepper mild mottle virus* (PMMoV), *Tomato mosaic virus* (TMV), *Tobacco mosaic virus* (ToMV), *Tomato spotted wilt virus* (TSWV) on tomato and/or pepper crops, as well as CMV, *Zucchini yellow mosaic virus* (ZYMV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV) in cucurbits have been reported to occur over the past decade in different regions of Azerbaijan (Huseynova et al. 2016; Huseynova et al., 2017; Verdin et al., 2018; Desbiez et al., 2018), and some of them have capacities to cause epidemics and significant yield losses to these crops, e.g., TYLCV (Moriones and Navas-Castillo, 2000).

The rapid increase in viral diseases in the modern world poses a serious problem for food security. Especially in plants of agricultural importance, viruses that cause disease are very dangerous and cause serious economic losses. The diagnosis, identification of pathogens and certification of seedlings have great importance for the timely prevention of viral diseases. Therefore, plant protection from pathogens is not only an economically important problem, but also one of the most important areas of modern scientific research. The issue of providing the population with eco friendly agricultural products has always been in the focus of our government's attention and is reflected in the most government programs. In recent years, the international exchange of seedlings has led to the spread of viral diseases in large regions. This is

becoming increasingly dangerous for tomato that is sensitive to viral diseases (Hanssen et al., 2012). Thus, the economic damage caused by various DNA and RNA genome viruses of tomato increases every year and is calculated in millions of manats. It is known that with severe infections, a higher loss of product is observed. From this point of view, it is important to study virus diseases of tomato and take preventive measures against them. The choice of struggling methods against them requires the initial diagnosis of pathogens, taxonomic characteristics, identification of the pathogenic spectrum of plants and insect vectors (Xu et al., 2017).

Therefore, the purpose of the research was to investigate the occurrence frequency and distribution of the main tomato viruses in tomato growing areas of Azerbaijan.

MATERIALS AND METHODS

Surveys and plant sampling. In order to identify viruses infecting tomato and evaluate the prevalence of viral infections, three surveys were conducted during June-July months of 2015, 2016 and 2017 growing seasons in important tomato areas of the southern part of Azerbaijan including Jalilabad, Masally and Lankaran (Figure 1). Total 12 fields were surveyed and 263 samples were collected from symptomatic plants as well as from non-symptomatic and healthy plants. Two non-cultivated (weed species) plants, showing TMV like symptoms were also collected around fields surveyed. Collected samples included young and fresh leaves and fruits of tomatoes with various symptoms. The plant material was transported to the laboratory on ice, immediately placed in plastic bags and kept at 4°C until they were processed. For long-term storage, leaf samples were stored at -80 ºC.

Detection and identification of viruses. Enzyme-linked immunosorbent assay (ELISA) and Polymerase chain reaction (PCR) which are the most common and widely used techniques for routine screening of pathogens were used for virus detection and identification.

Serological assays. Initially, rapid one-step assay AgriStrip which based on lateral flow immunochromatography and manufactured by Bioreba (Reinach, Switzerland) was performed to confirm the presence of TMV, T_oMV, CMV, TSWV in samples depending on suspicious virus symptoms. Midribs (area of the blade with primary veins) from basal (mature) and apical (young) leaf blades and petioles from infected tomato samples were analyzed. After the sample was homogenized a few drops of homogenate were transferred to a new cuvette. The strip was immersed into the extract, and the result was read within a couple of minutes. The positive result was considered as a color band on the test line, while the colored band on the control line always appeared.

Midrib and leaf petioles from symptomless tomatoes as well as from tomato with virus-like symptoms were also tested for TMV, T_oMV, CMV, TSWV, TYLCV, TEV, TRSV, AMV, BCTV by TAS-ELSA and DAS-ELISA using the ELISA kits developed by Bioreba AG (Reinach, Switzerland) and Agdia (USA) according to the manufacturer's instructions. All chemicals and buffers used in this assay, as well as negative and positive controls for each virus were provided by the company. Briefly, ELISA plates were coated with 200 µl of IgG (1:1000 dilution) diluted in carbonate coating buffer and incubated for 3 hours at 37ºC. Before 200 µl leaf extracts were added to the each well, plate was washed with washing buffer and incubated overnight at 4ºC. Then the plates were washed again and 200 µl of alkaline phosphatase-conjugated IgG diluted in conjugate buffer (1:1000) was added and incubated for 3 hours at 37ºC. After washing the ELISA plates were incubated with 200 µl of substrate (1 mg/ml of p-nitrophenyl phosphate in substrate buffer) at room temperature. Color reactions were measured at 405 nm (A405) after 2 h, using ELISA microplate reader (Stat Fax Microplate, Awareness Technology, USA). Each sample was analyzed in two wells, and samples were considered positive if the A405 nm values were more than three times those of the healthy control.

Molecular analysis. To confirm the presence of DNA and RNA genome viruses, leaf samples with a positive reaction in the serological assays with the virus were tested by PCR, RT-PCR methods using universal primers and primer pairs designed for the specific detection of the virus (Table 2). Total RNA was extracted from the leaf tissues using Trizol reagent (Invitrogen, USA) according to the manufacturer's instructions. Approximately 50-100 mg of fresh leaf tissue were placed in a sterile mortar and homogenized with 500 µl of grinding buffer (TRI-reagent). Aliquots of 500 µl of the extract were mixed with 100 µl of chloroform in a new set of sterile microfuge tubes and centrifuged at 13,500 rpm with refrigerated centrifuge for 15 min. Then, 300 µl of the supernatant were transferred to a new eppendorf tube containing 250 µl isopropanol and after shaking gently the tubes were kept for 15 min at room temperature. After centrifugation at 13,500 rpm for 20 min the supernatant was discarded and the pellet washed twice with 75% ethanol and dried at room temperature. The pellet was resuspended with 150 µl of RNase-free water and stored at –80°C until use. For TYLCV detection total DNAs were extracted from 1 g fresh leaf midribs of infected and

healthy plants (as control) following CTAB extraction protocol (Maixner et al., 1995). Purity degree and concentration of extracted DNA and RNA samples were determined spectro-photometrically (Ultrospec 3300 PRO, Amersham, USA). Then the RNA and DNA samples were analysed with RT-PCR and PCR. RT-PCR was performed in the reaction mix containing 4 µl of RNA, 1.5 µl of virus universal or specific primers, 1.5 µl of dNTPs (25 mM), 4 µl of RT (5x) buffer, 1 µl of M-MLV (RT enzyme), 8 µl of ddH2O. The reaction was carried out for 1 h at 42ºC. In order to stop the reaction, the samples were kept at 65ºC for 10 min. After the electrophoretic analysis, RT-PCR products were tested using PCR method. Amplification was carried out in a thermocycler (Multigene Gradient, "Labnet"company, USA) after preliminary denaturation at 95°C for 2 min, followed by 35 cycles at 94°C for 1 min, annealing for 45 s and extention at 70°C for 50 s, and a final extension at 72°C for 10 min. Following PCR, 10 µl of the product was electrophoresed on a 1% and 1.5% agarose gel, stained with ethidium bromide and recorded digitally using UV-Gel Doc system (UK).

RESULTS AND DISCUSSION

Identification and distribution of tomato viruses. We analyzed 263 tomato samples taken from commercial fields in three regions of the southern part of Azerbaijan (Figure 1). Samples were collected from 12 fields randomly selected in each geographical area surveyed. Selected fields were separated by about three km in the each surveyed areas. Each field was examined and sampled once in a year at the middle growth stage of the plants.

During these surveys, virus-like symptoms, including formation of yellowish and mosaic-red spots, in some cases rolling leaves, mottling, chlorosis, dwarfing, leaf deformation and roughness, necrotic spots on the leaves, reduced leaf size, boat shaped leaves, reduced fruit bearing and quality were frequently observed in tomato fields (Figure 2).

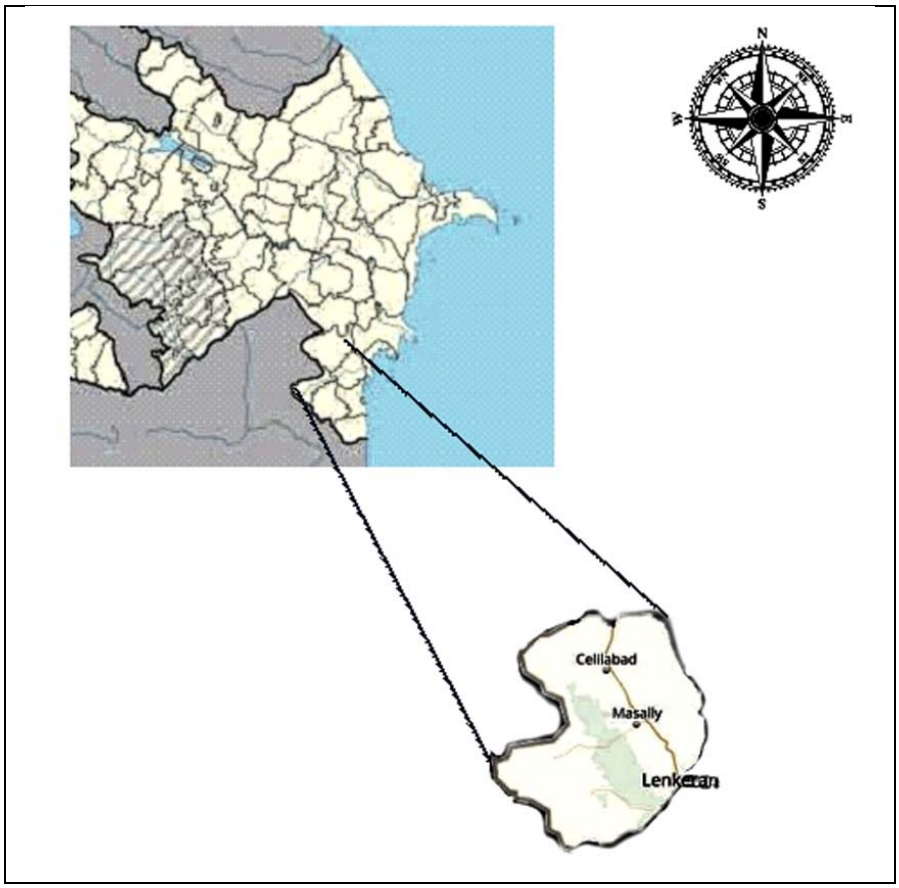

Fig. 1. Map of Southern part of Azerbaijan showing regions in which surveys were conducted during the 2015-2017 growing seasons.

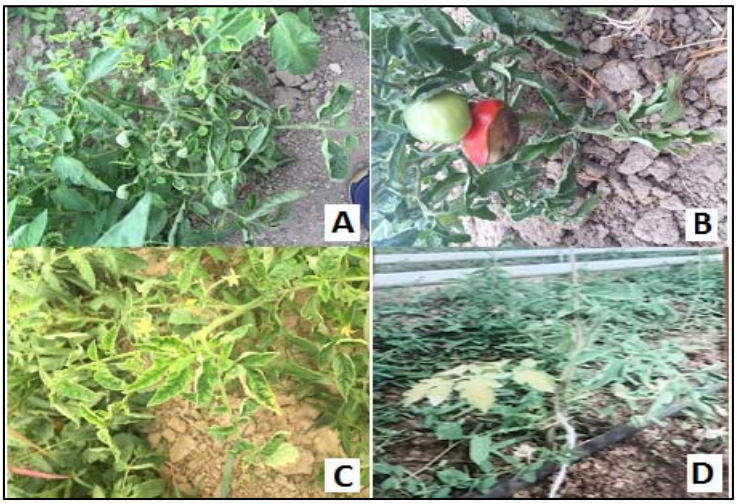

Fig. 2. Reduced leaf size and formation of boat shaped leaves (A), reduced fruit bearing and quality (B), mosaic and rolling of leaves (C), chlorosis and leaf deformation (D) symptoms associated with the virus infection on tomato.

Table 1. Incidence of nine* viruses in tomato plants collected in the southern regions of Azerbaijan.

Region of sampling	Year of sampling	No. of samples	TMV	TEV	TYLCV	ToMV	CMV	TSWV
	2015	36	6	0	0	9	12	0
Calilabad	2016	32	4	0	0	6	10	0
	2017	28	2	0	0	2	8	0
	2015	34	3	0	0	11	9	0
Lankaran	2016	20	2	0	0	8	5	0
	2017	12	0	0	0	3	2	0
	2015	48	8	3	4	6	9	8
Masally	2016	34	6	2	2	5	6	5
	2017	19	3	0	0	4	4	2
Total	2015-2017	263	34	5	6	54	65	15

*TRSV, AMV, BCTV were tested in tomato samples, but not detected in any of the samples tested.

During the surveys (2015, 2016 and 2017), TMV, ToMV, CMV, TSWV, TYLCV, TEV, TRSV, AMV, BCTV were analyzed in all tomato species by serological methods. TMV, ToMV and CMV were detected in all the regions, whereas TSWV, TEV and TYLCV were detected only in Masally (Figure 1 and Table 1). We failed to detect TRSV, AMV and BCTV in any of the samples tested. During the three-year survey, almost 68% of tested plants were infected by at least one of the viruses. In 2015, laboratory testing of 118 collected tomato samples indicated that the incidence of viral infection overall was 74.6%, eighty-eight out of 118 (Table 1). In 2016, laboratory testing of the 86 collected tomato samples indicated that the incidence of viral infection overall was 71%, sixty-one out of 86. CMV was the most common virus, infecting about 25% of the tomato samples. TMV and ToMV, infected tomato samples by about 21% and 13%, respectively, followed CMV in frequency (Table 2). The viruses TEV and TYLCV were detected in less than 2%, 2.5% of all samples, respectively. TSWV was followed in frequency by TMV and ToMV, which infected about 6% (15 out of 263) of the tomato samples (Table 1). During the surveys, the proportion of plants with symptoms of leaf chlorosis was visually estimated for each field. These observations, together with the analysis of the detected viruses in the tomato samples, gave an estimate of the frequency of CMV in the study areas. In 64% of the fields, the incidence of CMV was greater than 22%. The presence of CMV was also estimated in the weed hosts as natural sources of plant viruses. The incidence of tomato virus in weed hosts was recorded in Masally (22.2 %) and Jalilabad (10.4 %). Other tomato viruses were not detected in weeds.

Samples that were positive (TMV, ToMV, CMV, TSWV, TYLCV and TEV) according to ELISA results were checked by RT-PCR and PCR to confirm the presence of viruses and to identify them. The PCR and RT-PCR tests using the universal or specific primer pairs (Table 2) resulted in the amplification of one DNA product of approximately 320, 422, 529, 276 and 500 bp in size for ELISA-positive samples tested from tomato plants (Figure 3).

Table 2. List of primers used in this study.

Primers	Sequences (5'-3')	Reference
CMV1 CMV2	5'-GCCGTAAGCTGGATGGACAA-3' (5'-TATGATAAGAAGCTTGTTTCGCG-3')	Zitikaite I. et al., 2011
TMV-2 TMV1	5'-GAAAGCGGACAGAAACCCGCTG-3' 5'-GACCTGACAAAAATGGAGAAGATCT-3'	Silva R.M. et al., 2008
ToMV-6 ToMV-5	5'-GATCTGTCAAAGTCTGAGAAACTTC-3' 5'-CTCCATCGTTCACACTCGTTACT-3'	Silva R.M. et al., 2008
Tobamo-1 Tobamo-2	5`-CGAGAGGGGCAACAAACAT-3` 5`- ACCTGTCTCCATCTCTTTGG-3`	Kumar S. et al., 2011
TY-1 TY-2	5`-GCCCATGTA(T/C)CG(A/G)AAGCC-3` 5`-GG(A/G)TTAGA(A/G)GCATG(A/C)GTAC-3`	Accotto G.P. et al., 2000
MA13 F MA26 R	(5`-AATGCAATCTTCGTCACC-3`) (5`-CGCCCGTCTCGAAGGTTCG-3`)	Chinnaraja C. et al., 2016
L1 TSWV R L2 TSWV F	5`-ATC AGT CGA AAT GGT CGG CA-3` 5`-ATC AGT CGAAAT GGT CGG CA-3`	Milosovic S. et al., 2011
TEV-Poly2_F TEV-Poly2_R	5`-GTGTGCAAAGAAATTCCAGACTC-3` 5`-CACCACCAATTAACACAGACAAAG-3`	Lee J. et al., 2011
AMV1-F AMV1-R	5`-CCATCARGAGTTCTTCACAAAAG-3` 5`-TCGTCACGTCATCAGTGAGAC-3`	Al-Abrahim J.S. et al., 2014
TRSV F TRSV R	5`-CTTGCGGCCCAAATCTATAA-3` 5`-ACTTGTCCCAGGAGAGCTA-3`	Sneideris D. et al., 2012
BCTV-C1 2387R BCTV-C1 2097F	5`-TGCTCCAATAAGGTGCTTCCAGTG -3` 5`- TTTCCTCTGTCCTCATTCACAAACG-3`	Almasi M.A. et al., 2013

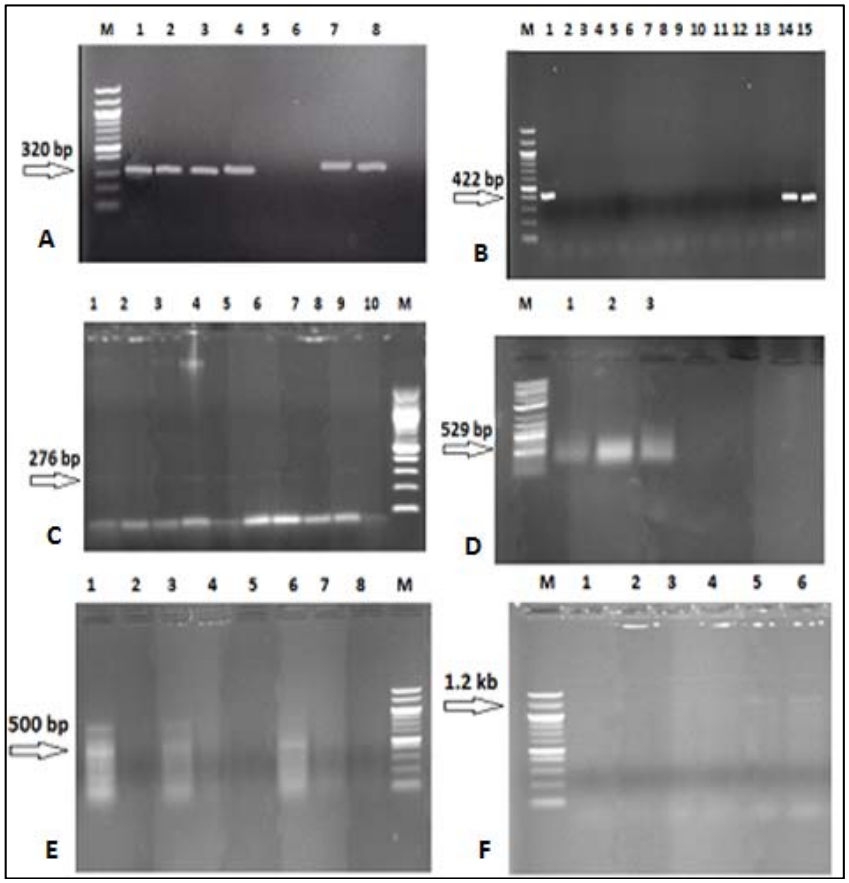

Fig. 3. RT-PCR and PCR analysis with universal (A) and specific primers of CP gene (B,C,D,E and F) of the tomato viruses. M-1kb and 100 bp DNA ladder. 1- 15 different tomato samples.

Obtained results confirmed the presence of ToMV, TMV, TEV, TSWV and CMV in tomato samples. No DNA product from healthy plant extracts was amplified. RT-PCR and PCR results confirmed that these tomato viruses occurred in all tested species and in regions surveyed. There was no mixed infection in collected tomato samples in the above-mentioned regions.

The expected fragments of approximately 320 bp (Kumar et al., 2011) were amplified by PCR from

total RNAs extracted from 88 ELISA-positive samples using the universal primers tobamo 1 and tobamo 2 (Figure 3A). The results of RT-PCR and PCR analyses confirmed the presence of tobamoviruses (TMV and ToMV) in samples of tomato. However, amplicons were not obtained from the other ELISA-negative samples. The DNAs extracted from this samples were subsequently tested by RT-PCR using the tobamovirus specific primers (TMV1 and TMV2; ToMV-5 and ToMV-6); an amplicon with the expected size of 422 bp (Silva et al., 2008) was obtained for tomato samples tested, showing severe yellowing and leaf deformation symptoms (Figure 3B). One DNA amplification product of approximately 276 bp or of 529 bp was observed in samples that were positive for TSWV or TEV, respectively, in DAS-ELISA (Figure 3 C,D). The expected fragments of approximately 500 bp (Zitikaite et al., 2011) were amplified by PCR from total RNAs extracted from 65 ELISA-positive samples using the spesific primers CMV 1 and CMV 2 (Figure 3E). Thus, the results of RT-PCR and PCR analyses confirmed the presence of tobamoviruses (TMV and ToMV) in samples of tomato. The primers used in this study, the sequences and reference data for ech primer are shown in Table 2. To confirm the presence of TYLCV infection, PCR was carried out with geminivirus specific primers, such as MA13/MA26 primers targeting 1292 bp of circular DNA-A of TYLCV (Chinnaraja et al., 2016). PCR amplicons for TYLCV were obtained from the six symptomatic tomato samples (Figure 3F).

Viruses always caused major losses in the quantity and quality of tomato crops worldwide and they exibit one of the most significant limiting factors for growers (Letschert et al., 2002; Hassani-Mehraban et al., 2010; Kaye et al., 2011). In Azerbaijan these crops have a high-level incidence of symptoms peculiar to viral infection (Huseynova et al. 2016; Huseynova et al., 2017; Verdin et al., 2018; Desbiez et al., 2018). Regardless of the significance of tomato crops in Azerbaijan, in previous studies only a limited number of samples from a few areas were tested for viruses, but their occurance and distribution in the major tomato grown regions were not estimated. This is the first report of an extensive survey using serological and molecular diagnostic procedures to identify the most important viruses of tomato crops and determine their incidence in the southern Azerbaijan. In the three years of the survey, symptoms such as mosaic, leaf chlorosis, leaf and fruit deformation were found in all tomato fields examined with a prevalence between 25 and 70% of plants. Only 68% of the collected symptomatic samples were found infected with a viruses. The remaining samples may be infected with viruses that have not been examined, or the observed symptoms could be caused by reasons other than a viral infection. CMV was the most prevalent virus in all surveyed tomato growing areas of the Jalilabad, Masally and Lankaran regions. It is also known that CMV causes a big threat to neighboring countries (Gallitelli, 2000). A high frequency of CMV symptoms was detected in tomato plants in Iran, where CMV causes a huge crop loss each year (Zitikaitė et al., 2011; Arafati et al., 2013). Tobamoviruses also have a serious economic impact leading to yield losses in many crops especially in solanaceous crops (Chitra et al., 1999; Chiemsombat et al., 2008). TMV has been reported to cause considerable reduction in Alburz, East Azarbaijan, Fars, Golestan and Tehran provinces, which are the major horticultural crop cultivation regions of Iran (Hu et al., 2012: Alishiri et al., 2013). ToMV is characteristic for eggplants in southern, northern and central Iranian regions (Aghamohammadi et al., 2011). Yazdani-Khameneh (2013) indicated the prevalence of tobamoviruses, especially ToMV, in vegetables in Iran. In recent years, *Pepino mosaic virus* (PepMV) has emerged as one of the fastest growing viruses, which is the most important viral agent in the tomato production worldwide (Hajiabadi et al., 2012). PepMV causes severe symptoms in greenhouse tomato plants in western Mediterranean in Turkey (Sevik et al., 2016). Depending on the host and virus isolate, plant deaths are observed with plant growth disorder, blight, fan leaf, ring stains, softening in fruits and necrosis (He et al., 2012; Koç et al., 2017). Tomato spotted wilt virus (TSWV) is one of the most devastating viruses of sweet pepper and tomato in Samsun province of Turkey (Golnaraghi et al., 2001; Ghotbi et al., 2005; Hajiabadi et al., 2009; Deligoz et al., 2014).

This study reports the natural prevalence of tomato viruses in the southern part of Azerbaijan, which can provide important basic information useful for virus control strategies for tomato growing in our country. The widespread occurrence of viruses showed that these regions of Azerbaijan are very susceptible. Conducting control over the vectors of insects that spread viral diseases should be of fundamental importance for preventing these viruses.

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Azərbaycanda Tomat Bitkisini Yoluxduran Viruslar

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2015-2017-ci illərin iyun-sentyabr ayları ərzində Azərbaycanın cənub bölgəsində yerləşən əsas tərəvəz çilik rayonlarına fitopatoloji monitorinqlər təşkil olunmuşdur. Cəlilabad, Masallı, Lənkəran rayonlarında yarpaq ayasının burulması, saralması, nekrozu, mozaikası və s. kimi simptomlara malik 263 tomat bitkisi (*Solanum lycopersicum* L.) nümunələri seçilərək toplanmışdır. Toplanmış simptomatik tomat nümunələrinin ilkin olaraq, seroloji (AgriStrip, DAS-ELISA), daha sonra isə molekulyar metodlarla (RT-PZR, PZR) diaqnostikası həyata keçirilmişdir. Seroloji testin nəticələrinə əsasən 179 tomat bitkisi nümunəsi (68%) pozitiv nəticə göstərmişdir. ELİSA testininə əsasən, pozitiv nəticə göstərən DNT və RNT genomlu viruslar, PZR və RT-PZR metodları ilə də yoxlanılmış və nəticələr üst-üstə düşmüşdür. PZR və RT-PZR metodları ilə aparılan amplifikasiya zamanı, universal və spesifik praymer cütləri tətbiq edilmiş və nəticədə gözlənilən 320, 422, 529, 276 və 500 bp ölçüdə amplikonların sintezi baş vermişdir. Yekun olaraq, toplanmış tomat bitkisi nümunələrində *Tomato mosaic virus* (T_oMV), *Tobacco mosaic virus* (TMV), *Tomato etech virus* (TEV)*, Tomato spotted wilt virus* (TSWV*)* və *Cucumber mosaic virus* (CMV) aşkar edilmişdir. Təqdim edilən tədqiqat işi Azərbacanın cənub bölgəsində tomat bitkisində virus infeksiyalarının növ müxtəlifliyini və yayılma areallarını əks etdirir.

Açar sözlər: *Tomat bitkisi, virus xəstəlikləri, Tomato mosaic virus, Tobacco mosaic virus, Tomato etech virus, Tomato spotted wilt virus, Cucumber mosaic virus*

Распространение Вирусных Заболеваний Томата в Азербайджане

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Наблюдения и фитопатологические исследования проводились в период вегетации растений в течение 2015-2917 гг. в самых значимых для овощеводства Азербайджана областях. В трех регионах (Джалилабад, Масаллы, Ленкорань) было собрано 263 подозрительных образца различных сортов томатов (*Solanum lycopersicum* L.) и некоторых некультивируемых растений-хозяев с симптомами листового завитка, карликовости, сужения листьев, некроза, мозаики или пожелтения листьев, с пониженным урожаем и качеством плодов. В зависимости от симптомов, собранные образцы сначала были подвергнуты скринингу с использованием серологических (быстрый одноэтапный анализ AgriStrip, DAS-ELISA), а затем молекулярных (RT-ПЦР, ПЦР) методов, с целью выявления основных вирусов, инфицирующих эти культуры. Согласно результатам серологических тестов, 179 образцов (68%) положительно реагировали с вирусными антителами. Инфекции ДНК и РНК геномных вирусов были проверены полимеразной цепной реакцией (ПЦР) и обратной полимеразной цепной реакцией (ОТ-ПЦР). Тесты ПЦР и ОТ-ПЦР с использованием универсальных или специфических пар праймеров привели к синтезу ожидаемых фрагментов размерами 320, 422, 529, 276 и 500 п.н., соответственно. Полученные результаты, подтвердили наличие *Tomato mosaic virus* (T_oMV), *Tobacco mosaic virus* (TMV), *Tomato etech virus* (TEV)*, Tomato spotted wilt virus* (TSWV*)* və *Cucumber mosaic virus* (CMV) вирусов в образцах томатов. В данном исследовании сообщается о естественной распространенности вирусов томатных растений в южной части Азербайджана.

Ключевые слова: *Томаты, вирусные заболевания, Tomato mosaic virus, Tobacco mosaic virus, Tomato etech virus, Tomato spotted wilt virus, Cucumber mosaic virus*

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Reactivation of the Oxygen-Evolving Function of Photosystem II Inhibited by Hydroxylamine

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The oxygen-evolving activity of photosystem II (PSII) was inhibited by extraction of the Mn-cluster using hydroxylamine. The activity was recovered partially by the photo-activation treatment applied after addition of the exogenous Mn^{2+} to the inhibited samples. The effect of Ca^{2+}, Cl^- and H^+ ions in the reaction medium on reconstitution of the oxygen-evolving activity of PSII was studied. The results can be useful for the understanding of the principles of artificial energy converters based on photosynthetic reaction centers and for the study of artificial photosynthesis.

Keywords: *Photosystem II, molecular oxygen, hydroxylamine, Mn-cluster, calcium, chloride, pH*

INTRODUCTION

Photosystem II (PSII) involved in the energy conversion in thylakoid membranes of oxygenic species, catalyzes a very important biological function – oxidation of water molecules and formation of molecular oxygen (Barber, 2006; Muh and Zouni, 2011). Structure of its photochemical core determined with high accuracy in cyanobacteria and red algae (Zouni et al., 2001; Ferreira et al., 2004; Umena et al. 2011; Ago et al., 2016). This molecular complex is composed of ~20 proteins and numerous cofactors. Among them transmembrane D_1 and D_2 proteins (32-34 kDa), cytochrome b_{559}, core antenna proteins CP47 and CP43 carrying Chl *a*, and peripheral proteins of 33-, 24- and 18 kDa located on the lumen surface of thylakoid membranes are the most important. D_1/D_2 heterodimer of the PSII complex consists of an initial electron donor P_{680} (chlorophyll *a* dimer), electron acceptors pheophytin (Phe) and plastoquinones (O_A, Q_B), redox active tyrosines Y_Z (D_1-Tyr^{161}) and Y_D (D_2-Tyr^{161}) (Barber, 2006; Muh and Zouni, 2011). The light absorption in the reaction center results in the electron transport from P_{680} dimer to Phe molecule and then consistently to plastoquinones Q_A and Q_B. The oxidized form of the initial electron donor P_{680} ($P_{680}^{\bullet+}$) is a strong oxidant (~1.1 V), which is eventually reduced at the expense of electrons transported from the water oxidation center (Klimov et al. 1977; Klimov and Krasnovski, 1981). The core of the water oxidation center composed of the Mn_4CaO_5 cluster. Tyrosine Y_Z residue of D_1 protein participates in the electron transfer between the Mn_4CaO_5 cluster and $P_{680}^{\bullet+}$.

When the water molecule is oxidized, the complex PSII passes through the states S_0, S_1, ..., S_4 of the hypothetical S-cycle. Formation of molecular oxygen occurs in the final $S_3 \rightarrow [S_4] \rightarrow S_0$ transition. It is assumed that the positive charges produced in the S-cycle, accumulate in the Mn_4CaO_5 cluster (Kok et al., 1970; Debus, 1992; Muh and Zouni, 2011). PSII kept in darkness for a long time is stabilized mainly at S_1 and S_0 (75% and 25%, respectively) states. S_0 and S_1 states are, respectively, semi-stable and stable, whereas S_2 and S_3 are energetically unstable. They return to the S_1 state in the dark. S_4 state is a transitional, and spontaneously passes to S_0. Molecular oxygen is formed in the S_3-S_4-S_0 transition and released to atmosphere (Debus, 1992; Muh and Zouni, 2011).

Recently, intensive investigations have been carried out on developing various biomimetic devices including artificial energy converters on the basis of the structure and functional principles of photosynthetic complexes (reviewed in: Barber, 2009; McConnell et al., 2010; Barber and Tran, 2013). Due to effective energy convertion (quantum yield of primary reactions of photosynthesis is ~1.0) and high oxidation potential PSII is considered to be useful for these investigations. A main goal of the investigations of artificial systems is the elucidation of principles of the assembly of components (antenna, reaction center, components of the oxygen-evolving machinery etc.) of energy converters (Hamarstrom and Styring, 2008; Brudvig, 2008; Dismukes et al., 2009; Kanady et al., 2011). Consequently, the study of the photosynthetic processes by inhibition and recovery of the reactions occurring in the natural system and

the role of various components in the natural phytosystems becomes important.

In our research the oxygen-evolving function of the water oxidation center was inhibited by extraction of the Mn_4CaO_5 cluster from the PSII complex. Then, this function was restored through photoactivation by adding exogenous Mn^{2+}. The effect of various factors such as Ca^{2+}, Cl^-, pH on effective reconstitution of the oxygen-evolving activity has been studied.

MATERIALS AND METHODS

PSII membrane fragments (BBY type) having a high oxygen-evolving capacity (~600 μmol O_2 (mg chl)$^{-1}$s^{-1}) were isolated from spinach, using Triton X-100 (Berthold et al., 1981; Völker et al. 1985). For extraction of the Mn_4CaO_5 cluster, PSII membranes were homogenized in 25 mM MES-NaOH (pH 6.5) buffer containing 400 mM sucrose, 20 mM NaCl (buffer A) at chlorophyll concentration ~0.5 mg/ml. After addition of hydroxylamine (NH_2OH), incubation was performed in darkness at room temperature (~25°C). At the end of incubation the homogenate was diluted 5-6 times with the cold (~4°C) buffer A and centrifuged at 18,000 g, at 4°C. The precipitate was washed 3 times through the homogenization in the cold buffer A and precipitation at 18,000 g, at 4°C. Reconstruction of the oxygen-evolving activity of PSII was performed in the buffer A, containing $MnCl_2$ at room temperature, under photoactivating light (~50 μmol photon m^{-2}s^{-1}). The same method was used to study the effect of various factors on photoactivation. The chlorophyll content was evaluated through spectrophotometric (JY-201, Jobin Ivon, France) determination of optical density of its 80% acetone extract at 663 nm and 645 nm (MacKinney, 1941). The rate of O_2 evolution of the PSII complex was measured amperometrically using the Clark electrode (Rank Brothers Ltd., UK).

RESULTS AND DISCUSSION

Hydroxylamine added PSII membranes lose their Mn cluster very rapidly. This treatment results in almost complete inhibition of O_2 evolution and electron transport activities of the PSII complex. The inhibitory effect of hydroxylamine depends on its concentration, content and temperature of the incubation medium. It is known that preparations treated with hydroxylamine are more sensitive to light inhibition compared with native PSII complexes. Reactivation of O_2 evolution and electron transport activities of Mn-depleted preparations are still an object of discussions. The effect of 1.0-3.0 mM hydroxylamine on the oxygen-evolving activity of PSII membranes has been examined in the presented research. As seen in figure 1, inhibition of the oxygen yield of PSII membranes was very fast during the first 5 minutes of incubation. Subsequent (>5 min) incubation with hydroxylamine demonstrates only slow inhibition. Consequently, the optimal effective concentration of hydroxilamine was found to be 2.0 mM and duration of effective action was 5 min.

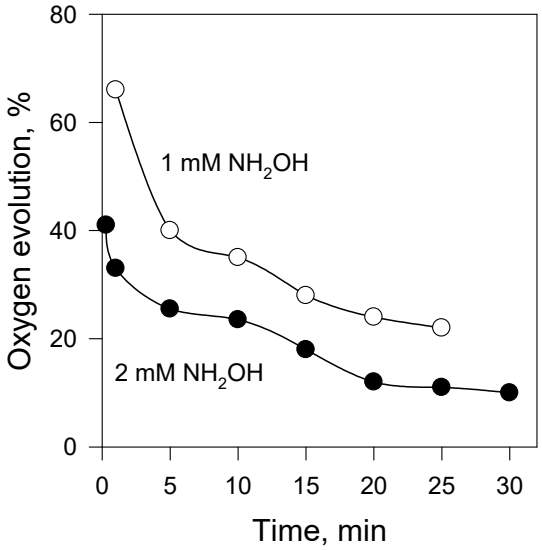

Fig. 1. The change in the oxygen evolution activity of the PSII membrane preparations in the presence of hydroxylamine. The maximum oxygen yield of the native preparations (600 μmol O_2 (mg chl)$^{-1}$s^{-1}) was assumed to be 100%. During the incubation with hydroxylamine the ambient temperature was 25°C, and chlorophyll concentration was 0.5 mg/ml. For the incubation and measurements 25mM MES-NaOH (pH 6.5) buffer, containing 400 mM sucrose and 20 mM NaCl was used.

Reactivation of the oxygen evolution activity through reassembling the Mn-cluster in inhibited PSII preparations, and its dependence on the pH, concentrations of Ca^{2+} and Cl^- ions have been studied. The oxygen-evolving activity of the inhibited PSII complex in preparations with the extracted Mn-cluster was partly restored through photoactivation (~20-35% in various preparations) after addition of exogenous Mn^{2+} ($MnCl_2$). The necessity of adding 1-3 mM Mn^{2+} into the reaction medium for restoration of the oxygen-evolving activity was established (Fig. 2). Maximum restoration of the oxygen-evolving function was ensured by 10-15 min illuminatuion with ~50 μmol photon m^{-2}s^{-1} white light.

Ca^{2+} is involved in the water oxidation center (Debus, 1992; Vrettos et al., 2001; Ugur et al., 2016).

The necessity of the presence of high Ca^{2+} concentrations in the reaction medium for effective reconstruction of the Mn-cluster was reported previously (Ghanotakis et al., 1984; Miller and Brudvig, 1989; Chen et al. 1995). We also established that to provide high yield oxygen evolution addition of 20-50 mM Ca^{2+} ($CaCl_2$) to the reaction medium is required. At higher concentrations of Ca^{2+} ions (>50 mM) an additional increase in the oxygen-evolving activity of reconstituted PSII preparations was not observed (Fig. 3). However, the amount of Ca^{2+} ions for restoration of the oxygen-evolving activity is much more higher compared with the native PSII (~1 Ca^{2+}/RC).

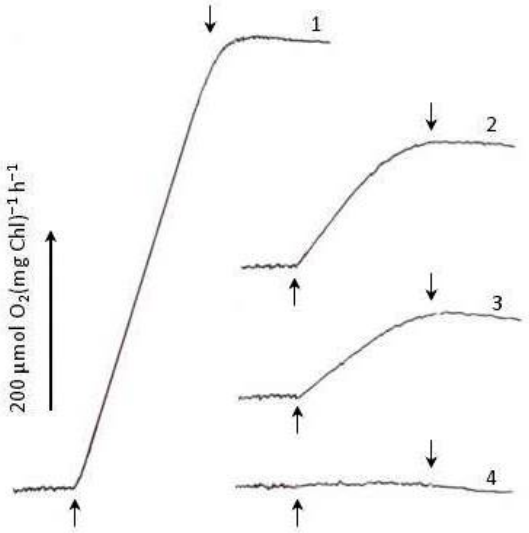

Fig. 2. Oxygen evolution in PSII particles inhibited by hydroxylamine and repaired by photoactivation: 1 – native, 2 – treated with hydroxylamine, 3 – in the presence of 2 mM $MnCl_2$, and 4 – kinetics of oxygen evolution in the photoactivated PSII preparations in the presence of 2 mM $MnCl_2$ and 20 mM $CaCl_2$. The medium 25mM MES-NaOH (pH 6.5), containing 400 mM sucrose and 20 mM NaCl was used for photoactivation and O_2-measurements Fotoactivation was performed at room temperature for 10 min, under illumination of ~50 µmol photon $m^{-2}s^{-1}$ white light. ↑ (↓) – measutring light on (off).

The presence of Cl^- ions is also required for the maximum oxygen-evolving activity of PSII (Sandusky and Yocum, 1986; Miyao and Inoue, 1991, Boussac and Rutherford, 1994; Olesen and Andreasson, 2003). However, in our experiments, high yield of oxygen evolution achieved after photoactivation treatment, required much higher concentration of Cl^- ions (≥1.0 M) compared with Ca^{2+} ions (Fig. 4). This fact may be attributed to the lower affinity of Cl^- ions to the water oxidation center compared with Ca^{2+} ions.

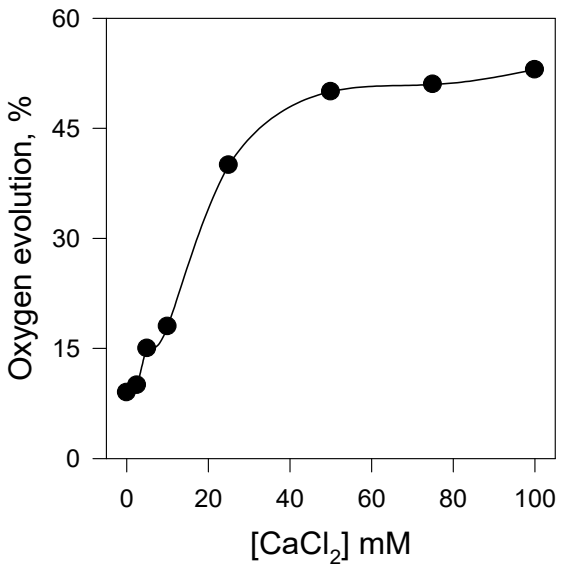

Fig. 3. The effect of Ca^{2+} ions on reactivation of the oxygen-evolving activity of PSII. The maximum oxygen yield (600 µmol O_2 (mg chl)$^{-1}s^{-1}$) was assumed to be 100%. The medium used for photoactivation and measurements as described in Fig. 2. In addition, photoactivation medium includes 2 mM $MnCl_2$. Photoactivation was performed at room temperature for 10 min, under illumination of ~50 µmol photon $m^{-2}s^{-1}$ white light.

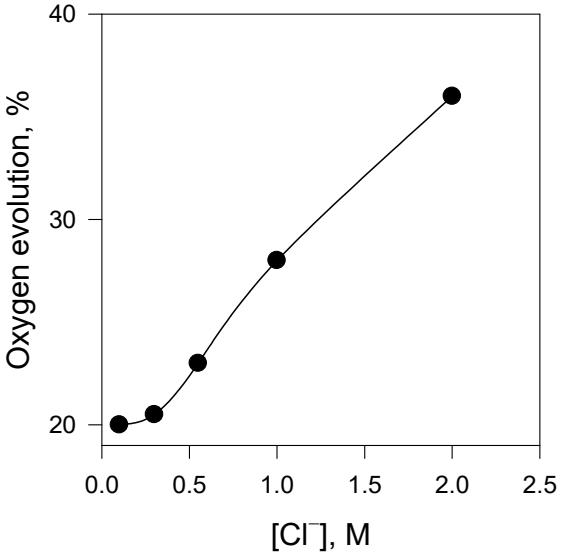

Fig. 4. The effect of Cl^- ions on reconstitution of the oxygen-evolving activity of the PSII complex. The medium used for photoactivation and measurements as described in Fig. 2. In addition, photoactivation medium includes 2 mM $MnCl_2$ and 20 mM $CaCl_2$. Fotoactivation was performed at room temperature for 10 min, under illumination of ~50 µmol photon $m^{-2}s^{-1}$ white light.

Considering importance of pH value for O_2-evolution, we studied reconstruction of the Mn-cluster in the pH range from 5.5 to 6.5. According to the results of our experiments, oxygen evolution enhanced with increasing pH (from 5.5 to 6.5). In other words, the pH-dependent change of the

restored value of the oxygen yield occurred in the following direction: pH 5.5<5.9<6.2<6.5 (Fig. 5). Photoactivation of O_2 evolution at higher values of pH was not studied, as higher values of pH were assumed to inhibit the oxygen-evolving complex.

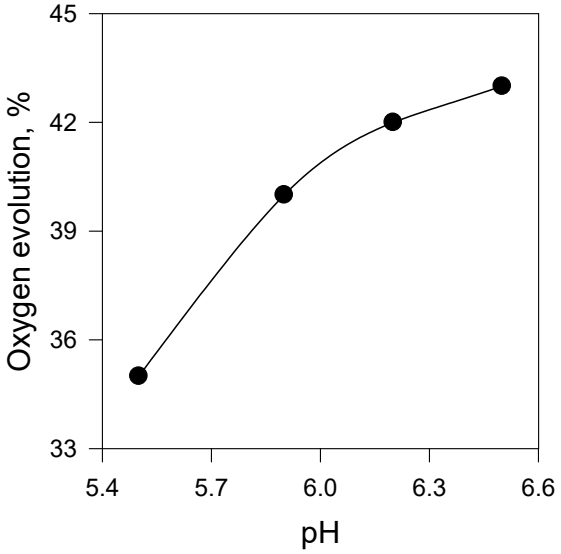

Fig. 5. The effect of pH of the reaction medium on restoration of the oxygen-evolving activity of the PSII complex. The medium used for photoactivation and measurements as described in Fig. 2. In addition, photoactivation medium includes 2 mM $MnCl_2$ and 20 mM $CaCl_2$. Fotoactivation was performed at room temperature for 10 min, under illumination of ~50 μmol photon $m^{-2}s^{-1}$ white light.

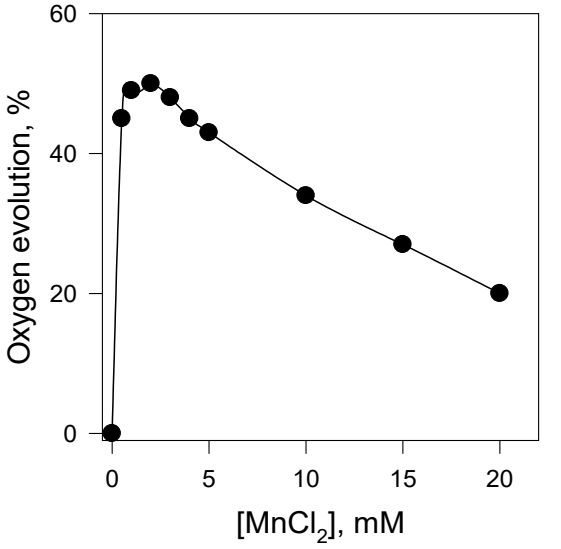

Fig. 6. Reconstruction of the oxygen-evolving activity of the PSII complex in dependence of concentrations of Mn^{2+} ions in the photoactivation medium. The medium used for photoactivation and measurements as described in Fig. 2. Besides Mn^{2+}, the photoactivation medium contains 20 mM $CaCl_2$ and ~100 mM Cl^- ions. Photoactivation was performed at 25°C, for 10 min, at light intensity 50 μmol photon $m^{-2}s^{-1}$.

Figure 6 presents the the oxygen evolving activity of PSII photoactivated at different concentrations of Mn^{2+} in the presence of 20 mM Ca^{2+} and 100 mM Cl^- ions, and optimum pH value of 6.5. As seen in the figure, the oxygen yield was higher at 1-5 mM concentrations of Mn ions and declined with increasing concentrations of these ions.

Thus, the partly restoration of the oxygen-evolving function of PSII inhibited by hydroxylamine has been established as a result of the experimental evaluation of the possibility of reconstruction of the PSII preparations with the extracted Mn-cluster.

Considering all the optimization mechanisms, maximum yield of oxygen evolution in the reconstituted PSII was less than 50% of that observed in the native complex.

Possible reasons for such a big difference in the oxygen evolving activity of the reconstructed and native complexes, observed in our experiments, may include: (i) initiation of the other inhibitory mechanisms (for example, removal of peripheral proteins from the binding sites, photoinhibition etc.) during the preparation process; (ii) incomplete reassembling of the Mn cluster; (iii) high concentrations of the ions in the reaction medium, required for the reversibility of oxygen evolution, and thereby possibility of the both activatory and inhibitory effects of these ions on the PSII electron transport. On the other hand, a very high value of Mn^{2+}/RC stoichiometry (~10^4 times compared with the native complex) for maximum photoactivation of the oxygen yield, also may be questioned.

Currently, the research on the identification of the inhibition mechanisms and optimization of restoration of the oxygen-evolving complex continues.

ACKNOWLEDGMENTS

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Hidroksilaminlə İnhibirləşmiş Fotosistem II Kompleksinin Oksigen Ayırma Funksiyasının Bərpası

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Fotosistem II (FS II) kompleksində, suyun oksidləşdiyi katalitik mərkəzin Mn-klasteri hidroksilaminlə ekstraksiya olunaraq, oksigen ayırma fəallığı inhibirə edilmişdir. Ekzogen Mn^{2+} əlavə olunduqda inhibirə olunmuş kompleksin oksigen ayırma fəallığı foto-aktivləşmə yolu ilə qismən bərpa olunmuşdur. Reaksiya mühitindəki Ca^{2+}, Cl^{-} və H^{+} ionlarının miqdarının, bərpa prosesinə təsiri öyrənilmişdir. Alınmış nəticələr FSII-nin iş prinsiplərinə əsaslanan süni enerji çeviricilərinin işlənib hazırlanması və süni fotosintez tədqiqatları üçün əhəmiyyətli ola bilər.

Açar sözlər: *Fotosistem II, molekulyar oksigen, hidroksilamin, Mn klasteri, kalsium, xlorid, pH*

Восстановление Кислородвыделяющей Функции Фотосистемы 2, Ингибированной Гидроксиламином

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Выделение кислорода комплексом фотосистемы 2 (ФС-2) ингибировалось после экстракции Mn-кластера центра окисления воды. После добавления экзогенного Mn^{2+}, активность выделения кислорода ингибированной ФС-2 частично восстанавливалась в процессе фотоактивации. Изучено влияние ионов Ca^{2+}, Cl^{-} и H^{+}, присутствующих в реакционной среде, на восстановление кислородвыделяющей активности ФС-2. Результаты могут быть полезными для разработки искуственных преобразователей энергии на основе принципов функционирования ФС-2 и для исследования искусственного фотосинтеза.

Ключевые слова: *Фотосистема 2, молекулярный кислород, гидроксиламин, марганцевый кластер, кальций, хлор, pH*

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Outlier Detection in Atomic Temperature Factor - B Value Distribution

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Several methods for outlier detection in Atomic Displacement Parameters – B value is applied to one particular macromolecular structure. It is demonstrated that outliers in B values give good indication errors in the atomic models. In this particular example it is demonstrated that a hypothesis that atomic B values distribution is shifted inverse gamma distribution. Removal of outliers from the set of B values improves the estimation of the parameters of the distribution. Local outliers in B values indicate errors in atomic models: validity of the assumption that neighbouring atoms must have similar B values has been verified. It is expected that local and global outlier detection program and modelling of B values as inverse gamma distribution will help to select the reliable atomic models. We suggest that such outlier detection and modelling should be part of model building and refinement of macromolecular structures using crystallographic diffraction data and single particle cryo electron microscopy maps.

Keywords: *Macromolecules, validation, macro-molecular crystallography, outlier detection, inverse gamma distribution*

1. INTRODUCTION

There are three main methods to derive atomic models of biological macromolecules (Berman et al., 2012; Cavagnero, 2003): crystallography, electron microscopy and nuclear magnetic resonance (NMR) (Rupp, 2010; Frank, 2006; Clayden et al., 2001). Both crystallography and electron microscopy use scattering of particles whereas NMR uses various spectroscopic measurements to derive structural information. Atomic models are derived using software packages that use different assumptions about the nature of experiment as well as molecules under study. The resulting atomic structures should be considered as statistical models and they have to be validated using as independent as possible validation tools (Rupp, 2010; Papageorgiou, Mattsson, 2014; Henderson et al., 2012). Validations must be done against experimental data as well as against prior knowledge about macromolecules. There is a number of software tools dealing with the problem of validation of atomic structures (Chen et al., 2010). In this work we would like to address one of the problems standard validation programs usually ignore – validation of atomic displacement parameters (ADP). After all, if atomic displacement parameters can indicate the level of accuracy of atomic positions, validation of atomic positions and interatomic distances should be adapted accordingly; there is no point of validating wrong atoms against chemical and structural information.

In crystallography and electron microscopy studies of molecules observed densities are modelled as a sum of Gaussians centred at the atomic positions (Rupp, 2010):

$$\rho(x) = \sum_{i=1}^{N} \rho_i(x - x_i)$$

with:

$$\rho_i(x) = \sum_{j=1}^{N_{Gauss}} \frac{c_{ii}}{(2\pi(u_i+u_{ij})^{3/2}} e^{-\frac{|x|^2}{2(u_i+u_{ij})}} \qquad (1)$$

Where u_i is the property of the observed atoms in the molecule, u_{ij} are properties of chemical elements or atom types. Each atomic type is described by N_{gauss} Gaussians, usually N_{gauss} = 5. c_i are weights of Gaussians. x_i are vectors of atomic positions, x is a vector of position in the three-dimensional space. These models, although are approximations to true densities, work sufficiently well in practice. They do not account such fine electronic details as bonding electrons or charge redistribution as a response to interactions with the environment. $\rho(x)$ has different meaning for different scattering methods: if X-rays are used then $\rho(x)$ is the electron density, if electrons are used then it is the electrostatic potential of the molecule. As it can be seen from the formula when u_i becomes large then the density corresponding to this atom become smeared out or blurred. If different atoms have wildly different ADPs then it can be expected that these atoms will have very different densities corresponding to their ADPs. If ADPs would represent only oscillation of atoms around their centre then it would reflect the relative mobility of atoms. In general, it can be

expected that oscillations are different in different directions resulting in anisotropic ADPs. In this work we consider only isotropic ADPs.

Fourier space counterpart of the expression (1) is:

$$F(s) = \sum_{i=1}^{N} f_i(s) e^{-2\pi^2 u_i |s|^2/4} e^{-2\pi \imath s x_i} \quad (2)$$

Where *F(s)* is the Fourier transformation of the density, *s* is the vector of positions in the Fourier space, f_i is the scattering factor of the atom:

$$f_i(s) = \sum_{j=1}^{N_{gauss}} c_{ij} e^{-2\,\pi^2 u_{ij} |s|^2}$$

In both representations we essentially assume that the contribution from atoms to the density is convolutions of Gaussians describing atomic mobility or uncertainty and atoms at rest. Uncertainty associated with the Gaussians is called atomic displacement parameters. There are several contributors to the ADP including: 1) dynamic and static disorder in crystals that combine such factors as atomic mobility, crystal lattice disorder: 2) errors in atomic positions, these have the effect of increasing atomic displacement parameters to compensate for errors in the atomic positions; 3) misidentified atom types, this can either increase or decrease ADPs. It would be very difficult to disentangle these contributions without additional information, therefore when dealing and trying to interpret atomic models with the models from the PDB we have to bear this in mind.

Since every atom is linked with the neighbouring atoms either via covalent bond or via non-bonding interactions we can assume that neighbouring atoms have similar oscillation, if they deviate from each other too much then such atoms should be considered as suspicious and they must be revised with care using the observed/estimated density. It should also be mentioned that ADPs of atoms define their relative contribution to the Fourier coefficients via Debye-Waller factor (Debye, 1913) which essentially states that this contribution can be described as a Gaussian in three-dimensional space.

Since ADPs are directly related to the oscillation or uncertainties of the atoms which are modelled as Gaussian that means that ADPs are proportional to the second central moments or variance of the normal probability distribution. In Bayesian statistics it is usual to model the distribution of variances of the normal distribution as an Inverse Gamma distribution (Witkovsky, 2001; Cook, 2008). This distribution has been used successfully as natural conjugate priors for Bayesian modelling of data with Gaussian population distribution (Murphy, 2007).

Since B values are related to the errors in the atomic model they sometimes are used to select reliable set of atoms for further analysis (Chen et al., 2010), therefore it is important to design a procedure that would allow reliable detection of atoms with unusual ADPs; if ADP is too high then it is likely that this atom has been wrongly placed, if it is too small then this atom may have been misidentified, i.e. it might be heavier than that in the PDB file.

In this paper we will describe several outlier detection algorithms for isotropic ADPs for a single entry from the PDB. We will discuss the global and local outliers. We will also demonstrate that removal of outliers improves the estimation of the parameters of shifted inverse gamma distribution proposed as a model for B value distributions.

2. METHODS

There is a number of outlier detection methods described in the literature (Barnett, 1994; Iglewicz, 1993; High, 2000). In this paper we will discuss the methods we managed to use successfully for analyses of ADP distribution.

*Tukey's box and whisker plot method (*Hartwig, 1979): It is a widely used method in descriptive and exploratory statistical data analysis. In this method such statistics as the median, 1st and 3rd quartile, lower and upper extreme values are plotted on the same plot. Such plots help to visually inspect the data and see if there are outliers. Tukey's rule of determining outliers consists of the following steps: 1) calculate the interquartile range as IQR = 3rd quartile– 1st quartile; 2) calculate the upper fence as UpperFence = 3rd quartile + k*IQR; 3) calculate the lower fence as LowerFence = 1st quartile – k*IQR. Points that fall below the lower or above the upper fence are considered outliers. Here k is a factor used to identify outliers with various severities: k=1.5 is used for "mild" and k=3 is used for "extreme" outliers. In our application we need to remove only extreme outliers.

Z-score: Another standard method is Z-score (Shiffler, 1988) that is used to detect outliers in the data with using standard deviation and mean. For each data point Z values are calculated using the formula:

$$Z_i = \frac{x_i - \bar{x}}{sd}$$

where $\bar{x}$ and *sd* are the and standard deviation of the data. Z-scores with an absolute value greater then k are generally considered as outliers. Usually k=3 is taken as default which works in practice sufficiently well. In this paper Z-score method was used with the parameter k=3. This method is not robust to outliers, existence of outliers affects the mean and the standard deviation calculated from the sample. Moreover this method works well with the data points sampled from the population with symmetric distribution.

Modified Z-score: Iglewicz and Hoaglin

(Iglewicz, 1993) recommend using:

$$M_i = \frac{0.6745(x_i - \tilde{x})}{MAD}$$

where $\tilde{x}$ and MAD are the median and the median absolute deviation respectively. MAD is the median of the absolute differences between the data points and the median of the data. These statistics are often used in robust statistical estimations: median replaces the mean and MAD replaces the standard deviation. If the population from which the data have been drawn has the normal distribution then median is equal to the mean and MAD/0.6745 is equal to the standard deviation (Venables, 1999). Although the authors recommend that modified Z-scores with an absolute value of greater than k=3.5 to be considered outliers, in practice to detect outliers with various severity different values of k should be used.

For local analysis first for each atom the list of its neighbours was calculated using the efficient cell algorithm (Mattson, 1999). For this 4.2Å radius was used, although the radius is a tuneable parameter. Then for each atom B values of its neighbourhood was analysed.

3. RESULTS

3.1. Global analyses and outliers. B values of the macromolecular structures are proxies for atomic mobility as well as errors in the model. The modelling of the B value distribution is important for understanding of fundamental properties of positional errors and atomic mobility. They can also be used for outlier detection and in future for map calculation. As it was mentioned in [Masmaliyeva, Murshudov 2017, Dauter 2006], the distribution of B values can be approximated by a shifted Inverse Gamma (IG) distribution. In this paper, as an example we used the protein structure with the PDB code 4XKT (resolution 1.82, R factor 0.17, Rfree 0.19) [Bradley et.al. 2015]. Since it is likely that the structures in the PDB have been under-refined, before any further analysis such as outlier removal and estimation of the parameters of the distribution, the structure was re-refined using the maximum likelihood refinement program Refmac5 (Murshudov et al., 2004) from the CCP4 (Wimm et al., 2011). We applied above described methods to determine outliers and to calculate the parameters of the distribution before and after removal of outliers. The results of the estimations are given on Table 1. Figure 1 illustrates the histogram and the fitted density plot of the initial B value distribution of the protein structure. Figure 1 and Table 1 show that the initial distribution has a long right tail and low shape parameter respectively. As it was mentioned in (Masmaliyeva, Murshudov, 2017) the shape parameter alpha should be around 3.5.

In Tukey's method for detection of “mild” outliers determined with the factor = 1.5 are too sensitive for B value distribution as shown in the Table 1 and Figure 2 (a). As it is described in (High, 2000; Hartwig, Dearing, 1979), for asymmetric distributions, data values below InnerFence are not always outliers and the values higher than OuterFences are almost always outliers.

It is known (Leys et al., 2013) that the methods based on median absolute deviation instead of mean and standard deviation are more robust to outlier methods because median and median absolute deviation themselves are not affected by few outliers in contrast to mean and standard deviation. Median is robust to 50% outliers meaning that its breakdown point is at 50%, MAD is robust to up to 40%. In our application the method using standard Z-score method seems to give more sensible answers. The reason for this will be a part of future detailed analysis.

The number of outliers detected with each method mentioned above is given on Table 1. There are 610 atoms with outlying B value which is detected by all methods mentioned above. In respect that these atoms were detected by all considered methods, we expect them to be true outliers. Figure 5 drawn by the model building program Coot (Emsley, 2010) shows the electron density of two the amino acid residues detected as outliers. It is clear there is no electron density for these atoms indicating that these residues have been modelled incorrectly. There are just 51 B values which determined as an outlier by just one method and all them are results of Tukey’s method with k=1.5 factor. This means that k=1.5 is very low and should be treated carefully.

Table 1. Number of outliers detected with different methods in B value distribution of 4XKT protein

4XKT	Outliers number	B_0	Min	Max	Mean	Median	Variance	Skewness	Kurtosis	1st Q	alpha	beta
Initial distribution	--	5.356	5.94	131.33	15.98	12.35	154.3	4.52	27.65	10.26	2.77	17.89
Tukey’s method (factor=1.5)	1271	4.828	5.94	26.78	13.22	11.96	17.621	1.11	3.64	10.12	4.5	30.23
Tukey’s method (factor=3)	610	4.9	5.94	36.68	13.96	12.16	29.99	1.63	5.64	10.2	3.87	26.45
Z-score method	383	5.01	5.94	53.24	14.39	12.22	42.16	2.22	9.36	10.22	3.54	23.99
Modified Z-score method	819	4.86	5.94	32.66	13.7	12.09	24.76	1.43	4.86	10.17	4.1	27.94

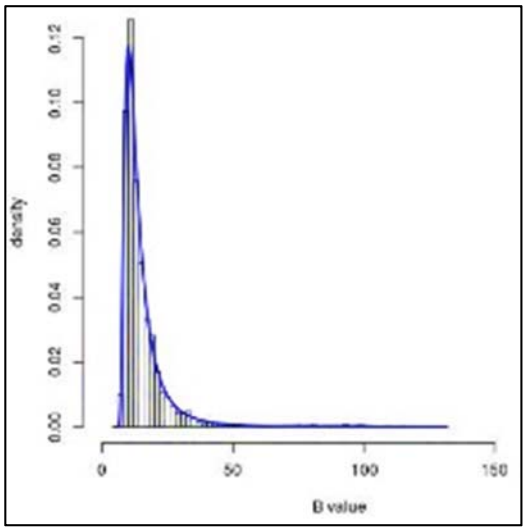


Fig. 1. Initial B value distribution of the protein 4XKT.

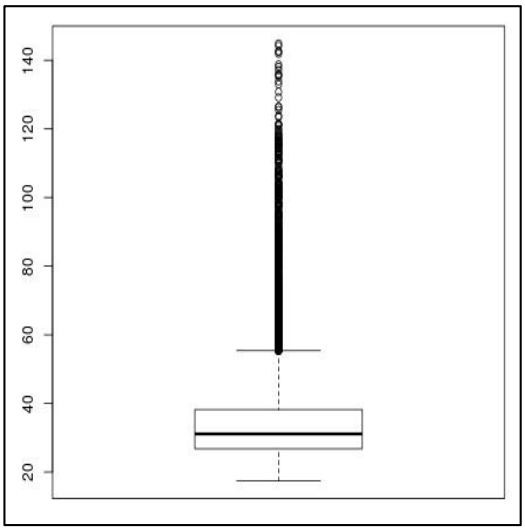
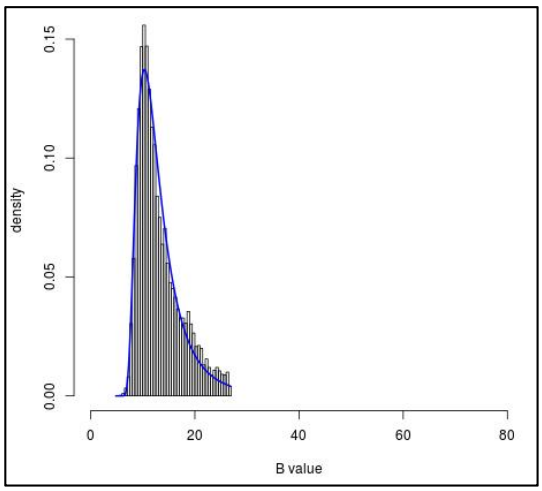
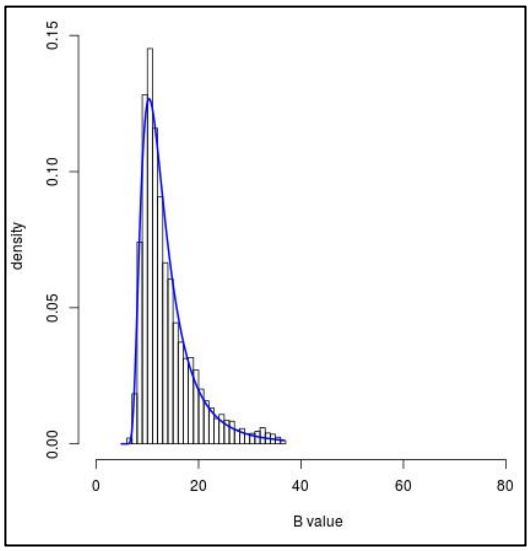


Fig. 3. Box-plot of initial B value distribution of the protein 4XKT.

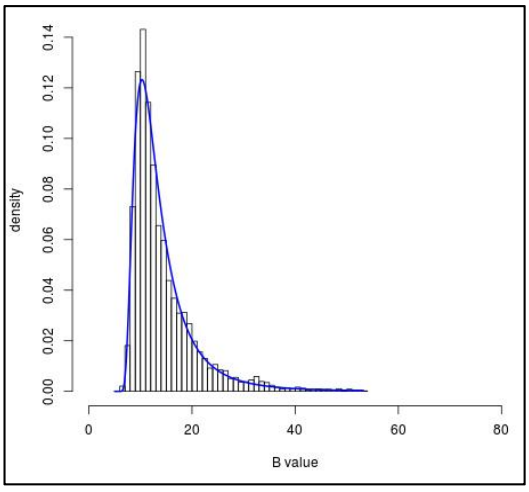


(a)

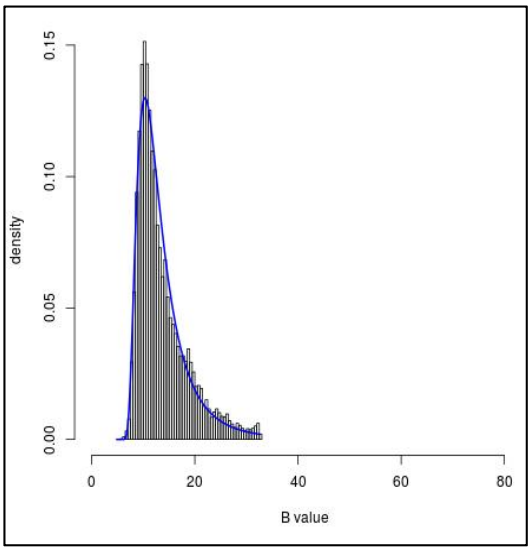


(b)

Fig. 2. B value distribution of the protein 4XKT after removing outliers with Tukey's method with **(a)** k=1.5 and (**b)** k=3.



(a)

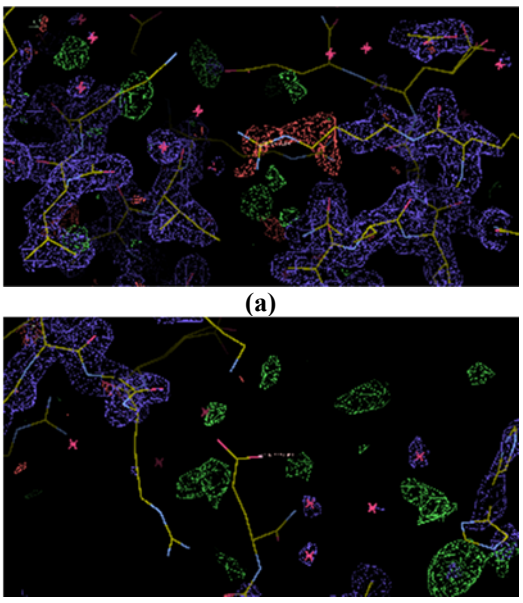


(b)

Fig. 4. B value distribution of the protein 4XKT after removing outliers with **(a)** Z-score and **(b)** modified Z-score method

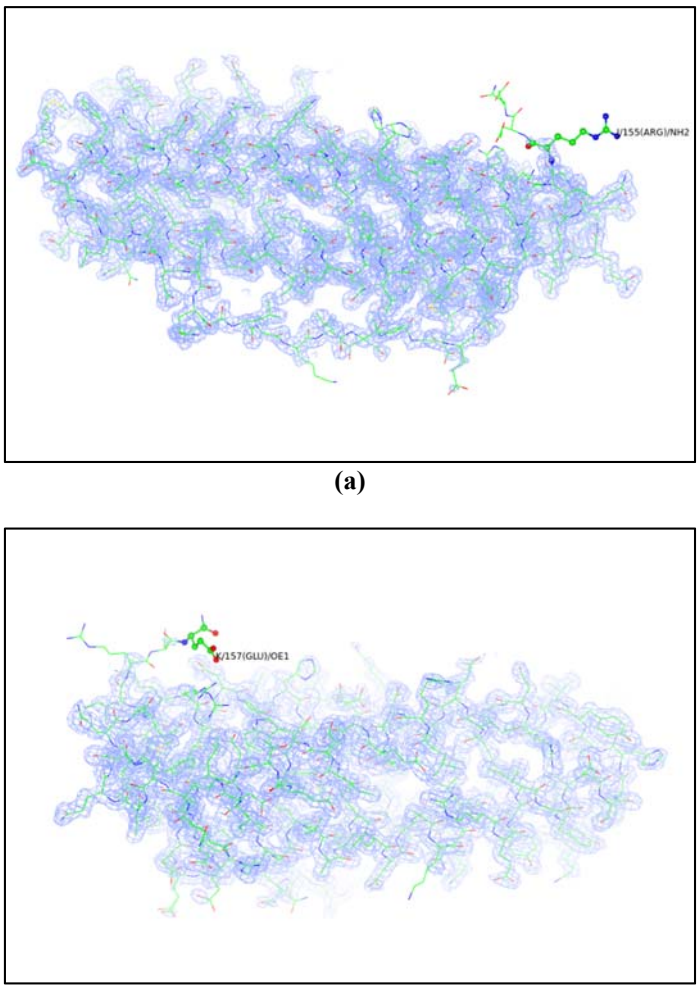
3.2. Local analysis and outlier detection. When atoms are incorrectly placed, the atomic B values become much larger than those of neighbouring atoms, reflecting errors in the model. It is generally expected that neighbouring atoms should have similar B values in regions where modelled atoms are positioned accurately. If neighbouring atom have wildly different B value after refinement, then it usually means that some of the atoms are either 1) in the wrong place; or 2) incorrectly parameterised, for example, occupancies and/or element types for some of the atoms are wrong (Masmaliyeva, Murshudov, 2017).

To detect outliers of atoms in their local environment modified standard deviation was used. As it is mentioned above $sd_{modified} \approx MAD/0.6745$ was used. In the local analysis we detected 4117 atoms with outlying value of B factor value. The largest outlier with B value $98.96 sd_{modified}$ corresponded to OE1 of 157th GLU residue of the chain D (Figure 5 b). In Figure 6 residues with a local outliers described in ball-and-stick mode. With 4.2 radius and modified SD 10.7, 4117 atoms with outlying B value were detected.



(a) **(b)**

Fig. 5. Electron density of some residues containing an atom with outlier B value. (a) 39th residue ARG of A chain; (b) 157th GLU residue of D chain. This figure was drawn using coot [Emsley 2010] (Map sigma = 0.343415).



(a) **(b)**

Fig. 6. Examples of local outliers; electron density of K and J chains of the protein 4XKT with labelled "outliers".
This figure was drawn using ccp4mg (Nicholas, 2011) (Map sigma = 0.49).

4. CONCLUSION AND FUTURE PERSPECTIVES

Outliers are the data points which strongly deviate from the centre of the distribution. In this paper, global and local B value outliers of three-dimensional structures of macromolecules are discussed. Outliers of B values in a structural model indicate errors and/or misinterpretation of the scattering data during model building and refinement. Several outlier detection techniques have been used. These are Tukey's boxplot, Z-score and modified Z-score methods. Removing the extreme values of B values improves statistical estimation of the B value distribution – shifted inverse gamma distribution. B value outlier detection should be used as a part of model building and refinement. It will ensure that atoms are positioned correctly resulting in more accurate atomic models that are usually used for drug design and bioinformatics analysis purposes.

When B values are larger than that of the rest of the atoms then it means that either these atoms are in wrong place or wrongly parametrized. However, during refinement of atomic models using scattering data it is better to assume that the B-values reflect atomic mobility. Therefore, in such cases it is better to restrain the B-values of neighbour atoms to be similar to each other. If they differ wildly it is usually an indication that model contains errors; these errors should be detected and corrected during modelling stage – if it is done on time and with care then accuracy of the resulting atomic models can be increased substantially.

The results of this paper will in future be implemented in a python language based program and distributed to the structural biology community to help them to correct atomic models during model building and refinement.

In future we also plan to extend of B value analyses for modelling of the distributions and detection of outliers for anisotropic B value cases. It seems that by analogy with the isotropic B value distribution the distribution of anisotropic B values should be modelled using the inverse Wishart distribution [Haff 1979] which is used as conjugate priors for multivariate normal distribution. We will also design new methods for anisotropic B value outlier detection: one potential candidate for this is BACON algorithm (Nedret, 2000) which seems to be able to detect with sufficient accuracy outliers in multivariate data.

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Atomik Temperatur Faktoru – B Qiyməti Paylanmasında Autlayerlərin Axtarışı

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Bu məqalədə atom yerdəyişmə parametrlərində (AYP) və ya B qiymətlərinə uyğun olan ehtimal paylanmasından kənar qiymətləri – autlayerləri müəyyən edən bir neçə üsul bir zülal quruluşuna tədbiq olunmuşdur. Bu məqalədə göstərilir ki, belə kənar qiymətlər atom modelində olan səhvləri müəyyən etməyə kömək edir. Bundan əlavə AYP-lərin sürüşən tərs qamma paylanmasına uyğun olması hipotezi də bir zülal tədbiq ilə təsdiqlənmişdir. Biz göstərdik ki, AYP-də olan kənar qiymətlərin aradan götürülməsi ehtimal paylanmasının parametrlərinin qiymətlərinin dəqiqliyini də artırır. AYP-dəki lokal kənar qiymətlər bu zülal quruluşunda olan səhvlərin harada olduğunu göstərir. Gələcəkdə kənar qiymətlərin tapılması nisbətən yaxşı zülal qurluşlarının seçilməsinə də kömək edəcək. Bundan əlavə əgər bu proqram kristalloqrafiya və tək hissəcik cryo elektron mikroskopiyası vasitəsi ilə model qurulması mərhələsində istifadə edliərsə onda alınan modelin etibarlılığı daha yüksək olar.

Açar sözlər: *Makromolekullar, validasiya, makro-molekulyar kristalloqrafiya, autlayet axtarışı, tərs qamma paylanması*

Обнаружение Выброса в Атомном Температурном Факторе - Распределения Значения “B”

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Несколько методов обнаружения выбросов в параметрах атомного смещения - значения B были применены к одной конкретной макромолекулярной структуре. Показано, что выбросы в значениях “B” указывают на ошибки в атомных моделях. Этот конкретный пример доказывает верность гипотезы о скользящем обратном гамма-распределении значений “B”. Удаление выбросов из набора значений “B” улучшает оценку параметров распределения. Локальные выбросы в значениях B указывают на ошибки в атомных моделях. Нами была проверена справедливость предположения о том, что соседние атомы должны иметь одинаковые значения “B”. Ожидается, что локальная и глобальная программы обнаружения выбросов и моделирование значений “B” в качестве обратного гамма-распределения помогут выбрать надежные атомные модели. Мы предполагаем, что такое обнаружение и моделирование выбросов должно быть частью построения модели и уточнения макромолекулярных структур с использованием данных кристаллографической дифракции и карт одноэлектронной криоэлектронной микроскопии.

Ключевые слова: *Макромолекулы, валидация, макромолекулярная кристаллография, обнаружение выбросов, обратное гамма-распределение*

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The PlantProm: A Database of Plant Promoter Sequences (Release 2016)

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Knowledge on promoter sequences and their characteristics is crucial for improving our basic understanding of gene regulation. In 2003, we launched the PlantProm database of 305 plant proximal promoter sequences for RNA polymerase II with experimentally determined transcription start site (TSS). Here, we present a new release of the PlantProm database that contains 576 entries including 150, 403 and 23 promoters of monocot, dicot and other plant genes, respectively, as well as high-throughput annotated and predicted promoters for five plant genomes. The database provides DNA sequences of promoters and their taxonomic/promoter type classification, occurrence of sequence motifs of known plant transcription factor binding sites in promoters, Nucleotide Frequency Matrices for two important promoter elements as TATA-box and Initiator element. In addition, the database includes computationally predicted TSS for 22,257 genes of *Oryza sativa*, 23,334 genes of *Zea mays*, 18,226 genes of *Medicago truncatula*, 38,702 genes of *Glycine max* and 11,037 genes of *Vitis vinifera*. The PlantProm DB is publicly available on http://www.softberry.com/plantprom2016/.

Keywords: *RNA polymerase II, plant promoter, transcription start site, database, promoter elements*

INTRODUCTION

Promoters occupy genomic regions upstream of and around transcription start site (TSS). Information on promoter sequences is fundamental for interpreting gene expression patterns, and constructing and understanding genetic regulatory networks. Transcription factor (TF) binding sites (TFBSs) that define specificity and rate of transcription are positioned in both proximal and distal promoter regions; TFBSs responsible for TSS selection are mostly localized in the proximal promoter, within a hundred nucleotides around the TSS (for review see: Solovyev et al., 2010; Hernandez-Garcia and Finer, 2014; Roy and Singer, 2015). To date, we are still far from complete understanding of genome architecture and functions. Experimental and computational approaches to this problem face significant challenges such as: (a) the mechanisms determining transcriptional status of gene(s) and choice of TSS are still mostly unclear and depend on cell/tissue type, developmental stage and environmental signals (Verona et al., 2008; Zou et al., 2008); (b) Experimental identification of TSSs is still quite expensive and time-consuming; (c) development of computational tools for predicting TSS(s) requires representative learning sets of experimentally validated promoters, but these data are still very limited (Hernandez-Garcia and Finer, 2014; Roy and Singer, 2015).

There are two types of available plant promoter collections: (1) Sets of promoters with TSS(s) determined by the genome-wide mapping of full-length cDNAs (FL-cDNA) and/or 5'-end tagging approaches, as CAGE, 5'-SAGE and TEC-RED (for review see Harbers and Carninci, 2005), presented in plant promoter databases (DB) such as RARGE DB (Sakurai et al., 2005; Akiyama et al., 2014) and ppdb (Yamamoto and Obokata, 2008; Hieno et al., 2014). In particular, the FL-cDNA technology provides valuable information on transcriptional units and facilitates identification of TSSs (Seki et al., 2002; Kikuchi et al., 2003; Ogihara et al., 2004; Sato et al., 2009; Soderlund et al., 2009; Matsumoto et al., 2011; Fukami-Kobayashi et al., 2014). (2) Sets of promoters with TSS(s) identified by direct experimental approaches, as the primer extension assay (Carey et al., 2013) and 5'-RACE (Rapid Amplification of cDNA Ends) assays (Scotto–Lavino et al., 2006), collected in Eukaryotic Promoter Database (EPD; Dreos et al., 2013, 2015) and PlantProm DB (Shahmuradov et al., 2003). EPD was the first representative collection of eukaryotic RNA polymerase II (Pol II) promoters with TSS(s) identified by direct experimental approaches (Praz et al., 2002). However, human and animal promoters prevail in this collection. Promoters of only two plant species, *Arabidopsis thaliana* and *Zea mays,* are currently represented in EPD (Dreos et al., 2013, 2015).

The latest release (version 3.0) of the ppdb

(Hieno et al., 2014; http://ppdb.agr.gifu-u.ac.jp/ppdb/cgi-bin/index.cgi) is the biggest source on TSS positions for plant species, providing information on experimentally mapped TSSs of four plant species, as Arabidopsis, rice, poplar and moss (*Physcomitrella patens*). In particular, the ppdb contains TSS information for all Arabidopsis (27,206) and 12,535 (out of 32,325) rice protein-coding genes annotated in these genomes. However, our analysis of these TSS positions relative to the start points of the annotated coding DNA sequences (CDS) indicates that in some cases the distance between TSSs and CDS start positions is less than 10 base pairs (bp). In particular, we revealed 7,878 (~29%) and 1,554 (~13%) such "TSS-CDS" pairs in Arabidopsis and rice, respectively. Although the minimum length of 5'-untranslated region (UTR) for mRNAs remains unknown, many studies conclude that 5'-UTR should be longer than 20 bp for the efficient binding of ribosomes and initiation of translation (Li and Wan, 2004; Chen et al., 2011; Kim et al., 2014; Hinnebusch et al., 2016). So, our findings indicate that some subset of TSSs collected in the ppdb remains to be verified in future studies.

With the development of advanced experimental techniques, significant progress has been made in the genome-wide identification of promoters/TSSs and analysis of gene regulatory sequences (for review see Mundade et al., 2014; Suryamohan and Halfon, 2015; Levati et al., 2016). Recently, Geng et al. (2014) developed a high-yield screening system in peanut by establishing a simple digital expression profile based on Illumina sequencing that allows, in particular, tissue-specific promoter cloning. However, TSSs identified by these techniques lie only approximately around the real start points of transcription and, therefore, remain to be verified by the other more precise methods such as 5'-RACE (Shiraki et al., 2003; Hashimoto et al., 2004). Therefore, such promoter collections are not suitable for retrieving position-specific promoter features adjacent to the TSS, which is often exploited in computational tools for TSS prediction. To date, the most accurate promoter prediction programs (e.g. see: Shahmuradov et al., 2005; Anwar et al., 2008) have been developed by using promoter sets from PlantProm DB and/or EPD databases that include experimentally verified exact TSS positions.

Previously, we developed PlantProm DB collecting 305 experimentally verified plant Pol II promoters from many published sources (Shahmuradov et al., 2003). It has been used to study a variety of plant biology problems, which include investigating differential expression of soluble pyrophosphatase isoforms in Arabidopsis (Oeztuerk et al., 2015), cis-regulatory elements in plant cell signaling (Priest et al., 2009), a functional role for DNA methylation in transcription (Aceituno et al., 2008) and transcription of nuclear organellar DNA in plants (Wang et al., 2014), as well as many studies of computational promoter identification (Shahmuradov et al., 2005; Pandey and Krishnamachari, 2006; Gan et al., 2009; Tatarinova et al., 2013). All these results demonstrate the importance of our promoter collection.

Here we present a new release of PlantProm DB with 576 experimentally verified promoter sequences, enlarging our collection of 305 promoters from the first release. We provide a structural classification of these promoters and Nucleotide Frequency Matrices (NFM) for their important functional elements, such as TATA box and Initiator element (INR). Applying TSSPlant promoter prediction program (see its description below), we performed the genome-wide search of putative TSSs for protein-coding genes from 5 plant species (*Oryza sativa*, *Z. mays*, *Medicago truncatula*, *Glycine max* and *Vitis vinifera*). Results of these studies are included in this release of the PlantProm DB. Moreover, the new release contains information on statistically significant motifs of 3,032 known plant TFBSs found in 576 experimentally verified promoter sequences and in [-1000:+101] promoter regions of 113,556 genes of 5 plant genomes. At last, we significantly improved the DB interface and its search capabilities.

METHODS

To collect plant promoters with TSS position validated by direct experiments, such as primer extension and 5'-RACE assays, we applied essentially the same rules as described previously (Shahmuradov et al., 2003). To select non-redundant promoter sequences, we used BLAST program (Altschul et al., 1997) for pairwise comparisons of [-50:+1] promoter regions and kept only promoters showing less than 90% sequence homology in these regions.

To classify promoter sequences into the TATA and TATA-less promoters, as well as to compute NFMs for TATA and INR elements, we applied the Expectation Maximization (EM) algorithm (Cardon and Stormo, 1992). Details of EM algorithm for this task were described previously (Shahmuradov et al., 2003).

To predict putative TSSs in genomic sequences we applied novel promoter prediction tool, TSSPlant (Shahmuradov et al., 2017). TSSPlant predicts both TATA and TATA-less promoters in sequences of wide spectrum of plant genomes. It demonstrated significantly higher accuracy compared to other known and available promoter prediction programs,

including TSSP program, trained on previous version of PlantProm DB (http://www.softberry.com/berry.phtml). TSSPplant tool is now available for online running (http://www.softberry.com/berry.phtml?topic=tssplant&group=programs&subgroup=promoter).

For genome-wide search of putative promoters (TSSs) in higher plants we selected protein-coding genes of 5 species: monocots *O. sativa, japonica* (35,655 genes; genome assembly IRGSP-1.0) and *Z. mays* (36,988 genes; genome assembly AGPv3), dicots *M. truncatula* (47,202 genes; genome assembly MedtrA17_4.0), *G. max* (53,151 genes; genome assembly v1.0) and *V. vinifera* (26,118 genes; genome assembly IGGP_12x) from Ensembl genome browser annotation system (http://plants.ensembl.org/info/website/ftp/index.html). For promoter analysis only genes with annotated 5'-UTR length of 20 bp or more were selected. If the selected gene had several gene (mRNA) start points, we consider further only a variant with the longest 5'-UTR. For promoter search we extracted [-1000:+101] regions from the above selected genes, where +1 corresponds to the gene annotated start position. In total, we obtained [-1000:+101] regions for 22,332, 23,467, 18,227, 38,718 and 11,079 genes from *O. sativa, Z. mays*, *M. truncatula*, *G. max* and *V. vinifera*, respectively.

Search for statistically significant motifs of 3,032 known plant TFBSs from the Regsite database (http://www.softberry.com/berry.phtml?topic=regsite) was performed by Nsite program (Shahmuradov and Solovyev, 2015; see also: http://www.softberry.com/plantprom2016/). Nsite executes searches for statistically non-random motifs of known TFBSs in a single DNA sequence. A predicted motif is considered as statistically significant if (i) the expected (by chance) number of such motifs in a given nucleotide sequence is less than an assigned threshold and (ii) the total number of identified motifs is equal to or greater than the upper limit of 95% confidence interval. The search and statistical estimations are performed separately on both strands of a query sequence.

PlantProm database was implemented using Apache WEB Server running on CentOS Linux. MySQL was used as a server database. The server part of Web interface was written in PHP. Modules for downloading gff3 (general feature format 3) annotations and sequence files for individual promoters were written in Perl. The "Search services" used to retrieve information from data tables were implemented using JavaScript library.

RESULTS

General Structure and Content of the PlantProm DB style

Fig. 1 shows the structure and content of PlantProm DB. It consists of seven main modules:

(1) Promoters from direct experiments;
(2) Putative TSS map for protein-coding genes;
(3) Classification of promoters;
(4) Canonical NFMs;
(5) Nucleotide composition;
(6) Regulatory motifs;
(7) Search services.

PlantProm DB release 2016.03 is available at http://www.softberry.com/plantprom2016/. It provides user-friendly interface: all data can be retrieved and downloaded.

Promoters from direct experiments

The module "Promoters from direct experiments" allows a user to retrieve and download 576 promoter sequences of 251 bp length from 87 plant species with TSS identified by primer extension assay and/or 5'-RACE assays, where position 201 corresponds to the experimentally validated TSS (+1). The set includes 305 promoters from the first release and 271 newly added promoters. If this module is chosen in the Main Menu, the sub-menu displayed in Fig. 2 appears. Here, depending on chosen option ("view" or "download") for the selected set of promoters, a user can view or download promoter sequences in FASTA format; with the "view" option, TATA-boxes and transcribed regions are displayed in upper case.

The module "Classification of promoters" is composed of functions to retrieve and download various taxonomic and promoter type (TATA or TATA-less) classes of 576 promoters. It consists of two sections: "Summary" and "Individual Characteristics". In the first section, a list of all species represented in the experimentally verified promoter collection and data on the total number and the number of promoters' of each class are given for each species. If the user visits the "Individual Characteristics" section that is organized as a table, many individual characteristics of genes/promoters and original data sources, including GenBank and PubMed links for every annotated promoter, will be displayed (http://www.softberry.com/data/plantprom/Links/Taxon_Table_2.htm).

The module "Canonical NFMs" allows database users to retrieve and download TATA-box and INR NFMs for various classes of promoters.

The module "Nucleotide composition" contains data on nucleotide composition of promoter regions of various classes, including sequences before the TSS, [-200:-1], and after the TSS, [+1:+51]; the user can view and download this information.

TSSs in five model plant genomes

The module "Putative TSS map for protein-coding genes" allows the user to retrieve and download locations of putative TSSs predicted by TSSPlant program in [-1000:+101] regions of 113,556 protein-coding genes of five plant species (*O. sativa*, *Z. mays*, *M. truncatula*, *G. max* and *V. vinifera)*. In this module, for every genome, 4 options are given (Fig. 3). The user can view and download data on predicted TSSs for every gene in gff or text formats, get information on every gene (gene name and product, genomic positions of a gene and mRNA and CDS starts, number of alternative mRNAs, length of longest 5'-UTR, etc.) and view/download [-1000:+101] region in FASTA format.

Regulatory motifs

The module "Regulatory motifs" contains data on statistically significant (E-value < 0.01; for details of the statistical estimations see Shahmuradov and Solovyev, 2015) motifs of 3,032 known TFBSs and their consensuses in both experimentally verified promoters and [-1000:+101] regions of protein-coding genes from five plant species (Fig. 4). For experimentally verified promoters, the user can view these data for every promoter (out of 576). For 113,556 genes from five species, *O. sativa*, *Z. mays*, *M. truncatula*, *G. max* and *V. vinifera*, a single Nsite output file for every genome is supplied.

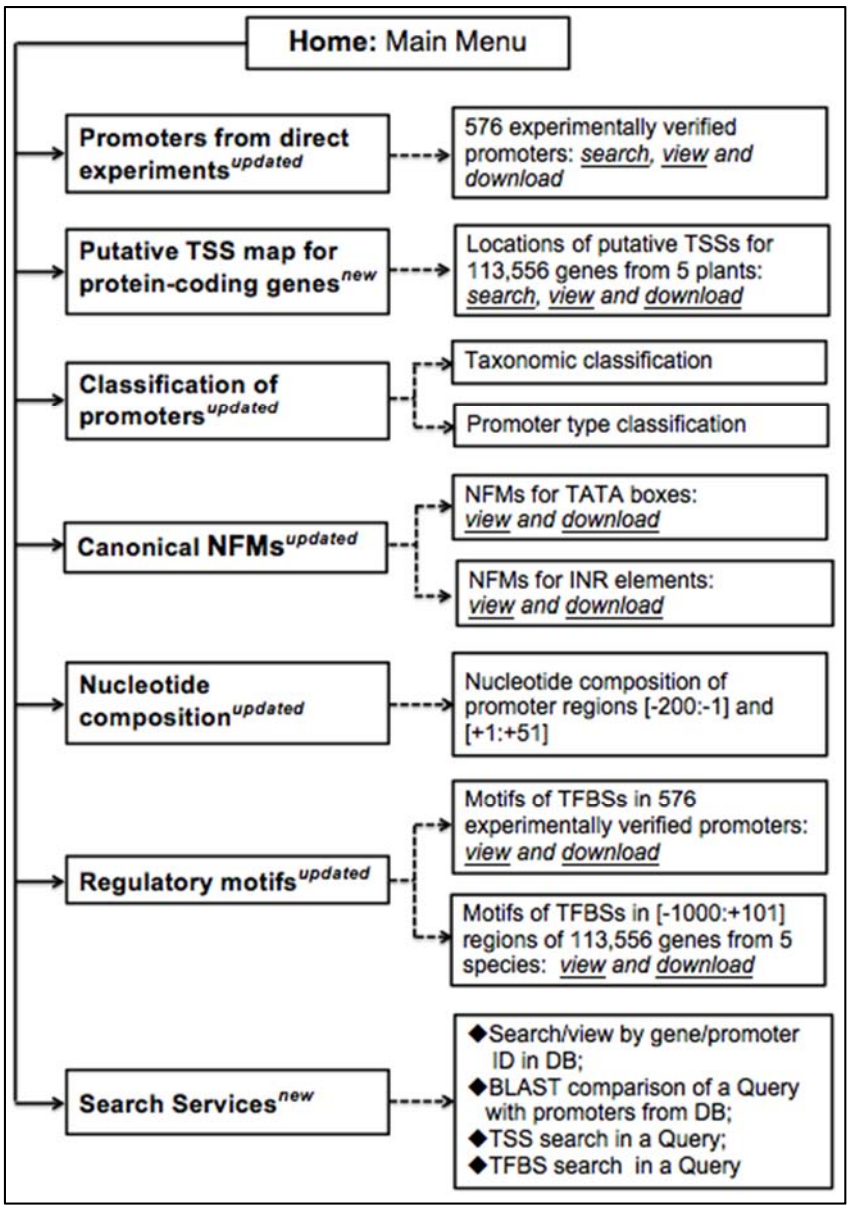

Fig. 1. The structure and content of PlantProm DB. New and significantly updated modules are marked ("*new*" or "*updated*").

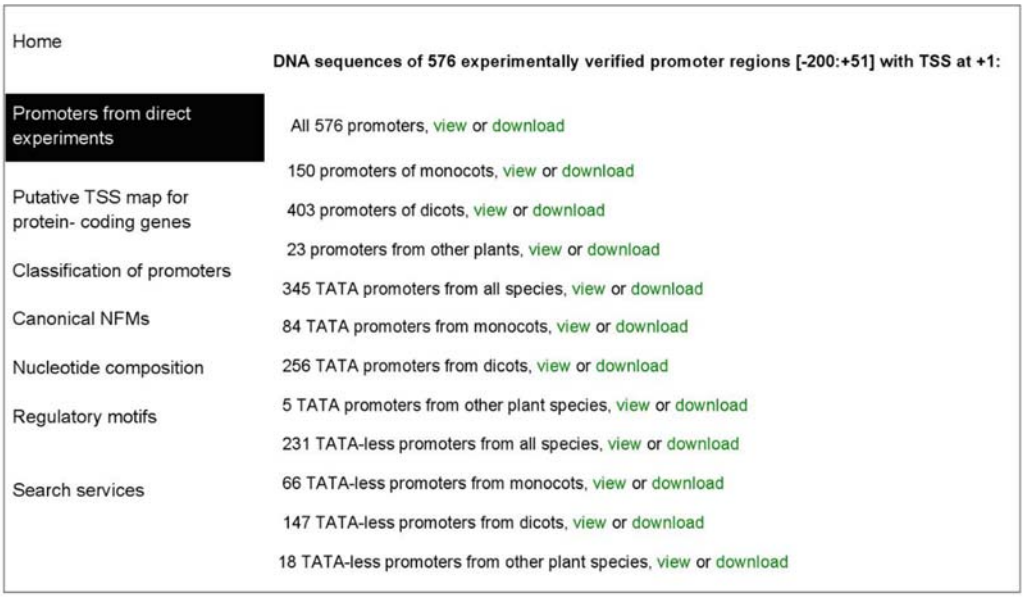

Fig. 2. The information content of the "Promoters from direct experiments" module.

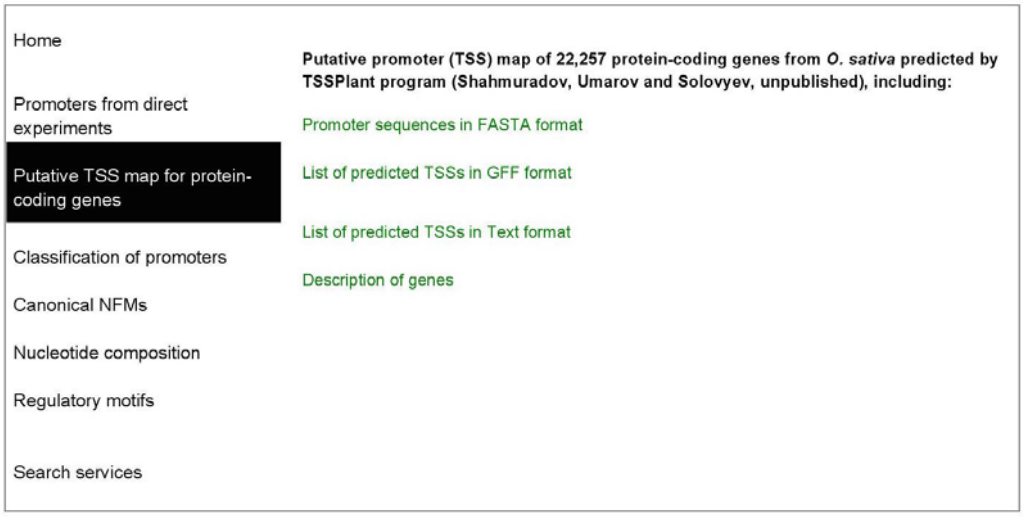

Fig. 3. The informational content of the "Putative TSS map for protein-coding genes" module for *O. sativa* genome.

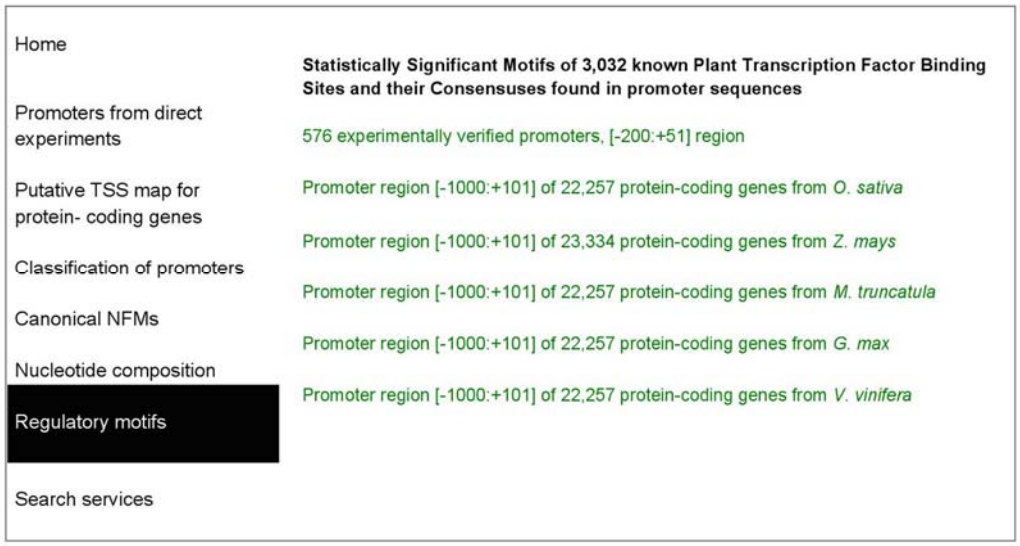

Fig. 4. The informational content of "Regulatory motifs" module.

Search services

Utilizing five options of "Search services" module, the user can retrieve, view and download promoters by their promoter identifier (ID; in set of 576 promoters) or gene ID (in set of 113,556 genes from five species), as well as perform comparison of a query sequence with promoter sequences from PlantProm DB, search for TSS and motifs of 3032 known plant TFBSs.

Option "Search for promoters from direct experiments"

The promoters of interest can be selected (a) by checking their corresponding boxes on the left side of the WEB page or (b) by performing a search using a keyword. Afterwards, if "Get fasta" button is clicked, a page with sequences of selected promoters in FASTA format will appear for a view and downloading. Moreover, promoters can be sorted by the GenBank accession number, organism name, gene name and product.

Option "Search for putative TSS map for protein-coding genes"

For this option the same search and sorting rules are used, as in the case of "Search for promoters from direct experiments". However, here, the selected promoters can be viewed in two popular (FASTA and gff) formats.

Option "BLAST search"

If the user chooses this option, the BLAST program search window will appear. To perform the BLAST search, the following steps are required: (i) paste a query sequence in FASTA format or browse and select a file from your local folder; (ii) choose a promoter set from the given list; (iii) choose the alignment option (**Pairwise** or **Tabular**) and (iv) click **Process** button.

Option "Nsite tool"

When the user chooses this option, the window of search of TFBS motifs by Nsite program is displayed; here, a set of known plant transcription regulatory motifs can be searched in a query sequence.

3.5.5 Option "TSSPlant tool"

If users choose this option, the window of search of putative TSSs by TSSPlant program in a query sequence will appear.

DISCUSSION

The described new release of PlantProm DB contains enlarged collection of experimentally verified promoter sequences and includes several novel additions, such as descriptions of functional motifs in promoter sequences, the computational promoter annotations of five plant genomes, and improved retrieval and search possibilities for different promoter and genome characteristics. In particular, comparison of nucleotide composition of promoter sequences upstream and downstream of TSS in dicots and monocots revealed a significant difference between them in the promoter upstream regions: in dicots they are significantly more A/T-rich.

For 113,556 out of 113,823 genes (99.8%) from 5 genomes, at least one TSS was predictd by TSSPlant program. We computed a distribution of distances between a TSS described in the Ensembl genome annotation (TSSan) and the closest predicted TSS (TSSpr). Such distribution for *G. max* is shown in Fig. 2 (for other genomes see: Supplementary Fig. S7, S8, S9 and S10,). For 55,864 out of 108,938 genes (51.2%), one of the predicted TSSs is located relatively close (at a distance ≤50 bp) to the annotated start site of transcription. However, for ≃49% genes, the predicted TSSs are observed at larger distances from the annotated gene starts. Of course, some of such cases can be explained by a limited prediction capacity of TSSPlant, which is true for all promoter recognition tools published to date. Beyond this possibility, we can consider the followings. We analyzed protein-coding genes with annotated 5'-UTR longer than 20 bp. Among them, for 1,826, 1,218, 1,064, 1,178 and 1,897 genes from *O. sativa, Z. mays, G. max, M.truncatula* and *V. vinifera* genomes, respectively, the annotated length of the longest 5'-UTR was less than 40 bp. To date, the minimal length of 5'-UTR required for proper processing and translation of mRNA is unknown. However, in the same genomes, the longest mRNAs for 8,145, 11,333, 4,606, 17,149, 5,640, 5,238 and 2,828 genes have 5'-UTR lengths of 300 nucleotides or more. This observation can suggest that for significant portion of analyzed genes the annotated 5'-UTRs are truncated, and therefore the distance between the predicted TSS and actual gene start is shorter than we currently observe. Thus, if we take 100 bp (the approximate length of a typical core promoter; Roym and Singer, 2015) as acceptable maximum discrepancy between the predicted TSS and the annotated gene start, then TSSpr for 70,352 genes (~65%) is localized within that range. Another observation of our studies is that the total number of predicted TSSs per gene varies between 2 and 3. It partially agrees with ppdb data for rice: if we consider TSSs separated by 300 bp or more, two TSSs for 257 genes and three TSSs for 15 genes will be presented in the database. So, multiple TSSs seem to be a typical trait of the plant promoter architecture.

All high-throughput promoter identification approaches have their limitations in accuracy of promoter localization, so it is important to support a manually created database with high quality TSSs

and promoter sequences derived from direct experimental studies of particular genes. At the same time, many genome annotation databases such as UCSC (Speir et al., 2016) and Ensembl (Yates et al., 2016) genome browsers contain experimentally discovered and predicted genes (from automatic annotations). It would be beneficial for various gene regulation studies to provide information on promoter location for each annotated gene, i.e. to add putative promoters derived by computational predictions to the current databases' content. We are currently preparing such information alongside with high-throughput promoter identification data for a set of sequenced plant genomes beyond the five already represented in this release.

PlantProm DB furnishes a representative learning set of promoter sequences that is essential for development of plant promoter prediction programs. Annotated regulatory motifs can be used for interpreting gene expression patterns and understanding genetic regulatory networks.

In animals (human, mice, Drosophila, etc.), many genes are regulated by multiple alternative promoters rather than a single promoter (Batut et al., 2013; Hernandez-Garcia and Finer, 2014). Study of alternative promoters has received little attention in plants, although recent advances in genomics and sequencing technologies would accelerate studies of alternate promoter usage in plants (Hernandez-Garcia and Finer, 2014). We are planning to update PlantProm DB regularly including available alternative promoter information.

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PlantProm: Bitki Promotor Ardıcıllıqları Üzrə Verilənlər Bazası (Buraxılış 2016)

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Promotor ardıcıllıqları və onlar səciyyəvi xüsusiyyətləri haqqında biliklər gen tənzimlənməsinin əsaslarının başa düşülməsi üçün həlledici əhəmiyyət kəsb edir. 2003-cü ildə biz RNA polimeraza II üçün transkripsiya start saytı (TSS) təcrübi yolla müəyyənləşdirilmiş 305 bitki proksimal promotor ardıcıllığı üzrə PlantProm verilənlər bazasını təqdim etmişdik. Bu işdə biz PlantProm verilənlər bazasının yeni buraxılışını təqdim edirik. Həmin bazaya birləpəli, ikiləpəli və digər bitkilərdən müvafiq surətdə 150, 403 və 23 promotordan ibarət 576 nümunə, həmçinin 5 bitki genomunun annotasiya olunmuş və güman olunan promotorları üzrə məlumatlar daxildir. Verilənlər bazasında promotorların DNT ardıcıllıqları və onların taksonomik/promotor sinifləri üzrə təsnifatı, promotorlarda transkripsiya faktorlarının birləşmə saytları, TATA-boks və Initiator kimi 2 mühüm promotor elementi üzrə nukleotid tezlikləri matrisləri verilir. Bundan əlavə, verilənlər bazasına *Oryza sativa, Zea mays, Medicago truncatula, Glycine max* və *Vitis vinifera* bitkilərinin müvafiq surətdə 22257, 23334, 18226, 38702 11037 geni üçün potensial TSS-lər üzrəməlumatlar daxildir. PlantProm vürilənlər bazası http://www.softberry.com/plantprom2016/ səhifəsində mövcuddur.

Açar sözlər: *RNT polyimeraza II, bitki promotoru, transkripsiya start saytı, verilənlər bazası, promotor elementləri*

PlantProm: База Данных по Промоторным Последовательностям Растений (Выпуск 2016)

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Знания о последовательностях промотора и их характеристиках имеет решающее значение для понимания основ регуляции генов. В 2003 году мы представили базу данных PlantProm по 305 проксимальным промоторным последовательностям растений для РНК-полимеразы II с экспериментально выявленным сайтом старта транскрипции (ССТ). Здесь мы представляем новую версию базы данных PlantProm, которая включает 576 записей, включая 150, 403 и 23 промотора генов однодольных, двудольных и других растений, соответственно, а также аннотированные и предсказанные промоторы для пяти геномов растений. В базе данных представлены последовательности ДНК промоторов и их классификация по таксономическим/промоторным классам, последовательности мотивов известных сайтов связывания факторов транскрипции растений в промоторах, матрицы нуклеотидных частот для элементов TATA-box и *Initiator*. Кроме того, база данных включает в себя предсказанные ССТ для 22257 генов *Oryza sativa*, 23334 гена *Zea mays*, 18226 генов *Medicago truncatula*, 38 702 гена *Glycine max* и 11 037 генов *Vitis vinifera*. База данных PlantProm доступна на http://www.softberry.com/plantprom2016/.

Ключевые слова: *РНК полимераза II, промоторы растений, сайт старта транскрипции, база данных, промоторные элементы*

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Alteration of Central Metabolism During Plant Adaptation to Abiotic Stresses (Review)

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Plant tolerance to environmental stresses is a polygenic trait, and adaptation to stressful environment is regulated at different levels of plant organization. Widely used gene expression profiling not always allows understanding the essence of the adaptive changes, because of multilevel regulatory processes involved in adaptation. Therefore, an analysis of alterations in metabolites in plants undergoing the adaptation to the stressful environment, probably, is the most adequate approach capable of revealing a complex picture of adaptive responses. Here the information about metabolic adjustments during plants adaptation to abiotic stresses is given with emphasis on waterlogging stress.

Keywords: C*entral metabolism, metabolite profiling, abiotic stresses, stress tolerance*

A large part of soils all over the world is subjected to flooding or waterlogging, which has a strong negative impact on the soils quality and crops. During temporary waterlogging, which is a very common scenario in agricultural practice, water saturates soil for a period of time, wherein roots and lower plant parts are subjected to excessive water while upper parts of plants remain above the water. All plant organs, including aerial, experience severe stress during waterlogging and after waterlogging is withdrawn. Waterlogging alters organic and inorganic composition of soil and accessibility of nutrient, changes the microbial environment, leads to the formation of toxic compounds, and, the most important, it leads to the limitation in available for roots oxygen. Sufficient root oxygen supply is a vital condition for the normal functioning of the plant. The decrease in the level of available oxygen results in alteration of whole-organism metabolism, hormonal status, re-programming of gene expression enabling plants to survive in stressful environment (Blokhina and Fagersted, 2010). Exposure to oxygen following submergence or waterlogging, induces a severe oxidative stress, Reactive Oxygen Species (ROS) formation, resulting in damages to cell structures. Roots responses to waterlogging are more studied and better understood, while the data describing processes occurring in leaves of waterlogged plants are very contradictory. Even in respect to oxygen availability leaf tissue is described by different authors as hypoxic, normoxic, or experiencing oxidative stress. In spite of the significant growing interest to this topic stipulated by its practical importance, not much known about the molecular mechanisms underlying waterlogging and flooding tolerance (van Dongen and Licausi, 2015; Loreti et al., 2017).

Oxygen limitation induces a certain metabolite profile alterations in plant tissue (Borisjuk and Rolletschek, 2009). Accumulation of proteins related to translation and antioxidant defense and an accumulation of a set of enzymes involved in serine, glycine, and alanine biosynthesis from glyceraldehyde-3-phosphate or pyruvate was observed in rice coleoptiles, and accumulation of these amino acids in anoxic rice (Shingaki-Wells et al., 2011). Formation of acetaldehyde and ethanol, accumulation of organic acids, pH lowering are main responses to hypoxia/anoxia. Induction of anaerobic metabolism allows plants to continue ATP supply during a short period of anaerobic conditions (for few hours to few days). Plant leaves are rich in the enzymes necessary for fermentation (Kimmerer and Macdonald, 1987), although till now no correlation between the ability to form acetaldehyde and ethanol and tolerance to flooding was proved, and the order of tolerance did not correlate with root and shoot oxygen content or initial amounts of shoot starch and total soluble sugars (Vashisht et al., 2011). There are contradictory data about oxygen deficiency in leaves of waterlogged plants. In some cases, leaves from root-waterlogged Arabidopsis showed no accumulation of alcohol dehydrogenase and pyruvate decarboxylase mRNA, allowing to assume that leaves did not experience oxygen deficiency (Juntawong et al., 2014). Even when fully submerged under normal illumination shoots tissues remained normoxic (Vashisht et al., 2011; van Veen et al., 2013). Some tolerant species delay or avoid accumulation of ethanol by diverting glycolytic intermediates to alternate end products such as lactate, malate, succinate, y-aminobutyrate,

and alanine (Hook and Crawford, 1978). Typical for anoxic conditions alterations in primary metabolites are characterized by an accumulation of amino acids such as alanine, GABA, and the phosphoesters, glucose-6-phosphate and glycerol-3-phosphate, and other minor sugars (van Dongen et al., 2009; Rocha et al., 2010). Usually, general downregulation of energy-consuming processes under oxygen-limited conditions occurs, when hypoxic signaling pathway is turned on.

Sucrose and glycolysis intermediates. Plant responses to various stresses overlap significantly, and many features of metabolic adaptation are observed in plants subjected to drought, temperature stress, waterlogging or other unfavorable environmental conditions. An increase in sucrose level is one of the most noticeable changes in metabolite profile of plants during adaptation to various stresses, including waterlogging. Levels of raffinose group olygosacharides are also increased in stress-treated plants, as it was shown in many studies. Raffinose, stachyose, and verbascose are osmoprotectants, stabilizers of cellular membranes, scavengers of hydroxy radicals protecting plants from oxidative stress (Nishizawa et al., 2008), are known to accumulate in response to drought, chilling, heat, and high-light irradiation, i.e. all stresses that give rise to excess concentrations of reactive oxygen species (Urano et al., 2009). Raffinose accumulation is regulated by the ABA-independent CBF/DREB1 cold-responsive pathway (Cook et al., 2004) and exhibited an enhanced correlation with dehydration-increased amino acids. Accumulation of raffinose group oligosaccharides in agricultural plants has to be controlled due to their antinutritional properties. The level of myo-inositol, the substrate for mentioned oligosaccharides biosynthesis is also increased in stressed plants. Besides being a substrate for biosynthesis of oligosaccharides and constituents of cell wall (Roberts and Loewus, 1966) inositol is an important metabolic and signaling compound simultaneously, since it plays a role in a phosphate storage, participates in cell-to-cell communication, regulates availability of active auxin in plant tissue and transport (Chen and Xiong, 2010), and coordinates plant responses to salt and dehydration stress (Nelson et al., 1998).

Sucrose synthase route was previously shown in connection with anaerobiosis response (Sturm and Tang, 1999). It was demonstrated that flux via SUS is more important in roots of waterlogged Arabidopsis plants in comparison to ATP-consuming Invertase/hexokinase, but not in seedlings grown on carbon source. Sucrose cleavage through the SUS pathway depends on balanced ratios of UDP/UTP, regulated by nucleoside diphosphate kinase (NDPK) (Bailey-Serres and Voesenek 2008). NDPK activity is enhanced in plant organs under low oxygen (Perata et al., 1996; Guglielminetti et al., 1995), its mRNA is highly expressed in tissues with a low-oxygen microenvironment and high energy demands, such as meristems (Dorion et al., 2006) and seed endosperm (Sanclemente et al., 2016). An increase in UDP-glucose level may be an indicator of hypoxia stress, while under normoxic conditions invertase/hexokinase pathway is a dominant rout.

Tricarboxylic acid (TCA) cycle. A major alteration in TCA cycle intermediates is an accumulation of Succinate. Succinate accumulation is a well-known general plant response to environmental stresses. The addition of succinate salt to the root medium prior to Cu treatment increased the capacity of the maize plants to partially overcome Cu toxicity (Doncheva et al., 2006). A decrease in 2-oxoglutarate level under stress conditions may be an indicator of N deficiency in plants, since the level of 2-oxoglutarate, a key regulator of carbon and nitrogen interactions, decreased under N starvation (Obata and Fernie, 2012). A decrease in the levels of isocitrate was previously observed in plants subjected to high light stress and low temperature. Isocitrate and citrate - an important metabolic branch point, which provides carbon skeletons for nitrogen assimilation and reducing equivalents for biosynthetic reactions, support the functioning of the glyoxylate cycle and the process of gluconeogenesis, and play an important role in the TCA and in energy metabolism, associated with production of secondary metabolites, fatty acid oxidation and biosynthesis (Popova and de Carvalho, 1998). Many enzymes of the tricarboxylic acid cycle such as aconitate, pyruvate-, and 2-oxoglutarate-dehydrogenase are known to be sensitive to oxidative inhibition (Verniquet et al., 1991; Sweetlove et al., 2002), so decrease in mentioned TCA metabolites may indicate that oxidative stress takes place in plants.

Amino acids. Amino acids are not only protein constituents, but also a bridge between primary and secondary metabolism, carriers of nitrogen between roots and above-ground part of plants, precursors of many secondary metabolites, which play role in formation of structural components, and defense (Pratelli and Pilot, 2014). Amino acids profile is altered significantly in stressed plants.

Increase in Ser level is also known to be a general stress response (Rai, 2002). Alanine,

lactate, and GABA were shown as major metabolites increased in 2 hours in response to oxygen deprivation (Mustroph et al., 2014). Increase in Ala and GABA or enzymes involved into these metabolites biosynthesis were described in many cases for oxygen-deprived plant tissue, even under mild hypoxia (Miyashita et al., 2007; Limami et al., 2008). Reverse conversion of alanine to pyruvate during recovery from low-oxygen stress plays a role in Ala reuse after re-aeration (Miyashita et al., 2007). The level of Ala correlates with the intensity of starch catabolism and decreases under oxidative stress conditions. Ala and lactate prevent the accumulation of Pyr in order to avoid inhibition of glycolysis and fermentation (Rocha 2010b). Branched-chain and aromatic amino acids are substrates for many secondary metabolites biosynthesis. Branched-chain amino acids regulate with back regulation hormones gibberellins and IAA (indole acetic acid) (Gao et al., 2009; Parsons et al., 2015). These amino acids (especially leucine) are able to participate in regulation of gene expression (Kimball and Jefferson, 2006; Binder, 2010; Obata and Fernie, 2012), increase in these amino acids level was detected in plants subjected to water deficiency stress, including tomatoes (Semel et al., 2006), grain cultures (Bowne et al., 2012; Witt et al., 2012). Valine is also used for synthesis of proteins and secondary metabolites, accumulation of Val was shown in connection to elevated CO2 concentration in drought-stressed plants, drought stress tolerance (Merewitz et al., 2012), heat shock (Kaplan et al., 2004), and heat tolerance (Jingjin et al., 2012). Shikimate pathway, derived from PEP, a precursor of Pyr, leads to the formation of aromatic secondary metabolites, such as lignin, flavonoids, alkaloids, phytoalexins. These metabolites also have antioxidant property. Aromatic amino acids derived from the shikimate pathway are used in biosynthetic pathways for production of pigments, hormones, and cell wall components. Lignin provides protection against oxygen loss (Bailey-Serres et al., 2012). Shikimate pathway is a major consumer of photosynthetically fixed carbon in vascular plants (Jorgensen et al., 2005; Vogt, 2010). Accumulation of aromatic amino acids in response to biotic and abiotic stresses was previously shown (Kim et al., 2007). Because their biosynthetic pathways have been lost in animal lineages, these amino acids are essential components of the human diet (Maeda and Dudareva, 2012). Both, aromatic and branched chain amino acids are substrates for glucosinolates biosynthesis, defensive compounds, involved in protection against biotic and abiotic stressors in cruciferous (Glawischnig et al., 2003).

Oxaloacetate family amino acids levels, including Asp and Asp-derived Asn and methionine, are decreased under conditions of oxidative stress. Thr and Gly levels remain unchanged under low oxygen conditions (Mustroph et al., 2014). A possible connection between Asparagine-family pathway and Lys-branch was discussed previously, and increase of Asp-family pathway flow into the Lys branch on the expense of flux towards the other branches which leads to the synthesis and further catabolism of Met and Ile was shown (Galili, 2011). Stresses usually suppress the expression of genes encoding biosynthesis enzymes of the Asp-family pathway and stimulate the expression of catabolic genes of the Asp-family pathway.

The observed decrease in the level of Deoxyadenosine, S-adenosyl methionine, S-adenosyl-L-homocysteine can be a result of the deficiency in S and N or intensified consumption of this amino acid. Methionine is a precursor of ethylene through S-adenosylmethionine (Ravanel et al., 1998). Flooding and waterlogging stresses are accompanied by the production of ethylene, which regulates many reactions in response to these stresses (Sasidharan and Voesenek, 2015). Probably, decrease in methionine and related to methionine amino acids levels is a result of these amino acids consumption for ethylene biosynthesis. Methionine also can be used for polyamines biosynthesis. In cruciferous Met and Ala can be also substrates for the major class of glucosinolates biosynthesis.

Most noticeable alteration in a profile of 2-oxoglutarate-derived amino acids is an increase in the level of proline, a well-known stress response metabolite, accumulation of which was documented in response to a range of abiotic stresses. Ornitine and citrulline are possible shuttles of nitrate and carbon between mitochondrion and plastids. Levels of these amino acids are increased in response to low temperature (Cook et al., 2004). Ornitine – can be a source of proline synthesis, since proline in non-stress plants can be toxic for plant cells, ornithine accumulates as a precursor, it is a "gatekeeper" in controlling polyamines and GABA biosynthesis (Majumdar et al., 2016). Citrulline is a nonessential amino acid that is reported to be an efficient hydroxyl radical scavenger and is a strong antioxidant (Akashi et al., 2001; Rimando and Perkins-Veazie, 2005), this amino acid is critical to the detoxification and elimination of unwanted ammonia within cells (Nelson and Cox, 2000). Citrulline accumulation correlates with tolerance to salt and drought stress (Yokota et al., 2002; Kusvuran et al., 2013). Oxidative stress results in a strong decrease in Proline and serine Gln levels, similarly Asp and Asp-derived Asn, homoserine

and Met linked to oxaloacetate and Ala linked to pyruvate is decreased. Adaptation to low-temperature stress is accompanied by increases in ascorbate, ornithine, and citrulline. The accumulation of shikimate, phenylalanine, and fructose, and the decrease of succinate are found in both low temperature and light stress-treated plants (Kaplan et al., 2004; Obata and Fernie, 2012).

His is a chelator of toxic ions (Kramer et al., 1996; Zemanová et al., 2014), important for plant reproduction and growth (Stepansky and Leustek, 2006). His biosynthesis is connected with the pentose phosphate pathway, de novo biosynthesis and salvaging of purines, pyrimidines and the pyridine nucleotide cofactors NAD and NADP (Alifano et al., 1996; Ingle, 2011). Not much is known about the regulation of His level in plant. His biosynthesis process is very susceptible to feedback inhibition. In fungi, regulation of His biosynthesis is tightly coordinated with that of purine biosynthesis and is regulated by adenine limitation (Springer et al., 1996).

Antioxidants. Virtually all biotic and abiotic stresses are accompanied by oxidative stress, including hypoxic/anoxic stress (Pucciariello et al., 2012), and the ability to detoxify activated oxygen species is related to a higher tolerance to environmental stresses. Ascorbate (Asa) and reduced glutathione (GSH) are the main antioxidant components present in most plant cell organelles like mitochondria, chloroplasts, and peroxisomes. Asa can react with reactive oxygen species, such as 1O2, HO· and can act as the substrate for the enzyme ascorbate peroxidase. GSH acts as a cell redox regulator and may act as a ROS scavenger. The balance between GSH and oxidized glutathione (GSSG) is critical for keeping a favorable redox status for the detoxification of H2O2 (Foyer and Noctor, 2011). NAD and NADP play a central role in maintaining plant energy status and redox homeostasis (Hashida et al., 2009). NAD is used primarily in respiratory ATP production whereas NADP is used in reductive biosynthesis. Furthermore, a decrease in the NAD/NADP ratio is tied directly to photosynthetic activity, at least in cyanobacteria (Tamoi et al., 2005). It is also assumed that NADP biosynthesis plays an important role in ROS scavenging.

An adverse effect of stress on plants is also associated with nutrient deficiency (Steffens et al., 2005). Available in the literature data describe an increase in P uptake under waterlogging conditions (Rubio et al., 1997). Severe phosphor (P) deficiency leads to increased levels of phosphorylated intermediates (glucose-6-P, fructose-6-P, inositol-1-P, and glycerol-3-P) and organic acids (2-oxoglutarate, succinate, fumarate and malate). P-deficient plants modify carbohydrate metabolism initially to reduce P consumption and salvage P from small P-containing metabolites, which consequently reduce the levels of organic acid in the TCA cycle (Huang et al., 2008). An increase in the levels of Succinate and malate is a typical feature for P-deficiency stress, also found in waterlogged samples. The observed decrease in purine and pyrimidine derivatives can be a result of N deficiency either. N-uptake could be an important factor in waterlogging tolerance (Kreuzwieser et al., 2002). In general, the combination of the nutrient deficiency and oxidative stress shape the metabolic profile of plant leaves under waterlogging conditions.

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Bitkilərin Abiotik Streslərə Adaptasiyasında Mərkəzi Metabolizmin Dəyişiklikləri (İcmal)

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Bitkilərin ətraf mühitin stress amillərinə davamlılığı poligen xarakter daşıyır. Ətraf mühitin əlverişsiz şəraitinə adaptasiya isə bitki orqanizmlərinin müxtəlif səviyyələrində tənzimlənir. Tənzimləyici sistemlərin çoxpilləli olması hesabına ekspressiya olunan genlərin profilinin geniş tətbiq olunan analizi baş veən adaptiv dəyişiklikləri başa düşməyə imkan vemir. Bu baxımdan, stresə adaptasiya prosesində bitki metabilitlərinin miqdarının dəyişməsinin analizi daha düzgün yoll olub, mürəkkəb adaptiv cavab reaksiyalarını aşkar etməyə imkan verir. Təqdim olunan işdə torpaqda su basmalarınını əsas götürməklə, bitkilərin abiotik stresslərə adaptasiyası zamanı baş verən metabolitik yerdəyişmələr haqda olan məlumatlar analiz edilir.

Açar sözlər: *Mərkəzi metabolizm, metabilitik profilləşmə, abiotik streslər, stresə tolerantlıq*

Изменения Центрального Метаболизма в Процессе Адаптации Растений к Условиям абиотического стресса (Обзор)

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Устойчивость растений к стрессам окружающей среды носит полигенный характер, а адаптация к неблагоприятным условиям окружающий среды регулируется на разных уровнях организации растительного организма. Широко применяемый анализ профиля экспрессируемых генов не всегда позволяет понять суть происходящих адаптивных изменений по причине многоуровневости регуляторной системы, отвечающей за адаптацию. В связи с этим, анализ изменений уровня метаболитов растений, происходящих в процессе адаптации к стрессовым условиям, представляется наиболее адекватным подходом, способным раскрыть сложную картину адаптивных ответов. В данной работе мы проводим анализ данных о метаболических перестройках, происходящих в процессе адаптации растений к условиям абиотических стрессов, с акцентом на стрессе, связанном с затоплением почвы.

Ключевые слова: *Центральный метаболизм, метаболитное профилирование, абиотический стресс, стрессоустойчивость*

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Influence of Growing Conditions on Chlorophyll Content, Photosystem II Activity and Productivity of Tomato Varieties

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The present work was carried out to determine the effects of manure and $CaCO_3$ on growth, content of photosynthetic pigments, activity of photosystem II (PS II) and yield parameters of six varieties of tomatoes. The plants were grown under conditions of closed (greenhouse) and open ground, with manure applied in the calculation of 500 g and $CaCO_3$ (chopped eggshell) 50 g per 1 m^2 of soil. It was revealed that the tomato varieties Tolstoy and Volgograd have a high photosynthetic apparatus activity and productivity, which can be used in breeding works.

Keywords: *Tomato varieties, manure, chlorophyll, carotenoids, PSII, yield*

INTRODUCTION

Tomato is one of the valuable vegetable crops grown all over the world to provide the needs of the population with valuable natural compounds, as well as for processing in canneries. Growing tomatoes in winter greenhouses is of enormous economic importance for providing the population with vitamins C, B1, B2, B3, PP, as well as elements of potassium, sodium, magnesium, phosphorus, iron, sugars, apple and citric acids and proteins. Tomatoes are demanding soil fertility, especially phosphorus, nitrogen and potassium. In the seedling period, tomato intensively consumes potassium and phosphorus, later nitrogen. Plants use nitrogen to form vegetative organs, especially in the period from sprouting to flowering. The consumption of phosphorus is mainly associated with the growth of the root system, fruit and seeds. Potassium is especially needed during the period of growth and maturation of fruit. Tomatoes also need other microelements: sulfur, iron, boron, manganese and others. To obtain a high yield, it is necessary to increase the concentration of carbon dioxide, which can be increased by adding manure to the soil where tomatoes will grow. It is considered that first of all it is important to select organic materials instead of using synthetic fertilizers in organic vegetable growing in order to increase soil productivity. Therefore, green manure, composts and other organic fertilizers should be used in cultivation of organic vegetables (Harun, 2017). Manure is an environmentally friendly and economically beneficial organic fertilizer. In experiments carried out using sandy soil, with the addition of organic fertilizers, plant growth was markedly accelerated in comparison with control plants (Zhang et al, 2011; Cui et al., 2002**)**. Organic fertilizers also neutralized the acidity of the soil (Cui et al., 2003) and increased the activity of catalase (Chen et al., 2003). The addition of various stimulants improves the quality of the crop (Yongxia et al., 2013), stress tolerance (Giri et al., 2003). It is known that calcium is one of the necessary elements for the growth and development of plants, and it also removes the toxic effect of harmful ions for plants, such as sodium ions. In earlier studies it was shown that providing additional Ca had reduced some of the detrimental effects of Na on tomato and other crops (Navarro et al., 2005; Francesco et al., 2009).

Based on this, the purpose of our studies was to study the effect of manure and $CaCO_3$ on the growth, photosynthesis and productivity of different varieties of tomato.

MATERIALS AND METHODS

The objects of study were six varieties of tomato, grown under the conditions of a greenhouse and open ground. The manure was applied with the calculation of 500 g and $CaCO_3$ (chopped eggshell) 50 g per 1 m^2 of soil. In the phases of plant development, leaf samples were taken to determine the content of chlorophyll and carotenoids. The efficiency of the photosystem (Fv/Fm) was determined using a photosynthesis analyzer (PAM, Germany). The activity of photosystem II (PS II) was determined on the polarograph (OH103) by releasing oxygen with application of the Clark electrode**.** (Grishina, 1971). The content of

chlorophylls and carotenoids was determined on the spectrophotometer (Multiscan GO, Germany) by trituration the leaves in 80% acetone, measuring the absorption at 645, 663, and 440, using the Wettstein and Arnon coefficients (Khanishova et al., 2008). Data analysis and statistical analysis were conducted using Microsoft Excel. Statistical analysis was performed with the aid of the Statgraphics Plus 5.1 statistical package. The means of values were compared by Duncan's multiple range test (p=0.05).

RESULTS AND DISCUSSION

The results of experiments on the effect of manure on the content of photosynthetic pigments and on fluorescence parameters are shown in Table 1.

As can be seen in Table 1, manure positively affects the content of chlorophyll a + b and carotenoids. The ratio a/b increased, which indicates an accelerated synthesis of chlorophyll a. The manure also contributed to an increase in the activity of the photosynthetic apparatus of tomatoes (Figure). Photosystem 2 activity increased by 65% in Tolstoy, that markedly exceeded the activity in other varieties. To measure the physiological state of plants on whole leaves, the values of the ratio Fv/Fm were measured. As can be seen in the table, the values of Fv/Fm in control and experimental plants are significantly different. Inter-variety differences are also observed. Our data are consistent with generally accepted opinions that the values of the parameter Fv/Fm above 0.74 reflect the favorable state of the plants.

To study the effect of calcium on the growth and development of tomatoes, we used a chopped eggshell as organic calcium. The results of experiments obtained using organic calcium are given in figure.

According to several authors (Mahmoud et al., 2014; Saidu et al., 2011; Tiamiyu et al., 2013; Ayoub, Afrah, 2014) manure when decomposed increases both macro and micro nutrients as well as enhances the physical and chemical properties of the soil; this led to its high vegetative growth. The nonsignificant difference observed in the treatments supplied with goat and cow dung with control treatment could be either there were some nutrients already present in the soil or the plants need were satisfied with the quantity of nutrients present in the soil. Tomato grown on poultry manure and sown at the right time performed better in terms of the height of the plant than the other sources of organic manure and sowing date. This shows that poultry manure was readily available and in the best form for easy absorption by the plant roots, hence there was a boost in the morphological growth of the plant. The obtained results corroborated the finding of in okra (*Abelmoschus esculentus* L.) production in which they reported that organic manure, especially poultry manure could increase length of crops when compared with other sources of manures and sowing dates.

Table 1. Effect of manure on content of chlorophyll, carotenoids and the efficiency of the photosystem II

Variety	Chlorophyll(a+b)mg/g		Carotenoids mg/g		F_v / F_m	
	Control	Experiment	Control	Experiment	Control	Experiment
Rally	0.97*	1.5	15.2	16.8	0.7	0.8
Tolstoy	0.97	1.8	15.2	16.9	0.7	0.8
Volgograd spring	0.79	1.7	10.9	13.2	0.8	0.8
Volgograd autumn	0.81	2.0	12.3	14.5	0.7	0.8
22-74	0.50	1.1	21.1	23.2	0.5	0.6
Falkon	1.10	1.4	20.8	22.4	0.5	0.6

* Each value represents the mean ± SD (standard deviation) for the mean n = 3 independent experiments p = 0.05.

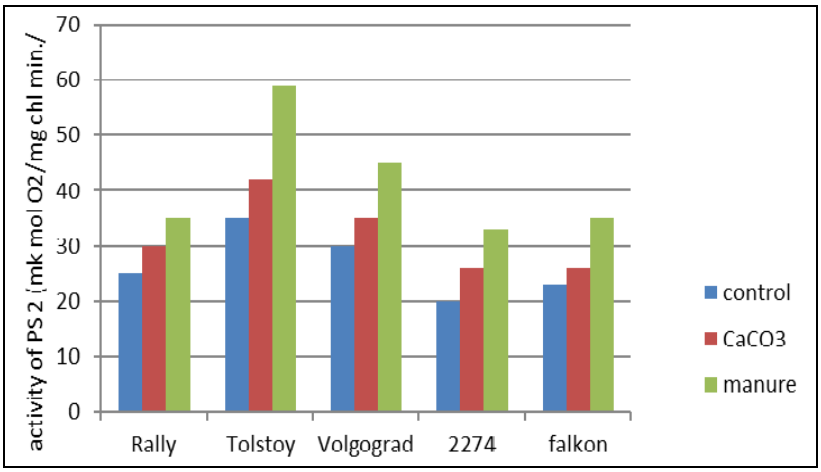

Fig. Effect of $CaCO_3$ and manure on activity of PS2 of tomatoes.

Table 2. Effect of manure on height and yield of tomato plants

Variety	Variant	Height, cm	Harvest of a single plant, g	Harvest, m^2 / kg	average fruit weight, g
Rally	control	51	750 ± 61	15	40
	manure	60	840 ± 68	17	45
Tolstoy	control	65	1200 ± 72	19	46
	manure	74	1370 ± 75	23	50
Volgograd spring	control	62	1300 ± 68	21	48
	manure	66	1450 ± 71	25	52
Volgograd autumn	control	55	1060 ± 65	19	44
	manure	60	1270 ± 68	22	49
22-74	control	45	550 ± 34	15	42
	manure	52	670 ± 45	18	45
Falkon	control	48	630 ± 46	16	43
	manure	64	750 ± 55	19	46

* Each value represents the mean ± SD (standard deviation) for the mean n = 3 independent experiments p = 0.05.

The non-significant effect of manure sources on fruit length could be due to the effect of these sources of organic manure on enhancing vegetative growth. All the nutrients supplied by the different manure sources might have been diverted to vegetative growth. This could be due to their bulkiness and higher amount of nutrients already present in the soil could contribute to this phenomenon.

The organic fertilizer affected the morphometric parameters of plants- stem diameter, wet weight of the aboveground parts of plants (Table 2). As can be seen in Table 2, there are differences between the varieties. The tomato variety Tolstoy has the highest morphometric parameters. Our studies have shown that the application of organic fertilizer has unequivocally increased the growth, the diameter of the stem, the wet weight of the aboveground and underground parts, as well as the productivity of tomatoes. According to the literature data, organic fertilizer improves the water potential of the soil, facilitates the entry of elements of mineral nutrition into the roots of plants (Chen et al., 2003). During the drought, manure prevents evaporation of water and promotes moisture retention in soil capillaries around the root system of plants. In drought conditions, varietal characteristics are also revealed: some varieties use mineral elements more intensively, others more slowly. In our experiments the Volgograd and Tolstoy varieties were the most intense, which, under identical conditions of supply with organic fertilizer, proved to be the most productive.

CONCLUSION

When growing 6 different varieties of tomato with the introduction of organic fertilizer the most productive were the varieties Tolstoy and Volgograd, which can be used in breeding for obtaining more highly productive varieties.

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Becərilmə Şəraitinin Tomat Sortlarında Xlorofilin Miqdarına, Fotosistem II -nin Fəallığına və Məhsuldarlığa Təsiri

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Təqdim olunan işdə peyinin və $CaCO_3$-ın 6 tomat sortunda fotosintez piqmentlərinin miqdarına, fotosistem II -nin fəallığına və məhsuldarlığına təsiri öyrənilmişdir. Bitkilər qapalı (istixana) və açıq torpaq şəraitində 1 m^2 sahəyə 500 q peyin və 50 q $CaCO_3$ (üyüdülmüş yumurta qabığı) verilməklə əkilmişdir. Müəyyən edilmişdir ki, Tolstoy və Volqoqrad tomat sortları yüksək fotosintez fəallığına və məhsuldarlığa malikdirlər və onlar seleksiya işlərində istifadə oluna bilərlər.

Açar sözlər: *Tomat sortları, peyin, xlorofil, karotinoidlər, fotosistem II, məhsuldarlıq*

Влияние Условий Выращивания на Содержание Хлорофилла, Активность Фотосистемы 2 и Продуктивность Сортов Томата

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Настоящая работа проводилась с целью определения влияния навоза и $CaCO_3$ на рост, содержание фотосинтетических пигментов, активность фотосистемы 2 (PS 2) и урожайные показатели шести сортов томатов. Растения выращивали в условиях закрытого (тепличного) и открытого грунта с внесением на 1 $м^2$ почвы 500 г навоза и 50 г CaCO3 (расколотая яичная скорлупа). Выявлено, что сорта томата Толстой и Волгоград обладают высокой активностью фотосинтетического аппарата и продуктивностью, и могут быть использованы в селекционных работах.

Ключевые слова: *Томаты, навоз, хлорофилл, каротиноиды, фотосистема 2, продуктивность*

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Polypeptide Pattern of Mesophyll and Bundle Sheath Thylakoids of Maize Chloroplasts

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The energy converting apparatus of the photosynthesizing oxygenic species organized in several different multisubunit protein complexes is associated with thylakoid membranes. A characteristic feature of C_4 plants is the differentiation of the photosynthetic leaf tissues into two distinct cell types, mesophyll (M) and bundle sheath (BS) cells. In this study, polypeptide patterns of mesophyll and bundle sheath thylakoids of maize (*Zea mays* L.) have been analyzed. The amount of the PSI core apoprotein (68 kDa) was found to be higher in bundle sheath compared with mesophyll thylakoids. α and β subunits (with molecular masses of 55 kDa and 52 kDa) of CF1 domain of the ATPase complex were present in both subcellular fractions. But the amount of α subunit was smaller in the bundle sheath thylakoids. The protein of 45 kDa belonging to the core antenna of PSII was more intensive in mesophyll thylakoids. Polypeptides (with molecular masses in the region of 28-24 kDa) in the composition of the light-harvesting complex II were present in both types of thylakoids. However, in the thylakoids of bundle sheath cells their amounts were reduced.

Keywords: *C_4 plants, mesophyll, bundle sheath, chloroplasts, thylakoids, Zea mays*

INTRODUCTION

In higher plants, the photosynthetic apparatus is compartmentalized in the specialized chloroplast organelle. The molecular machinery for the primary photosynthetic processes, the sunlight-driven generation of metabolic energy equivalents, is harbored in a thylakoid membrane system within the chloroplasts (Dekker and Boekema, 2005; Austin and Staehelin, 2011; Kirchhoff et al., 2013). An essential feature of the thylakoid membrane system is its high flexibility, which is required for adaptability and maintenance of the photosynthetic machinery in plants. Highly responsive to environmental conditions, the molecular membrane composition can change remarkably to optimize, protect, and maintain the photosynthetic apparatus (Melis, 1991; Walters, 2005; Jonson et al, 2011).

The protein complexes that catalyze electron transfer and energy transduction are unevenly distributed in thylakoids. The majority of the Photosystem II (PS II) complexes and light-harvesting complex II (LHC II) are largely found in the grana stacks while photosystem I (PS I) and ATP-synthase are located in the stroma exposed regions and the cytochrome b_6/f complex is evenly distributed in granal margins (Melis, 1991; Ke, 2001; Nelson and Yokum, 2006; Seibert, 1993; Stachelin and van-der Stay, 1996; Bukharov, Abdullayev, 1990; Andersson and Anderson, 1980; Mathis and Rutherford, 1987; Süss et al, 1993).

Photosystem II has an outer antenna dominated by light harvesting complex II (LHC II), which binds chlorophyll a, chlorophyll b, carotenoids and inner antenna of chlorophyll a binding proteins CP 47 and CP 43. The D1 and D2 polypeptides from the heterodimer of the PS II reaction center core that carries most of the cofactors are involved in electron transfer. Most proteins in the PS II complex are membrane spanning, but three extrinsic proteins involved in oxygen evolution are located on the lumenal side of the thylakoid membrane. In higher plants and green algae these proteins are nuclear encoding subunits of PsbO (33 kDa), PsbP (23 kDa) and PsbQ (16 kDa), which together form the lumenally exposed water splitting center.

A characteristic feature of C_4 plants is the differentiation of the photosynthetic leaf tissues into mesophyll (M) and bundle sheath (BS) cells. PS II complex is expressed in a tissue–specific manner in the NADP-ME type of C_4 plants (Edwards et al, 2001), predominantly in the mesophyll cells. Chloroplasts isolated from BS cells contain PS I activity, but do not photoreduce NADP from water, and cannot evolve oxygen similarly to the stroma thylakoids of C_3 plant chloroplasts (Lavergne and Leci, 1993). It was demonstrated that PS II in bundle sheath was inactive due to the absence of polypeptides participating in water oxidation and/or the light harvesting complex of PS II (Lu and Stemler, 2002). Moreover, it was shown that BS chloroplasts contained LHC II polypeptides but peptide

composition and amounts were different in both types of cells (Vainstein et al, 1989).

In this study, we determined the polypeptide pattern of mesophyll and bundle sheath thylakoid membranes, isolated from chloroplasts of maize.

MATERIALS AND METHODS

A cultivar of maize (*Zea mays* L.) named Zagatala 420 was used as an object of the research. The plants were grown under the controlled condition (photoperiod -14 h light/ 10 h dark, t=26°C/14°C and the light intensity -3000 lux). It was used 28-day-old seedlings of maize. The separation of the assimilative tissues (M and BS) into subcellular fractions was done according to the method (Guliyev et al., 2003). For this purpose, the buffer solution (buffer A), which consists of 25 mM HEPES buffer (pH 7.8), 0.3 M sucrose, 1 mM EDTA – Na, 0.2% BSA and 15-20 mM 2–mertaptoethanol was used.

Chlorophyll concentration was determined spectrophotometrically in the 80% asetone extract according to the Sims and Gamon method (Sims and Gamon, 2002). The pigments were extracted from the assimilating tissues of maize (M and BS) with 80 % -acetone–Tris solution (80:20, pH 7.8), the chlorophylls a and b were spectrophotometrically measured (Ultrospec 330 Pro "Amersham", USA) at wavelengths of 663 and 647 nm in accordance with absorption spectrums.

A high-resolution gradient-electrophoresis method was developed and used in the experiments. Electrophoresis was performed in the PU-2/4LS apparatus (“Farmacia”, Sweden) using a vertical system, at 4°C, 12 mA current, 450 V, for 16 hours. Upper concentrating gel was 6% acrylamide. Samples for electrophoresis was prepared as follows: 1% 2-mercaptoethanol and 2% Ds-Na detergent were added to the medium (detergent: chlorophyll=20:1 (mg)) and incubated at room temperature for 30 min. Samples corresponding to 50µg protein were applied to each slot.

Thylakoid membrane proteins were analyzed according to Laemmi using a 10 to 25% (w/v) linear gradient polyacrilamide gel in the presence of SDS as described earlier (Guseynova et al., 2006). To each slot, 20-45 µl of samples (an equal Chl content) were applied. After electrophoresis, the gels were stained for 30 min (before boiling) with a solution of 0.04% Coomassie Brilliant Blue G-250 (France) prepared in 3.5% perchloric acid ($HClO_4$). The gels were scanned using an Ultroscan 2202 Densitometer (LKB, Sweden) with a 633 nm laser as the light source. If necessary gels were dried in a special device (Slab Gel Dryer – 2003, LKB, Sweden). A set of standard proteins consisting of bovine serum albumin (66 kDa), glyceraldehyde-3-phosphate dehydrogenase (36 kDa), carbonic anhydrase (29 kDa), trypsinogen (24 kDa), trypsin inhibitor (20.1 kDa), and lactalbumin (14.2 kDa) (sigma, USA) was used for the determination of the molecular masses of polypeptides.

RESULTS AND DISCUSSION

The polypeptide patterns of the mesophyll (M) and bundle sheath (BS) thylakoids isolated from maize chloroplasts is shown in Fig.1. About 25 polypeptides ranged from 68 kDa to 10 kDa were observed using the gradient (10-25%) electrophoresis method. Laser densitogram of the gel is presented in Figure 2. Protein contents of photosynthetic membranes of mesophyll and bundle sheath chloroplasts were found to differ in both quantity and quality.

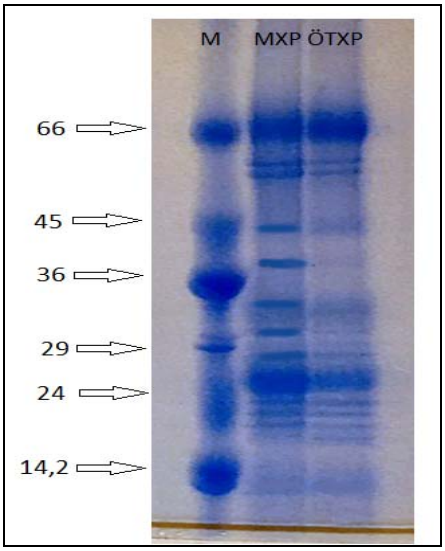

Fig. 1. Electrophoregram of thylakoid membranes of mesophyll and bundle sheath chloroplasts of maize on 10-25% PAAG in the presence of 0.1% DS-Na. MChl-thylakoids of mesophyll chloroplasts, BChl-thylakoids of bundle sheath chloroplasts, M-protein markers, kDa.

As seen in electrophoregram (Fig.1) and densitogram (Fig. 2) the protein composition of mesophyll thylakoids is similar to that of chloroplasts of typical higher plants (C_3 plants). Contrary to mesophyll thylakoids, some polypeptides lack in the protein content of bundle sheath thylakoids and amounts of others are reduced. As seen in the figures the amount of the PS I core apoprotein with molecular mass of 68 kDa is greater in bundle sheath thylakoids compared with mesophyll cell thylakoids. α and β subunits (with molecular masses of 55 kDa and 52 kDa) of CF1 domain of the ATPase complex are present in both subcellular fractions.

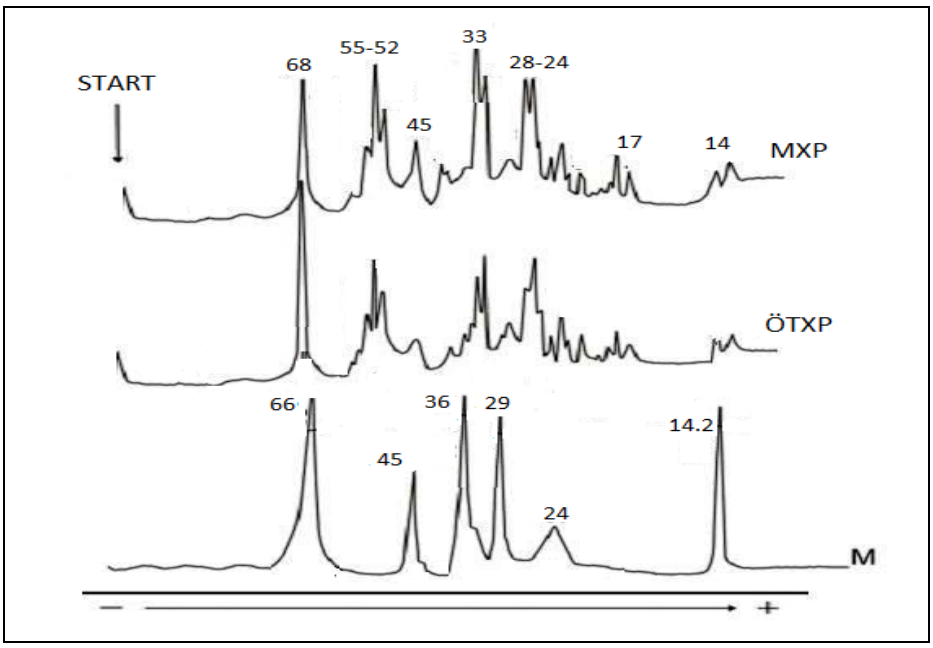

Fig. 2. Densitogram of thylakoid membrane proteins of mesophyll and bundle sheath chloroplasts of maize on 10-25% PAAG in the presence of 0.1% DS-Na. MChl-thylakoids of mesophyll chloroplasts, BChl-thylakoids of bundle sheath chloroplasts, M-protein markers, kDa.

However, the amount of α-subunit is smaller in thylakoids of BS cells. The protein of 45 kDa belonging to the PS II core antenna is more intensive in mesophyll thylakoids. Polypeptides (with molecular masses of 28-24 kDa) of the light-harvesting complex (LHC) of PSII are observed in both types of thylakoids, though their amounts are reduced in BS cells. Moreover, 33 kDa and 23 kDa proteins in the composition of oxygen-evolving complex (OEC) are observed in mesophyll cells and in relatively less amounts in thylakoids of BS cells. This confirms that chloroplasts of BS cells contain PS II complex, 33 kDa and 23 kDa polypeptides of oxygen-evolving complex. According to literature data immunoblot analysis revealed the existence of α-subunit in CF_1 domain of ATP-syntase complex, 33 kDa and 23 kDa proteins of the oxygen-evolving complex and polypeptides of LHCII and D1, D2 proteins in BS chloroplasts.

Thus, according to the obtained results, thylakoid membranes of mesophyll and bundle sheath chloroplasts in maize leaves have been found to differ in polypeptide contents.

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Qarğıdalı Xloroplastlarının Mezofil və Örtüktopa Tilakoidlərinin Zülal Tərkibi

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Fotosintez edən oksigenli növlərin bir neçə, müxtəlif multisuvahiddən ibarət zülal komplekslərində təşkil olunmuş enerji çevirən aparatı tilakoid membranları ilə assosiasiya olunur. C_4 bitkilərin xarakteristik xüsusiyyəti fotosintetik yarpaq toxumalarının iki müxtəlif hüceyrə tipinin olmasıdır: mezofil (M) və örtük topa (ÖT) hüceyrələri. Bu tədqiqatda qarğıdalının (*Zea mays* L.) mezofil və örtük topa tilakoidlərinin polipeptid tərkibi analiz edilmişdir. Müəyyən edilmişdir ki, FSI-in nüvəsinə daxil olan apozülalının miqdarı (68 kDa) mezofillə müqayisədə örtüktopa tilakoidlərində daha çoxdur. ATP-sintaza kompleksinin CF_1 domeninin α və β - subvahidlərinə aid olan 55 kDa və 52 kDa molekul kütləli zülallar hər iki tip subhüceyrə fraksiyalarında vardır, lakin α subvahidinin miqdarı örtüktopa tilakoidlərində bir qədər azdır. FSII-nin nüvə antennasının molekul kütləsi 45 kDa olan zülalı mezofil tilakoidlərində daha intensivdir. İşıqtoplayıcı kompleks II-nin (LHC II) tərkibinə daxil olan (28-24 kDa) polipeptidlər hər iki tip tilakoidlərdə müşahidə edilir, lakin örtük topa hüceyrələrinin tilakoidlərində onların miqdarı reduksiya edilmişdir.

Keywords: *C_4 plants, mesophyll, bundle sheath, chloroplasts, thylakoids, Zea mays*

Белковый Состав Мезофильных и Обкладочных Тилакоидов Хлоропластов Кукурузы

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У видов кислородного фотосинтеза аппарат преобразования энергии, сформированный из нескольких белковых комплексов, содержащих различные мультисубъединицы, ассоциирован с мембранами тилакоидов. Характерной особенностью С4 растений является наличие у фотосинтетических тканей листа двух типов клеток: мезофильных (М) и обкладочных (О). В данном исследовании проведен анализ полипептидного состава мезофильных и обкладочных тканей тилакоидов кукурузы (Zea mays L.). Установлено, что по сравнению с мезофильными клетками, в клетках обкладки содержание апобелка (68 kDa), входящего в ядро ФС 1, намного выше. В обеих субклеточных фракциях присутствуют белки с молекулярной массой 55 kDa və 52 kDa, относящиеся к α və β субъединицам CF_1 домена АТФ-синтетазного комплекса, однако содержание α субъединицы в обкладочных тилакоидах несколько меньше. Белок ядерной антенны ФС 2 с молекулярной массой 45 kDa более интенсивен в мезофильных тилакоидах. Полипептиды (28-24 kDa), входящие в светособирающий комплекс II, наблюдаются в обоих тилакоидах, однако в обкладочных тилакоидах их число редуцировано.

Ключевые слова: *C_4 растения, мезофилл, обкладка, хлоропласты, тилакоиды, кукуруза*

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Determination of the Activities of Some Photosynthetic Enzymes in *Sorghum Bicolor* Depending on Environmental Factors

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During the natural day-night cycle, light is the most important environmental factor controlling the diurnal changes of photosynthesis in C_4 plants. Activities of NADP-MDH and NADP-ME have been determined in *Sorghum bicolor* leaves at various stages of the plant development in relation to environmental parameters. A positive correlation has been found between the plant age and NADP-MDH activity.

Keywords: *C_4 photosynthesis, photosynthetic enzymes, temperature, NADP-MDH, NADP-ME*

INTRODUCTION

C_4 plants are more productive under hot climatic conditions compared with C_3 plants. Photosynthetic apparatus in leaves of these plants is distributed between bundle sheath and mesophyll cells with contrasting anatomic and biochemical properties. These cells differ in morphological and structural properties, as well as in the differentiation of thylakoid membranes) (Igamberdieva and Bykovab, 2018; Friso et al., 2010). *Sorghum bicolor* is a NADP-malic enzyme type C_4 plant, having high photosynthetic activity. This plant is of great agricultural importance. Sorgo is the fifth most important cereal plant in the world after rice, wheat, maize and barley, it is rich in nutrients, fibers, and biocomponents.

The main aim of the research was the diel dynamics of the activities of photosynthetic enzymes. To this end, the activities of NADP-malate dehydrogenase and NADP-malic enzyme have been determined in leaves of the mature *Sorghum bicolor* plant depending on the plant age and environmental parameters.

MATERIALS AND METHODS

Plant material and growth conditions: *Sorghum bicolor* was cultivated in the experimental field of the Institute of Molecular Biology and Biotechnologies. Activities of the photosynthetic enzymes were determined at the various stages of the plant growth. Diel dynamics of the NADP-MDH and NADP-ME activities was studied during the reproductive stage of the plant development.

Extraction of plant materials: To determine the activities of the enzymes leaves were ground using pestle and mortar. Homogenization was performed by adding 2 ml of 50 mM Tris-HCl (pH 8.0) buffer, containing 0.01% BSA, 0.5% Triton, 14 mM β-ME, 1 мM ethylenediaminetetraacetic acid (EDTA), and 0.5% polyvinyl pyrrolidone to 0.5g leaves in the presence of quartz sand. Homogenization continued for 5 min, at 10,000g. Supernatant was used for the enzyme activity assays.

Enzyme activity assays: Tris-HCl buffer (100 mM, pH 8.0) containing 10 mg/ml BSA, 0.5 M EDTA, 20 mM $MgCl_2$, 0.2 mM NADP·H and 50 µl activated enzyme preparation was used to determine NADP-malate dehydrogenase activity. The reaction was initiated by adding 1 mM oxaloacetate. To activate NADP-MDH, the enzyme preparation was kept in the reaction medium containing 1 M Tris-HCl (pH 8.0), 1M DTT and 50 µl enzyme preparation for 15 min (Scheibe and Stitt, 1988).

NADP-ME activity was determined spectrophotometrically by following NADPH production at 340 nm in the spectrophotometer Ultrospec 3300 pro. The standard assay medium contained 50 mM Tris-HC1, (pH 8.0), 10 mM $MgCl_2$, 0.5 mM NADP, and 4 mM L-malate in a final volume of 1 ml (Maurino et al., 1997).

RESULTS AND DISCUSSION

Activities of NADP-MDH and NADP-ME have been determined in *Sorghum bicolor* leaves at various stages of the plant development in relation to environmental parameters. Photosynthetic enzymes in C_4 plants are regulated by a number of factors, including light. The photosynthetic enzyme activity *in vivo* in plant leaves at any given point during photosynthesis reflects the combined effects of light, metabolites and other factors at that time (Cousins et al., 2003).

NADP-malate dehydrogenase (NADP-MDH; EC

1.1.1.82) is a crucial enzyme of the C_4 pathway. Playing an important role in the photosynthetic carbon assimilation, this enzyme catalyzes the conversion of oxaloacetate into malate. In Sorghum leaves, NADP-MDH is activated in the light and inactivated in the dark and this apparently depends on interconversion between dithiol and disulfide groups on the enzyme. *In vivo*, the light activation (reduction) process probably occurs via thioredoxin reduced in turn by the photosynthetic electron transport system through ferredoxin (Rebeille and Hatch, 1987). In C_4 plants such as sorghum and maize, it is located in the chloroplasts of mesophyll cells where the produced malate is exported to the bundle-sheath cell chloroplasts, thus delivering reducing equivalents that are needed for the photosynthetic fixation of carbon dioxide into organic molecules. Among all the malate dehydrogenases, the chloroplastic NADP-dependent form exhibits the unique property of being strictly regulated by light, while the NAD-dependent MDHs are permanently active. It is totally inactive in the dark and activated by the ferredoxin-thioredoxin system only when the chloroplasts are illuminated (Johansson et al., 1999).

Malic enzymes catalyze the oxidative decarboxylation of L-malate to yield pyruvate, CO_2, and NAD(P)H in the presence of a bivalent metal ion. In plants, different isoforms of the NADP-malic enzyme (NADP-ME) are involved in a wide range of metabolic pathways. The C_4-specific NADP-ME has evolved from C_3-type malic enzymes to represent a unique and specialized form of NADP-ME as indicated by its particular kinetic and regulatory properties. The photosynthetic C_4 NADP-ME, which is involved in the CO_2 concentrating mechanism that increases the photosynthetic yield of NADP-ME type C_4 plants, is compartmentalized in bundle sheath chloroplasts. In NADP-ME type C_4 plants, these organelles show a gradation of structure from chloroplasts with rudimentary grana (in maize and crabgrass) to completely agranal (in sugarcane and sorghum) (Detarsio et al., 2000).

Sorghum development has been separated into three major divisions: vegetative (GS-1), reproductive (GS-2), and grain fill (GS-3), with about a third of the life cycle spent in each (Wood et al., 2006). Stages shown and discussed range from emergence until physiological maturity. Time required to reach each stage depends both on the hybrid and the environment in which it is growing.

Dynamics of the activities of NADP-MDH and NADP-ME has been studied in the leaves of *Sorghum bicolor* at various stages of the plant development (Figure 1).

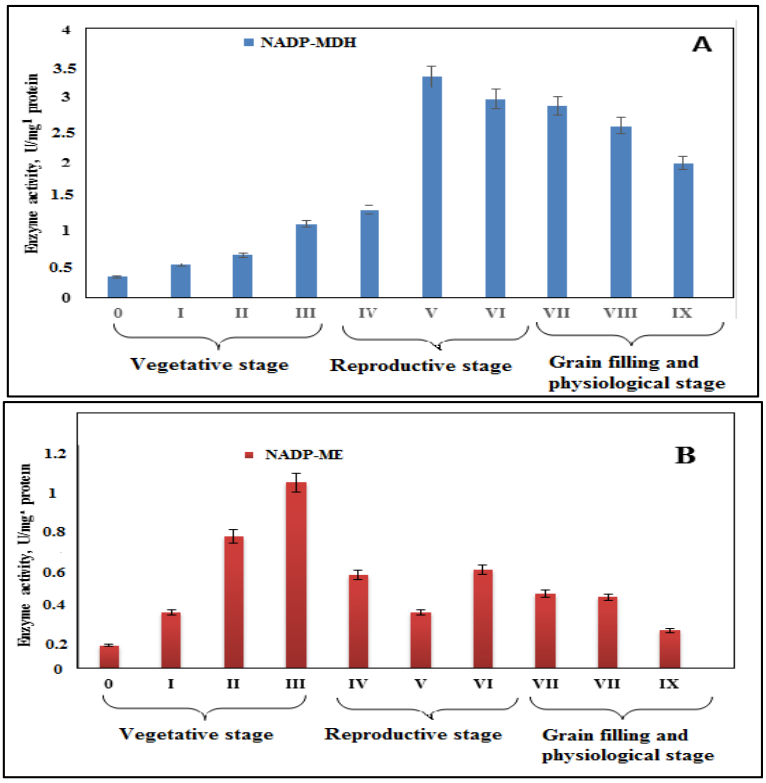

Fig. 1. Dynamics of the activities of NADP-MDH (A) and NADP-ME (B) in the *Sorghum bicolor* plants of various ages. ((0-Emergence; I- 3 three–leaf stage; II- five–leaf stage; III- Growing Point Differentiation)- vegetative stage; (IV- Final Leaf Visible in the Whorl; V- Boot Stage; VI- Half Bloom)- reproductive stage; (VII- Soft Dough; VIII- Hard Dough; IX- Physiological Maturity))- grain filling and physiological maturity stage.

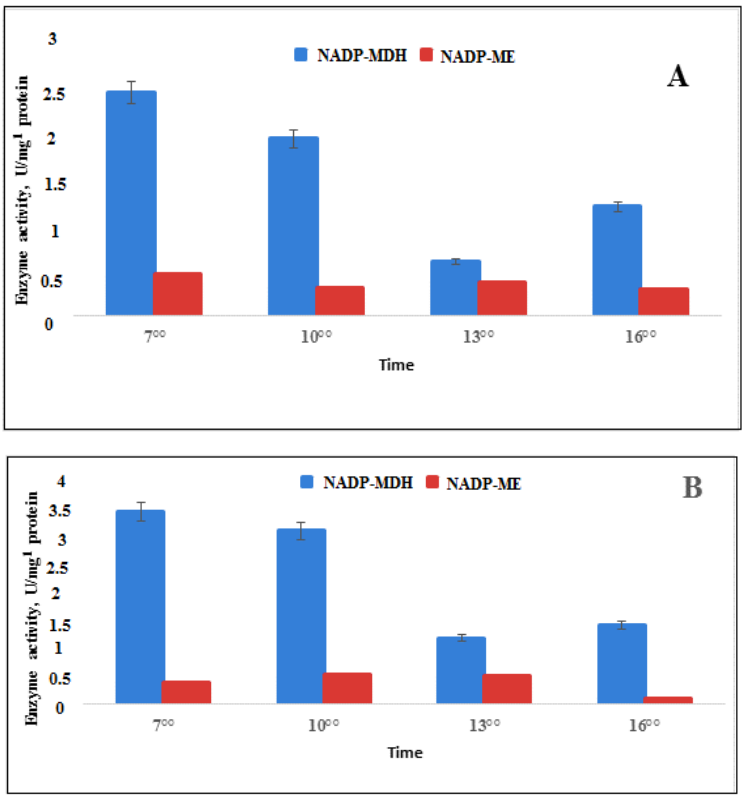

Fig. 2. Time-dependent dynamics of the NADP-MDH and NADP-ME activities in *Sorghum bicolor* leaves. A- Final Leaf Visible in the Whorl (reproductive stage); B- grain filling and physiological maturity stage.

According to the results of the research, a positive correlation exists between the NADP-MDH activity and the plant age. As seen in Figure 1 (A), the highest activity of the enzyme was observed in the tube formation, i.e. reproductive stage. Thus, the enzyme activity during flag-leaf formation stage was 3 times higher compared with the five–leaf stage, and 1.5 times higher compared with the grain formation and physiological maturity stages. The positive correlation observed between the enzyme activity and the plant age can be related to the plant development and the grain formation process.

As seen in Figure 1(B), the NADP-MDH activity during the vegetative stage (Growing Point Differentiation Stage) was higher than in the reproductive stage. Thus, the enzyme activity was 0.5 times higher in the last phase of the vegetative stage compared with the flag leaf formation phase.

Time-dependent dynamics of the activities of photosynthetic enzymes in the leaves of the mature *Sorghoum biclor* plant is shown in Figure 2.The activity of NADP-MDH was found to be higher during both flag leaf formation and physiological maturity stages, in the morning hours (7.00). The enzyme activity was 2 times higher at 7.00 compared with 16.00. Moreover, during both stages the enzyme activity gradually decreased until 16.00 with a subsequent increase. However, there was no pronounced difference in the NADP-ME activity during both stages.

The higher activity of NADP-MDH observed in the morning hours is suggested to relate to the high PEPCase activity. Thus, it is known that oxaloacetate, which is the product of PEPC, is converted into malate by NADP-MDH. According to previous reports, under hot climatic conditions the activity of PEPC in C_4 plants was higher during the morning hours and it decreased in the afternoon hours (Du et al., 2000). The decrease in the PEPC activity under hot is assumed to cause a decrease in the oxaloacetate amount, which is the product of PEPC. This is accompanied by the decrease in the NADP-MDH activity that catalyzes the conversion of oxaloacetate into malate.

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Ətraf Mühit Amillərindən Asılı Olaraq *Sorghum Bicolor* Bitkisində Bəzi Fotosintetik Fermentlərin Aktivliklərinin Təyini

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Təbii gecə və gündüz tsikli ərzində işıq C_4 bitkilərdə fotosintez prosesini gündəlik tənzimləyən ətraf mühitin mühüm faktorlarından biridir. İnkişafın müxtəlif mərhələlərində Sorghum bicolor bitkisinin yarpaqlarında ətraf mühit parametrlərindən asılı olaraq, NADP-MDH və NADP-ME fermentlərinin aktivliyi təyin olunmuşdur. Aparılan tədqiqatlar nəticəsində müəyyən olunmuşdur ki, NADP-MDH fermentinin aktivliyi ilə bitkinin yaşı arasında müsbət korrelyasiya mövcuddur.

Açar sözlər: *C_4 fotosintez, fotosintetik fermentlər, temperatur, NADP-MDH, NADP-ME*

Определение Активности Некоторых Фотосинтетических Ферментов в *Sorghum Bicolor* в Зависимости от Факторов Окружающей Среды

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Свет является одним из самых важных факторов окружающей среды, регулирующих процесс фотосинтеза в С4 растениях в период естественного дневного/ночного цикла . Активность ферментов NADP-MDH и NADP-ME была определена в растении *Sorghum Bicolor*. Установлена положительная корреляция между возрастом растений и активностью NADP-MDH.

Ключевые слова: *C_4 фотосинтез, ферменты фотосинтеза, температура, NADP-MDH, NADP-ME*

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Effect of Various Concentrations of NaCl on the Growth of Seedlings of Bread Wheat (*Triticum Aestivum* L.) Genotypes With Contrasting Productivity and Drought Tolerance

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The effect of various NaCl concentrations on germination ability and morphophysiological indices of bread wheat (*Triticum aestivum* L.) genotypes, which differ in productivity, drought tolerance and heights, has been studied. Wheat seeds were germinated at 0 mM, 150 mM and 200 mM concentrations of NaCl using the roll method. With increasing salt concentration retardation in the growth of shoots and root system was observed in all samples. Salt effects were found to be different in various genotypes. Maximum germination ability was detected in the bread wheat varieties 12nd FAWWON № 97, Daghdash-94 and Gyrmyzygul-1, treated with 200 mM NaCl. Only in the highly productive variety Gobustan and drought tolerant Pirshahin-1 germination energy was relatively lower (16%-17%) at 200 mM NaCl. There were no marked differences in the lengths of shoots and roots of the studied varieties. The Daghdash-94 variety was found to be the tallest among the studied varieties and the highest chlorophyll content was detected in Gyrmyzygul-1.

Keywords: *NaCl, wheat varieties, germination energy, germination percentage, chlorophyll*

INTRODUCTION

Soil salinization is one of the significant environmental factors that limit the growth, development and productivity of plants. Currently, about 20% of all irrigated areas of the world are saline (Minus and Richard, 2003). Salinity of soils constantly increases due to the rising groundwater level and improper irrigation in agriculture (Flowers, 2004).

Salinization of soil leads to water deficiency in the plant. Influencing the stomatal conductance of plants, water deficiency can affect the CO2 fixation rate and, consequently, the intensity of photosynthesis (Marler and Zozor, 1996). The decrease in the content of photosynthetic pigments-chlorophyll a and b, carotenoids, and in the activity of photosystems located in thylakoid membranes are assumed to relate to weakened assimilation of carbon dioxide ()Yordanov et al., 2000). Under salt stress the chlorophyll b content was found to decrease more than chlorophyll b content, regardless of the plant genotype. Salt stress, depending on the degree of plant tolerance, leads to a significant change in the activity of antioxidant systems of the cell (Atoyev et al., 2011, 2014; Klimova, 2013).

Salts have a double effect on the plant. First, they create a high osmotic pressure in the soil solution, providing a strong bond with water. This complicates water absorption by roots, causing osmotic stress. Second, ions of salt absorbed with water exert a negative impact on the plant metabolic processes (Minus and Termaat, 1986). Disturbance of growth and development of plants under salt stress is a consequence of some physiological responses of plants, including changes in the ion balance, mineral nutrition, stomatal conductance, photosynthesis rate and, ultimately, fixation and utilization of carbon dioxide (Bongi and Loreto, 1992).

Salinity is the major factor affecting plant metabolism, thereby causing changes in morphological, anatomical structure, physiological and biochemical conditions of plants. The first morphological response of plants to salt stress is the limitation in the development of roots and leaves. If salinity continues the plant development stops completely and eventually the plant perishes.

The study of salt effects on plant growth and development, evaluation of plant adaptation mechanisms to salt stress are very important issues for the effective use of saline soils. Adverse environmental conditions cause structural and functional changes affecting, first of all, vital activity of the organism (Aliyev et al., 2014). An active reconstruction of intracellular connections occurs under adverse ambient conditions. Moreover, negative conditions lead to pivotal changes in physiological and biochemical processes proceeding in plants. Therefore, the comprehensive study of these processes is necessary for the evaluation of plant stress tolerance.

Considering the above-mentioned issues, the

main purpose of the presented work was the comparative study of salt tolerance of bread wheat genotypes with contrasting productivity, drought tolerance and height based on their morphophysiological indices and establishing changes in leaf water regime, amounts of photosynthetic pigments and PSII activity.

MATERIALS AND METHODS

The objects of the study were bread wheat (*Triticum aestivum* L.) genotypes: high productive Gobustan, low productive 12nd FAWWON № 97, drought tolerant Pirshahin-1 and drought sensitive Tale-38, tall Daghdash-94 and short Gyrmyzygul-1. For the assessment of the morphometric and physiological parameters of drought tolerance, seeds of bread wheat varieties were germinated at various NaCl concentrations (0 mM, 150 mM, 200 mM) using the roll method (Shikmuradov, 2011; Belozerova and Rome, 2014). Seeds of each sample were maintained on the wet filter paper for 3 days in darkness and then in a 12h-light/ 12h-dark photoperiod for 11 days at 20-22°C. Germination ability of the wheat embryo was examined during 7 days (Aliyev et al., 2014). Based on some morphophysiological indices such as average root length, RWC, concentration of photosynthetic pigments and chlorophyll fluorescence indices, salt tolerance of the studied varieties were assessed on the 10th day of the germination stage.

RWC in leaves was determined according to the method of Tambussi et al. (Tambussi et al., 2005). Chlorophyll was extracted from leaves using 96% ethyl alcohol and quantification of chlorophyll a, chlorophyll b and carotenoids was conducted at 665 nm, 649 nm and 440 nm, respectively, using the spectrophotometric method of Wintermans et al. (Gavrilenko and Zhigalova, 2003). Leaf fluorescence indices were measured using the MINI-PAM (photosynthesis yield analyzer, Germany) device. The energy conversion efficiency of PSII was calculated using the formulas $F_v = F_m - F_0$ and F_v / F_m (Maxwell and Jonson, 2000).

RESULTS AND DISCUSSION

In spite of the negative impact of salt, a development relative to control variants was observed for bread wheat (*Triticum aestivum* L.) genotypes with contrasting productivity, drought tolerance and height during 10 days (Figure 1, A, B and C).

Various physiological methods are known for the determination of plant stress tolerance, which based on germination ability (Aliyev et al., 2014). For the initial assessment of salt tolerance of bread wheat genotypes, germination ability of control and salt-treated variants was compared (Figure 2 A, B). As seen in the figure, a decreasing trend in germination ability was observed in the all wheat genotypes germinated at various salt concentrations. Germination ability of the studied varieties changed in the following ranges: 100% - 92% in the control variants, 100% - 75% at 150mM NaCl and 83% - 33% at 200mM NaCl.

In 3-day-old wheat seedlings treated with NaCl, germination energy changed in the ranges: 92% - 58% in the control variants, 58%-33% at 150mM NaCl and 83%-33% at 200 mM NaCl. However, maximum germination percentage was observed in both variants of the all studied varieties. Maximum germination showed the varieties 12nd FAWWON № 97, Daghdash-94 and Gyrmyzygul-1. Germination energy was relatively low (16%-17%) only in high productive Gobustan and drought tolerant Pirshahin at 200 mM concentration of NaCl.

Seeds are known to experience high osmotic pressure of the environment during germination and certain physiological properties of plants are determined by absorption ability of seeds. Absorption ability of seeds facilitates the formation of a strong root system, which provides plant development under water deficiency.

The changes in the linear parameters of the growth process is a more reliable assessment of plant tolerance than seed germination indices. Therefore, during the initial stages of ontogenesis, the average length of roots and shoots of wheat genotypes grown at various concentrations of salt is of a great interest. Diagrams in Fig. 3 A, B present development indices (lengths of roots and shoots) of bread wheat varieties, grown at various concentrations of salt for 10 days. Thus, on the first 10 days the development of the studied wheat genotypes continued and then a decline relative to the control occurred. The development of roots and shoots of the all varieties was retarded as the concentration of NaCl increased. Thus, 2 times decline was observed in the length of shoots and 3-4 times decline in the length of roots relative to the control. The study of the effect of various NaCl concentrations on the growth of shoots and roots showed that, first of all, salt stress damaged the root system and then above ground organs of the plant. However, the varieties did not significantly differed in the lengths of roots and shoots (Figure 3 A, B). The variety Daghdash-94 was found to be tall in both variants.

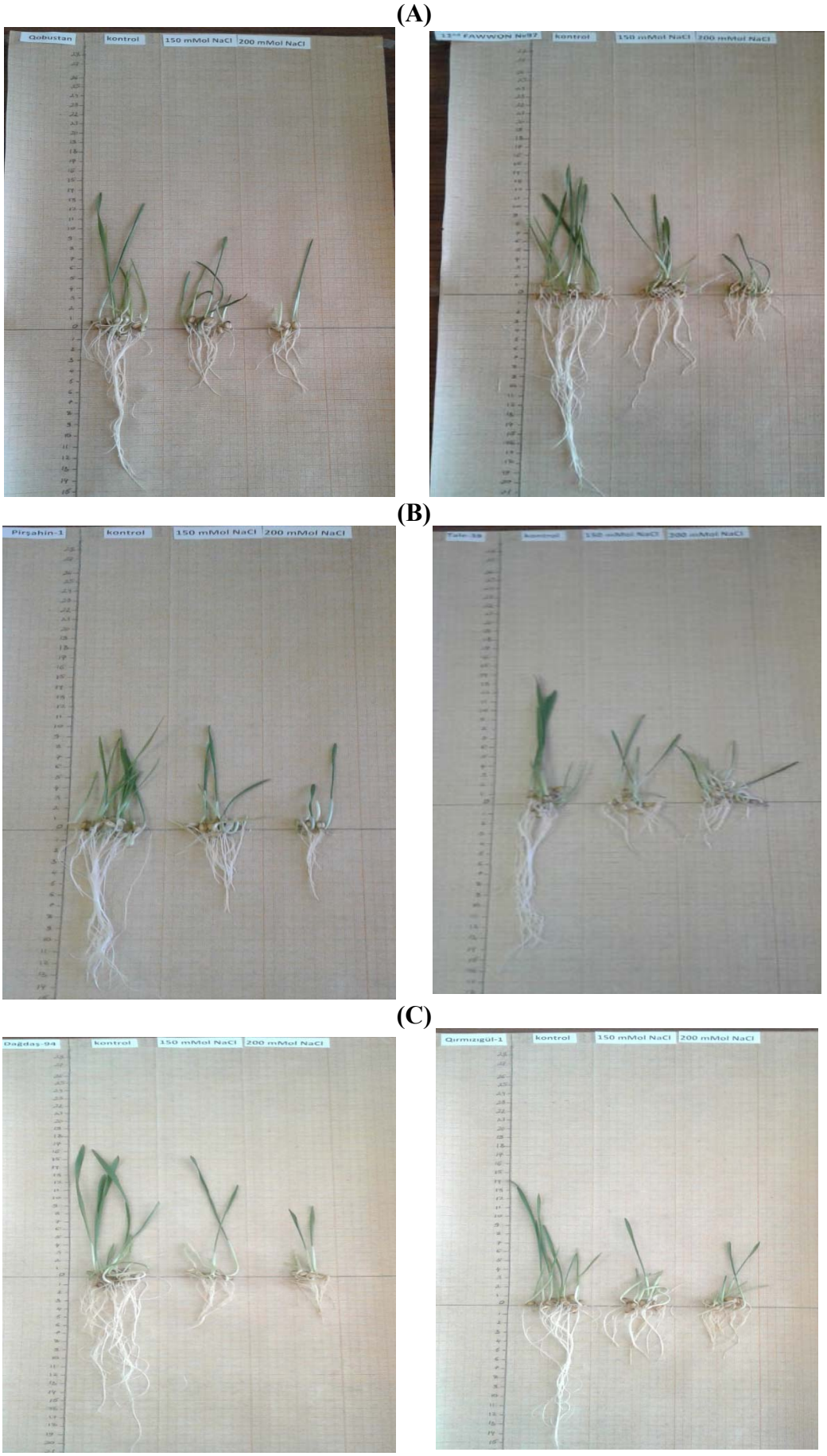
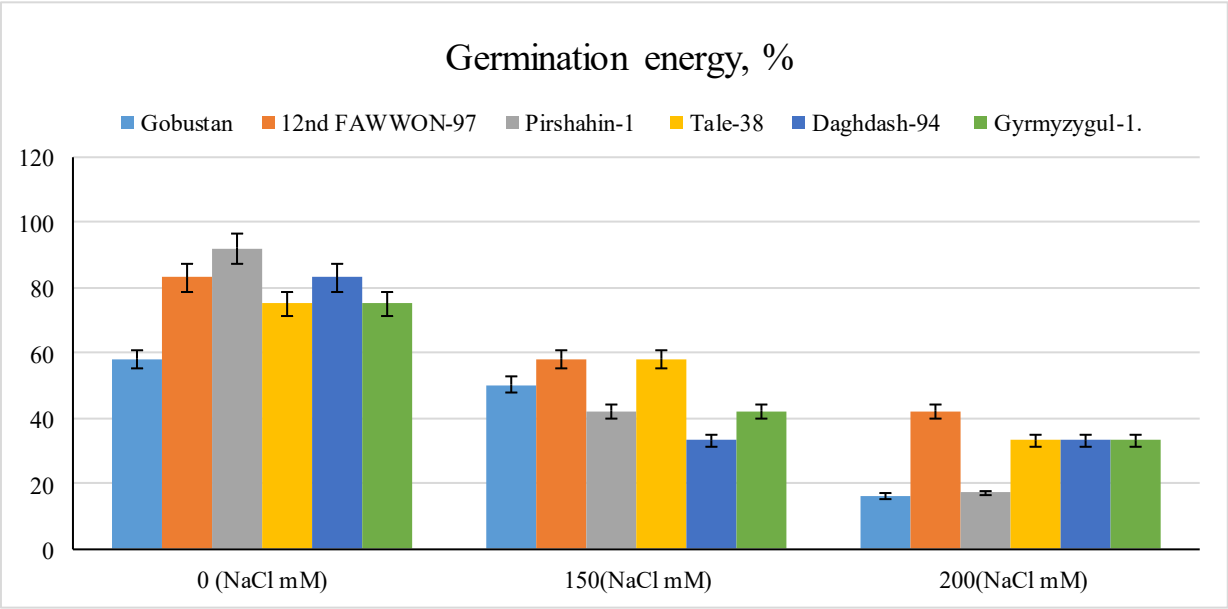
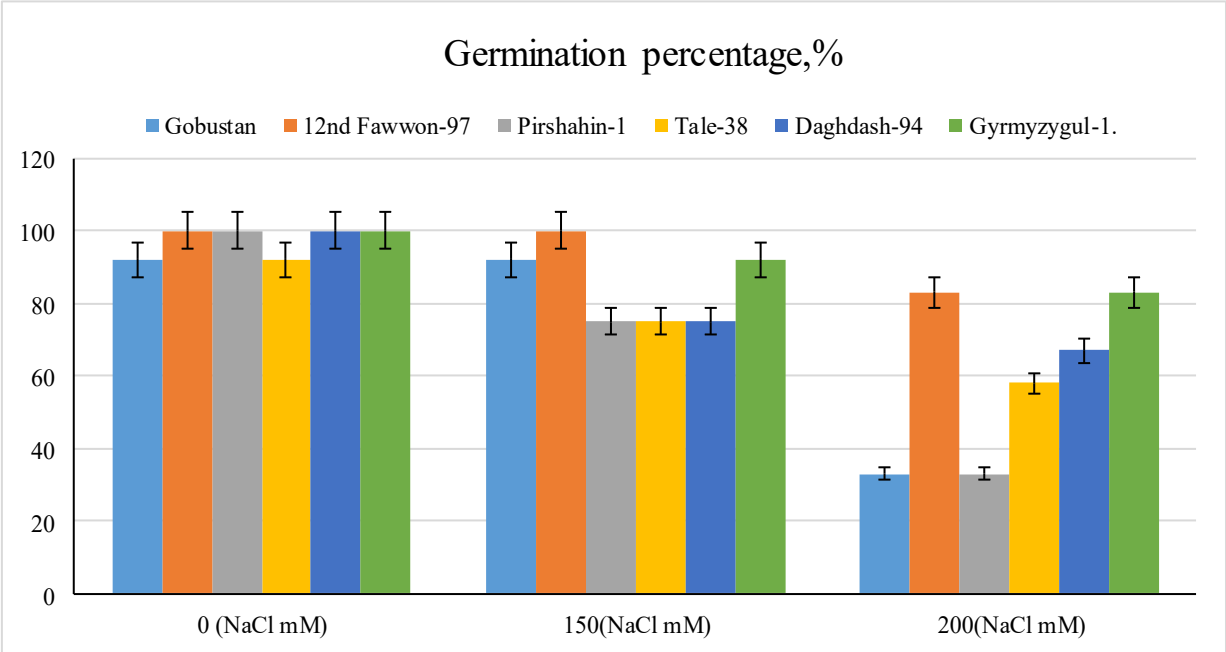


Fig. 1 (A, B, C). Development of 10-day-old seedlings of bread wheat genotypes (*Triticum aestivum* L.) with contrasting productivity (A), drought tolerance (B) and height (C) treated with different NaCl levels



(A)



(B)

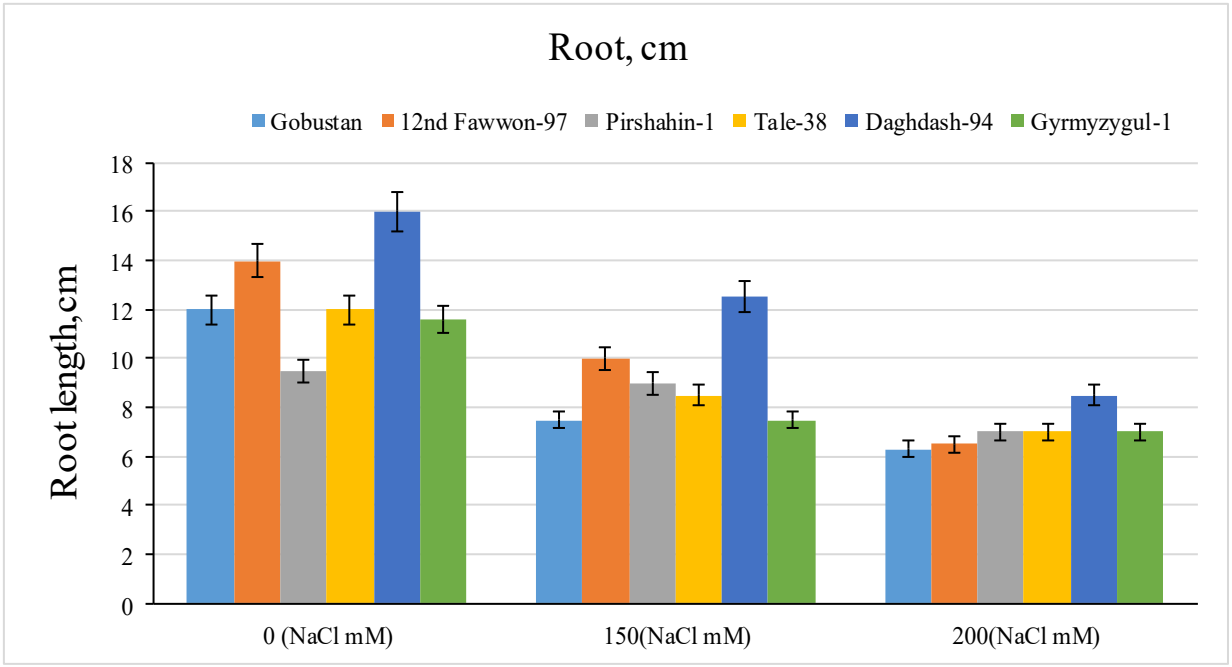
Fig. 2 (A, B). Effects of various NaCl levels on germination ability of bread wheat genotypes. A - germination energy, B - germination percentage.

The effect of various salt concentrations on RWC of 10-day-old seedlings of bread wheat genotypes was studied (Figure 4). RWC was found to decrease significantly as salt concentration increased. As seen in the figure, RWC changed in the ranges 99% - 86%, 96% -79% and 94% -66% in the control variant, and at 150mM and 200mM concentrations of salt, respectively. However, there was no pronounced difference in the dynamics of the changes in RWC in the wheat varieties Daghdash-94 and Gyrmyzygul-1 depending on NaCl concentrations. A marked negative impact of 200mM NaCl was observed in the variety 12nd FAWWON № 97.

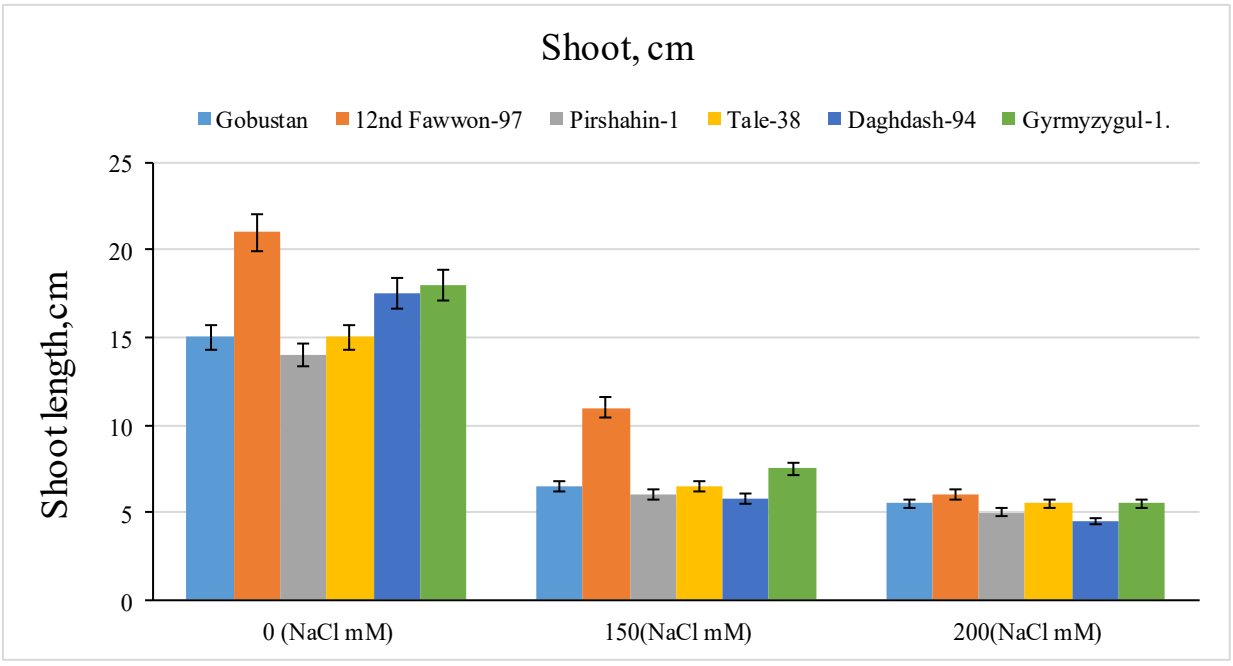
The content of leaf photosynthetic pigments was found to play a significant role in the function of photosynthetic apparatus and its productivity. A complex relation exists between photosynthetic productivity and amounts of the chlorophyll pigments. Salt stress disturbs chlorophyll structure and chloroplast membranes, leading to the violation of the structure and the decline in photochemical activity and light intake ability. Chlorophyll loses a part of its energy through the heat and fluorescence. But the energy waste increases due to the structural changes. Therefore, chlorophyll index is considered

as the main parameter in experiments related to salinity (Aliyev et al., 2014). Chloroplasts of the sensitive plants are destructed more under salt stress and therefore, the study of salt effects on photosynthetic apparatus is of great importance for the assessment of plant tolerance to stress factors and its relation to physiological parameters.

The changes in the content of pigments provide an important information about physiological status and adaptation of plants to changing environmental conditions (Gang et al., 2010). The aim of our study was to evaluate the degree of stress effect based on the change in the pigment content of wheat leaves. According to the results of the experiments performed with leaves of 10-day-old seedlings of bread wheat varieties, the general amount of chlorophyll decreased with increasing salt concentration in the all varieties compared with the control. However, the highest chlorophyll content was observed in the variety Gyrmyzygul-1 (Table).



(A)



(B)

Fig. 3 (A, B). The length of 10-day-old seedlings of bread wheat genotypes treated with various NaCl concentrations.

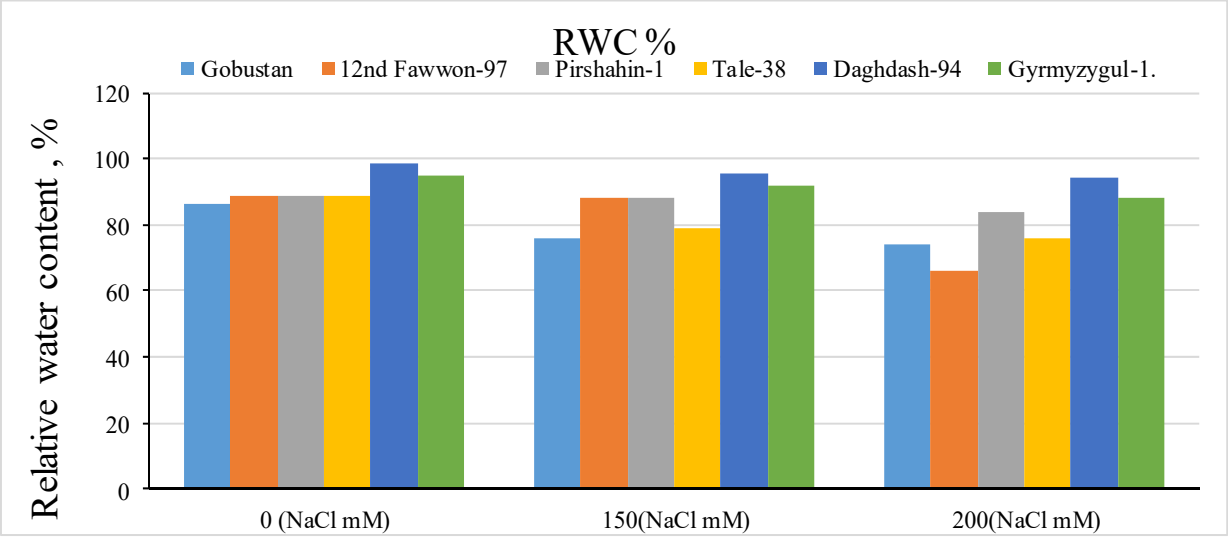


Fig. 4. RWC in 10-day-old leaves of bread wheat varieties treated with various NaCl concentrations.

Table. The influence of various NaCl concentrations on the content of chlorophyll and carotenoids (mg/g), and the energy conversion efficiency of PS II (Y).

№	Varieties	NaCl, mM	C_a	C_b	C_{a+b}	C_{kar}	Y
1	**Gobustan**	0	0.56	0.23	0.79	4.9	0.86
		150	0.55	0.23	0.78	4.6	0.84
		200	0.22	0.18	0.40	2.0	0.87
2	**12nd FAWWON № 97**	0	0.64	0.30	0.94	4.7	0.89
		150	0.42	0.27	0.69	4.5	0.90
		200	0.10	0.21	0.82	1.9	0.92
3	**Pirshahin-1**	0	0.67	0.46	1.13	4.2	0.79
		150	0.55	0.17	0.72	3.7	0.83
		200	0.54	0.15	0.69	1.6	0.83
4	**Tale-38**	0	0.61	0.23	0.84	4.8	0.81
		150	0.50	0.25	0.75	2.0	0.84
		200	0.25	0.11	0.36	1.7	0.81
5	**Daghdash-94**	0	1.12	0.38	1.50	4.8	0.82
		150	0.33	0.16	0.49	1.7	0.82
		200	0.24	0.27	0.51	1.0	0.73
6	**Gyrmyzygul-1**	0	1.16	0.58	1.74	2.2	0.81
		150	0.82	0.28	1.10	0.8	0.83
		200	0.72	0.21	0.93	0.4	0.83

As seen in the table, chlorophyll a+b and amounts of carotenoids decreased and a slight decrease occurred also in the energy conversion efficiency of PS II as salt concentration increased.

According to some authors, plants experience stress effects mainly due to the weakening function of the root system. Our results suggest that the manifestation of stress effects begins with the changes in seeds. The study of morphological and physiological effects of salinity would contribute to overcoming multiple issues related to negative effects of salt stress.

So salt stress was found to exert a negative impact on germination ability, leaf RWC, photosynthetic pigment amounts and PSII activity. The obtained results confirm that plant tolerance to stress conditions is a result of various adaptive responses.

Among the studied wheat genotypes 12nd FAWWON № 97, Daghdash-94 and Gyrmyzygul-1 were found to have high germination ability at 200 mM concentration of NaCl.

ACKNOWLEDGEMENT

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NaCl Duzunun Müxtəlif Qatılıqlarının Məhsuldarlığına və Quraqlığa Davamlılığına Görə Fərqlənən Yumşaq Buğda Genotiplərinin (*Triticum Aestivum* L.) Cücərtilərinin İnkişafına Təsiri

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NaCl duzunun müxtəlif qatılıqlarının məhsuldarlığına, quraqlığa davamlılığına və boylarına görə fərqlənən yumşaq buğda genotiplərinin (*Triticum aestivum* L.) cücərmə qabiliyyətinə və cücərtilərin morfo-fizioloji göstəricilərinə təsiri öyrənilmişdir. Buğda toxumları NaCl duzunun 0, 150, 200 mM qatılılıqlı məhlullarında rulon metodu ilə cücərdilmişdir. Duzun qatılığı artdıqca bütün nümunələrdə cücərtilərin və kök sistemlərinin böyüməsində ləngimə müşahidə edilmişdir. Duzun müxtəlif qatılıqlarının ayrı-ayrı cortlara təsiri müxtəlif olmuşdur. Duzun 200 mM qatılığında isə maksimal cücərmə ilə fərqlənən sortlar 12nd FAWWON № 97, Dağdaş-94 və Qırmızıgül-1 yumşaq buğda sortları olmuşdur. Yalnız yüksək məhsuldar Qobustan və quraqlığa davamlı - Pirşahin-1 sortlarında NaCl-un 200mM qatılığında cücərmə enerjisi nisbətən az (16% -17%) olmuşdur. Lakin köklərin və cücərtilərin uzunluguna görə sortlar arasında kəskin fərq müşahidə edilməmişdir Dagdaş-94 sortu bütun variantlarda hündürboylu sort olaraq özünü doğrultmuşdur. Qırmızıgül-1 sortu isə xlorofilin yüksək miqdarına malik olmaqla digər sortlardan fərqlənmişdir.

Açar sözlər: *NaCl, buğda sortları, cücərmə enerjisi, cücərmə faizi, xlorofil*

Влияние Различных Концентраций NaCl на Развитие Проростков Генотипов Мягкой Пшеницы (*Triticum Aestivum* L.) Отличающихся по Продуктивности и Засухоустойчивости

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Изучено влияние различных концентраций NaCl на способность к прорастанию и морфофизиологические параметры генотипов мягких пшеницы (*Triticum aestivum* L.), отличающихся друг от друга по росту, продуктивности и засухоустойчивости. Семена пшеницы выращивались по методу рулон в 0, 150, 200 мМ растворах NaCl. При увеличении концентрации соли у всех образцов наблюдалась задержка в росте проростков и корневой системы. Различные концентрации соли оказывали разное воздействие на изученные сорта пшеницы. При концентрации соли 200 мМ максимальное прорастание было обнаружено у сортов 12nd FAWWON № 97, Дагдаш 94 и Гырмызыгюль 1. Только у продуктивного сорта Гобустан и засухоустойчивого Пиршахин энергия прорастания при концентрации 200мМ NaCl была относительно низкой (16% -17%). Однако, в длине корней и проростков не между сортами существенной разницы не наблюдалось. Во всех вариантах Дагдаш 94 оказался высокорослым. Гырмызыгюль 1отличался от других сортов по высокому содержанию хлорофилла.

Ключевые слова: *NaCl, сорта пшеницы, энергия прорастания, процент прорастания, хлорофилл*

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Validation of QTL for the Flag Leaf Senescence in Wheat Under Drought

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The study of physiological senescence of the flag leaf playing the pivotal role in the uptake of solar energy and stipulating plant productivity in wheat is very important for providing high productivity under stress. Therefore, using RAPD OPH13 marker, the existence of a gene-locus linked to the physiological flag leaf senescence was examined in wheat genotypes under drought stress. Based on the analysis of PCR profiles, 450 bp diagnostic fragments were identified in 31 bread wheat and in 15 durum wheat genotypes. This result shows that there is a gene locus that provides physiological rejuvenation of the flag leaf, which is an indicator of the resistance to drought stress in those genotypes. The existence of the studied locus was not confirmed in 19% of the genotypes.

Keywords: *Wheat genotypes, flag leaf senescence, drought, PCR, RAPD*

INTRODUCTION

Drought is the crucial environmental stress that has pernicious consequences on agriculture. One of the main goals of breeding programs is to develop varieties exhibiting high tolerance to drought conditions. It is very important to achieve sustainability in agriculture. Despite, fundamental research has provided substantial gains in the understanding of the physiological and molecular responses of plants to drought stress, there is still a large lack between yields in optimal and stress conditions. In cereal crops, the upper three leaves on the stem, especially the top-most leaf, flag leaf are the main source of carbohydrate production (Al-Tahir 2014).

Leaf senescence is a physiological process that causes cell death and is regulated by age under the impact of other endogenous and environmental factors. Leaf senescence is an active and strictly regulated degeneration process. During this process, leaf cells are exposed to strong changes in cell structure, metabolism, and gene expression (Zhao et al., 2018). Leaf senescence generates the change in the leaf cell metabolism. Basically enhanced catabolism leads to a declined assimilation rate e.g., the photosynthetic capacity decreases, and macromolecular material degrades (Lira et. al., 2017). The earliest and most notable modification in the cell structure is the disintegration of the chloroplast, the organelle that contains up to 70% of the leaf protein. Metabolically, carbon assimilation is replaced by catabolism of chlorophyll and macromolecules such as proteins, membrane lipids, and RNA (Lim et al., 2007). Increased catabolic activity is responsible for transforming the cellular materials collected during the growth stage of leaf into exportable nutrients that are directed to developing seeds or to other growing organs. Hence, although leaf senescence is a detrimental process for the whole leaf organ, it can be accepted as a protection process (Senapati et al., 2018). Thus, leaf senescence is an evolutionarily selected developmental process and has a key role in the plant life cycle. According to agricultural reports, leaf senescence may decrease yield in crop plants by reducing the growth phase and may also cause post-harvest spoilage such as leaf yellowing and nutrient loss in vegetable crops.

Programmed cell death (PCD) is a self-destruct cellular process controlled by several factors and mediated through an active genetic program. This process is regulated by numerous active genetic programs. Molecular markers related to quantitative trait loci (QTL) for drought tolerant patterns could increase in breeding for drought conditions. Molecular markers can be used to research germplasm through segregation and association mapping to detect useful alleles in both cultivated and wild relatives. Moreover, association mapping is naturally more powerful than 'classical' genetic linkage mapping because it analyzes the results of generations of recombination and selection, most of the data available currently on water deficit are based on segregation mapping and QTL analysis. An advantage of the RAPD technique is that it gives rapid outcome but at the same time has limitations such as low reproducibility (Fernandez et al., 2002).

The main purpose of this research is the identification of QTL for the flag leaf senescence in wheat by RAPD marker.

MATERIALS AND METHODS

***Plant materials*:** Wheat genotypes (38 genotypes

of bread (*Triticum aestivum* L.) and 19 genotypes of durum (*Triticum durum* Desf.) wheat genotypes collected in the Gene Pool of the Research Institute of Crop Husbandry (Baku) acted as a research object. Plants were cultivated in field conditions.

***Extraction of plant DNA*:** DNA extraction was carried out using the CTAB method with some modifications (Murray and Thompson, 1980). Fresh plant tissue as a fragment of leaf was minced in liquid nitrogen, suspended in 1000 µl of CTAB extraction buffer (100 mM Tris-HCl, pH 8.0; 20 mM EDTA, pH 8.0; 1.4 mM NaCl; 40 mM β-mercaptoethanol), and pre-warmed in a water bath at 60ºC. Homogenization was completed by intense Vortex shaking. Then 400 ml of chloroform (99.8%) was added into each tube and the tubes were gently mixed. Next the tubes were placed in a water bath and incubated for 10 min at 60 ^{0}C. After incubation, the tubes were centrifuged in an Eppendorf type benchtop centrifuge (15,000 g) for 10 min at room temperature. After centrifugation the supernatant was carefully selected (taking care not to capture sediment particles) and transferred to clean 1.5 ml Eppendorf type tubes and 600 ml of cold isopropanol was added, mixed well and left at room temperature for 3-5 minutes. At this stage we can observe the dispersed DNA precipitate. The tube contents were centrifuged at room temperature in the Eppendorf type benchtop centrifuge (15,000 g) for 10 min.

The precipitate was washed several times with 70% ethanol, dried in a thermostat at 56 ^{0}C for 5 minutes and dissolved in TE buffer (10 mM Tris-HCl, pH 8; 1 mM EDTA). Samples were left in a refrigerator at 4^{0}C for the complete dissolution of DNA in a buffer.

***DNA quantification*:** After dissolution of DNA the quantity was determined by optical density (OD) at λ=260 using the ULTROSPEC 3300 PRO spectrophotometer ("AMERSHAM", USA).

Purity of the genomic DNA was determined by the ratio of absorptions at A260/A280. DNA quality was checked on the basis of performance of the extracted DNA samples on 0.8% agarose gel stained with 10 mg / ml of ethidium bromide in 1 × TBE (Tris base, Boric acid, EDTA) buffer. The gel was developed and photographed under ultraviolet light using "Gel Documentation System UVITEK" (UK).

***DNA amplification*:** Polymerase chain reaction was performed by Williams (1990). DNA amplification was performed in a 25 µl reaction mixture volume, containing 10 × buffer, 20 ng of the genomic DNA, 0.2 µM primer, 200 µM of each of the following: dATP, dCTP, dGTP and dTTP, 2.5 mM $MgCl_2$, and 0.2 units of Taq-polymerase in the incubation buffer. PCR was performed in the "Applied Biosystems 2720 Thermal Cycler" (Singapore) thermocycler under the following conditions: 1 cycle - 3 minutes at 94ºC; 38 cycles - 1 min at 94 ºC, an annealing step at variable annealing temperatures depending on the primer pairs for 1 min, 2 minutes at 72 ºC; the final elongation cycle was performed at 72 °C for 10 min, then kept at 4°C.

The reaction products were separated by electrophoresis on a 3% agarose gel in the HR-2025-High Resolution («IBI SCIENTIFIC» U.S.) horizontal electrophoresis machine with addition of ethidium bromide and documented using «Gel Documentation System UVITEK». Sizes of amplified fragments were determined with respect to 100 bp DNA marker. Statistical analysis included binary matrix compilation for each of the primers, in which "presence" (1) or "absence" (0) of fragments with equal molecular weight on the electropherogram were noted.

RESULTS AND DISCUSSION

Drought stress is the main factor affecting grain yield and leaf senescence in wheat. Leaf senescence causes significant changes at the cellular, tissue, organ, and organism levels. In this work QTL for flag leaf senescence has been researched under drought stress using RAPD marker. 57 wheat genotypes collected in the Gene Pool of the Research Institute of Crop Husbandry acted as research objects. 38 of them were bread wheat, and 19 were durum wheat genotypes. Plants were cultivated under field conditions. RAPD marker OPH13 (5`GACGCCACAC3`) linked to the QTL for flag leaf senescence (Milad et al. 2011) was used for the screening

As can be seen in Table 3, RAPD OPH13 gives a positive result in 46 genotypes, this is approximately 81% of all genotypes used for this analysis. In more detail, 450 bp diagnostic fragments were identified in 31 bread wheat and in 15 durum wheat genotypes. This result shows that there is a gene locus that provides physiological rejuvenation of the flag leaf, which is an indicator of the resistance to drought stress in those genotypes. Amplification products were absent in 11 genotypes: 7 samples among them were bread wheat, the remaining 4 were durum wheat. The Primer OPH13 (5` GACGCCACAC 3') produced a strong polymorphic band at 450 bp (Fig. 1).

Wheat flag leaf has a key role during photosynthesis in absorption solar energy and therefore, flag leaf senescence is one of the main parameters to provide high productivity. Leaf senescence is induced not only by hormonal factors due to plant aging, external environmental factors, such as high temperature and drought can also be the reason of premature senescence (Chandler 2001).

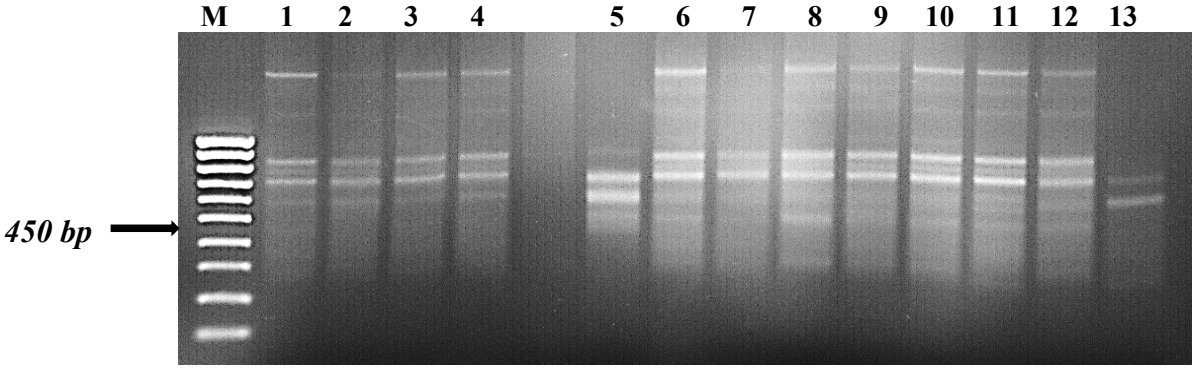

Fig. 1. PCR-profiles of wheat genotypes for RAPD OPH13. M - 100 bp DNA ladder, 1- Gilavar, 2- Sevinj, 3- Gyzyl bughda, 4- Garabagh, 5- Garagylchyg 2, 6- Yagut, 7- Shirvan 3, 8- Tartar, 9- Parvin, 10- Saba, 11- Ugur, 12- Aran, 13- Shiraslan 23, Arrow shows ~ 450 bp band.

Table 3. Results of PCR analysis using RAPD markers OPH13. [+] – presence of the expected locus, [-] – absence of this locus.

***T.aesivum* L.**			
Genotypes	OPH13 (450bp)	Genotypes	OPH13 (450 bp)
Parvin	+	Dagdash	+
Gilavar	-	Giymatli 2/17	+
Saba	+	Farandole	+
Layaqatli 80	+	Saratovskaya 29	+
Bayaz	+	Tale 38	+
Mahmud 80	+	A2	+
Murov 2	+	Miranovka	+
Pirshahin	+	Ruzi 84	+
Aran	+	1st WWEERYT	+
Ugur	+	Mirbashir 128	+
Zirva 85	+	Renan	+
Nurlu 99	+	11thFAWWON №	-
№97 12th FAWWON	-	Gyrmyzy gul 1	+
№50 4th FEFWSN	-	Akinchi 84	+
Parzivan	-	Azamatli 95	+
Fatima	+	Murov	+
Shaki 1	+	Sevinj	-
Farahim 2012	+	Gyzyl bugda	-
Qualite	+	Fransa	+
***T.durum* Desf.**			
Shirvan 3	-	Tigre	+
Mirvari	+	Gyrmyzy bugda	+
Turan	+	Garabagh	-
Vugar	+	Sharg	+
Tartar	+	Ag bugda	+
Yagut	+	Kakhraba	+
Mirbashir 50	+	Garagylchyg 2	+
Mugan	+	Shiraslan 23	-
Asgaran	+	Sarychanak 98	+
Barakatli 95	-		

. Biochemical and physiological events lead to leaf senescence, which is the final stage of leaf development. In wheat flag leaf senescence occurs when redistributing resources from the source to the sink during grain filling. The onset and rate of senescence are main determinants of yield potential (Evans 1993), because flag leaf photosynthesis in wheat contributes about 30–50% of the assimilates for grain filling (Sylvester-Bradley et al. 1990). Four classes of late senescence or 'stay-green' were described by Thomas and Smart (1993). Two of these classes relate to delayed onset of senescence or slower rate in progress of senescence, and the remaining two relate to cosmetic effects that lack photosynthetic capability. There were some reports on the inheritance of flag leaf senescence in wheat

under optimal conditions, where additive gene effects were demonstrated (Simon 1999). It was found that delayed onset of leaf senescence in sorghum (*Sorghum bicolour* L.) (Borrell et al. 2000a, 2000b), maize (*Zea mays* L.) (Baenziger et al. 1999) and durum wheat (*T. durum* L.) (Benbella and Paulsen 1998; Hafsi et al. 2000) increased plant productivity under water stress conditions. In sorghum a slower rate of senescence was also associated with increases in genetic yield under drought (Borrell et al. 2000a, 2000b).

It is necessary for plant breeders to understand the genetics of leaf senescence for increasing yield under drought. Moreover, this would allow the scientist to elucidate how genes and biochemical pathways controlling leaf senescence are regulated.

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Buğda Bitkisində Quraqlıq Stresi Şəraitində Flaq Yarpağın Qocalması ilə Əlaqədar QTL-in Tədqiqi

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Buğda bitkisində fotosintez zamanı günəş enerjisinin udulmasında əsas rol oynayan və bitkinin məhsuldarlığını şərtləndirən flaq yarpağın fizioloji qocalmasının tədqiq olunması stress şəraitində yüksək məhsuldarlığın təmin edilməsi üçün vacib parametrlərdən biridir. Bu baxımdan, RAPD OPH13 markerindən istifadə etməklə buğda genotiplərində stress şəraitində flaq yarpağın fizioloji qocalması ilə əlaqəli gen-lokusunun mövcudluğu tədqiq edilmişdir. PZR nəticələrindən əldə olunan elektroforetik profillərin analizinə əsasən, 31 yumşaq və 15 bərk buğda genotipində 450 bp diaqnostik fraqmentlər aşkar edilmişdir. Bu nəticə onu göstərir ki, genotiplərdə quraqlıq stresinə qarşı davamlılığın göstəricisi hesab olunan flag yarpağın fizioloji yaşılqalmasını təmin edən gen lokusu mövcuddur. Araşdırılan gen lokusu genotiplərin 19 %-də aşkar edilməmişdir.

Açar sözlər: *Buğda genotipləri, flag yarpağın qocalması, quraqlıq, PZR, RAPD*

Исследование Связанного со Старением Флангового Листа QTL у Пшеницы при Условиях Засухи

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Изучение физиологического старения флаговых листьв, играющих ключевую роль в поглощении солнечной энергии и определении продуктивности растений пшеницы, очень важно для обеспечения высокой производительности при стрессе. В генотипах пшеницы при условиях засухи, с помощью маркера RAPD OPH13, было исследовано наличие ген-локуса, связанного с процессом старения флаговых листьев. Основываясь на анализе ПЦР, в 31 генотипе мягкой и 15 – генотипах твердой пшеницы, были идентифицированы диагностические фрагменты 450 bp. Этот результат указывает на наличие локуса, обеспечивающего физиологическое озеленение флагового листа, который является критерием устойчивости к стрессу засухи у этих генотипов. Изученный локус не был обнаружен у 19% генотипов.

Ключевые слова: *Генотипы пшеницы, старение флагового листа, засуха, ПЦР, RAPD*

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Molecular Marker Technology: Current State and Prospective Directions (Review)

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The article provides detailed information on molecular marker technologies that are widely used in various fundamental and applied fields of science. Characteristics of the majority of existing types of molecular markers, their advantages and disadvantages are given. By being part of the genome, molecular markers carry a lot of information about its changes during ontogenesis and phylogenesis. This circumstance turns them into an excellent tool in studying evolution, systematics, genetic and epigenetic mechanisms of adaptation, as well as in creating more stress-resistant forms, in genetic mapping, genetic tagging, "genome editing", determining the quality of the surrounding environment, addressing issues of eco - and genotoxicology, diagnostics, forensic-medical expertise, etc. The results of numerous studies in which Hybridization or PCR-based molecular markers used for some of the above-mentioned purposes have been discussed as well.

Keywords: *Molecular marker, restriction fragment, polymorphism, amplification, cpSSRs, ESTs*

INTRODUCTİON

Biotechnology is one of the scientific areas open to the novelty. Molecular marker technologies - a practical achievement of this field - are very important both in scientific and practical terms. Development of these technologies started recently and within a few decades they began playing a great role in solving the problems of fundamental and applied sciences. There have been developed of many types of molecular markers, and combined with sequencing technologies, they aim to improve diagnosis, increase yields, etc. Progress in areas such as molecular selection, molecular genetics, genomic selection, genome editing has contributed to a more thorough understanding of molecular markers, and providing their greatest variety. Molecular markers are one of the powerful tools for analyzing genomes and allow us to establish the relationship between hereditary characteristics and the underlying genomic variability. Genotyping based on next-generation sequencing technologies contributes to the development of new markers for complex and unstructured populations.

The first markers, of course, were classical and, in the first place, morphological ones, according to which the state of the organism, in particular, plants, is evaluated. The following classical, no less informative and widely used, along with modern molecular ones, are cytological, biochemical markers. A molecular marker is usually called some gene in the genome or some region in the gene belonging to some fragment of DNA , a molecular marker is a nucleotide sequence associated with a trait of interest.

Molecular or DNA markers are classified based on different criteria. These criteria can be:

- a method of detection (on this basis they are divided into hybridization-based (historically the first) and based on PCR (Hybridization-based and PCR-based);
- the nature of the action on genes (dominant and co-dominant);
- the method of transmission to the offspring (on the paternal line by organoids, on the maternal line by organoids, by the nuclei of both parents, on the maternal line by the nucleus).

RFLP is the first and only method developed on the basis of hybridization. After the discovery of PCR, a large number of molecular marker methods that are widely used have been developed. These marker technologies are effectively used in diverse areas such as genetic diversity analysis, the design of genetic maps, genetic tagging, cloning, the creation of more stress-resistant variations, the analysis of the genotoxicity of substances, the study of epigenetic mechanisms of adaptation both during ontogenesis and phylogenesis, the study of systematics of various taxa, phylogenetics, evolutionary genetics, etc.

Thus, all the existing diversity of molecular

marker technologies can be divided into 2 main groups (based on the method of detection):

- based on hybridization
- based on the PCR system.

RFLP (Restriction fragment length polymorphism) is the first and perhaps the only hybridization-based molecular marker system that has been extensively used at the beginning of the molecular biology era. However, hybrid systems such as microarrays and diversity array technology (DArT) are currently being intensively used for detection of one nucleotide polymorphism (SNP - single nucleotide polymorphisms). Conversely, a lot of molecular-marker detection methods based on PCR have been developed. For example, amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), and sequence related amplified polymorphism (SRAP), inter-simple sequence repeat (ISSR), sequence tagged site (STS), and sequence characterized amplification region (SCAR) are mainly used for genomic analysis.

All molecular-marker systems have their advantages and disadvantages. For example, RFLP markers can be applied to closely related species and this is an advantage in comparative genomics. But in RFLP, the detection procedure is relatively complex and expensive, and therefore it is not easy to automate the procedure for thousands of individuals. AFLP is a widely used molecular marker system because it can detect multiple genetic loci in the genome and this is its advantage. On the other hand, there are many stages in the AFLP procedure that limit its use in marker assisted selection - when thousands of DNA samples need to be analyzed in a short time. SSR systems provide a high level of polymorphism in plant genomes and are commonly used in most genomic applications. Yet, the SSR technology detects only repeats in the sequence, while the number of SSRs in the genome is relatively limited compared to the number of SNPs. RAPD is easily performed in one round of PCR, although the low reproductive rate of RAPD amplification limits its wide use in genomic assays, etc. (Nadeem et al., 2018)

Molecular-marker technology is ideal when it has the following characteristics:

1) polymorphism, which makes possible the use of the entire genome;

2) the ability to detect genetic variations;

3) the ability to apply many reliable and independent markers;

4) simplicity, speed and low cost of production of markers;

5) a small amount of DNA or tissue is required;

6) has such functions as correlation to various phenotypes.

But no molecular-marker technology has all these features at once. Therefore, in many cases, a combination of different markers is used. For example, Amar et al., (2017) presents the results of a comprehensive analysis of the genetic diversity of local and international garnet varieties in Egypt, based on ISTR, ISSR and SRAP markers profiles. The set of ISTR, ISSR and SRAP combinations of the primers was compared. The comparison was made according to the degree of resolution, the effectiveness of the discriminating ability of the method (along with the level of genetic polymorphism) in order to determine which varieties evolved from others, i.e. evolutionary relationships. During the study of the SRAP results, the analysis proved to be the best in almost all parameters and it gives much more evidence about the total number of effective alleles, the number of polymorphic amplicons, whereas ISTR markers showed an average level of polymorphism.

The genetic stability of soybean seedlings was studied under allopathic treatment of them, fallen and mixed with soil in a different ratio of eucalyptus leaves (Abdelmigid and Morsi, 2018). The combination of RAPD and ISSR-PCR markers showed that the DNA of such soybeans demonstrates a general tendency to decrease GTS (genetic template stability) and with increasing dose of exposure, a decrease in GTS occurs more. Low GTS of plants subjected to stress reflects their high genetic instability. The results allowed the authors to offer the most appropriate combination of RAPD and ISSR markers for determining the allopathic tolerance of Glycine max (soybean) and for application in breeding programs.

1. Hybridization-based markers.

RFLP (Restriction Fragment Length Polymorphysm).

The main stages of RFLP:

- obtaining a pure DNA preparation
- treatment of DNA preparations with restriction enzymes that cut a DNA molecule into certain loci (in recognition sites), resulting in a mixture of a large number of DNA fragments with different lengths
- separation of the mixture into separate fragments in EF (in agarose or PAGE gel).
- transfer of fragments from the gel to the membrane
- hybridization with radio-labeled DNA samples.

Fragments form a polymorphic bands in the EF-gel. Each band is a population of DNA molecules with the same length. The reason for such a diversity between the fragments of two DNA

preparations (for example, extracted from the cells of different individuals of the same species) can be the loss or addition of DNA sites (InDel mutations), or the change in recognition site by other mutations (point mutations, translocations, duplications, inversions etc.). As a result of such changes, new recognition sites for restriction enzymes are lost or, on the contrary, new ones are added, which in turn leads to polymorphism between fragments. Individuals of the same species may differ from each other, i.e. they can exhibit polymorphism. If the genome of the individual is heterozygous, polymorphism is manifested only in relation to one chromosome.

2. PCR-based molecular technologies.

There are many more of them. The efficiency and sensitivity in their implementation are mainly determined by the correctly chosen primer (annealing temperature, stability of the duplex primer-template, primer length, GC%, melting and annealing temperature of primer, etc.) (Williams et al., 1990; Jones et al., 1997). If all the PCR conduction rules are followed but the primer is not selected correctly, then the PCR protocol results in inadequate results. Thus, all PCR-based technologies, apart from everything else, depend on the correct choice of the primer.

RAPD (randomly amplified polymorphic DNA). RAPD technology was developed independently by two groups of scientists (Williams, et al 1990, Welsh and McClelland, 1990). In this method, DNA amplification occurs using a random, short and single-stranded primer. Amplicons fully depend on the size of target genome and primer (Jiang, 2013). The reproducibility of RAPD markers depends on such factors as the amount of DNA, PCR buffer, concentration of $MgCl_2$, annealing t^o, Tag-Polymerase, etc. (Wolff et al., 1993).

The influence of various chromium concentrations on the phenotypic growth characteristics of T.aestivum L. was studied (Rai and Dayal, 2016). The pollution of the environment by chromium compounds is relevant for all countries of the world, it occurs in connection with the development of the metal processing industry, the use of fungicides, the production of paints, cleaning of petroleum products, etc. Plants growing in contaminated areas and phenotypically altered were analyzed for the presence of polymorphism in PCR with RAPD primers. Based on the results, the authors came to the conclusion that, firstly, Cr at a concentration of 80 ppm is capable of causing a mutagenic effect in wheat, and secondly, several primers must be used to maximize detection of DNA changes.

RAPD markers can be used for early identification of plant tolerance. So, RAPD analysis was applied (Saleh, 2016) to assess DNA damage in 4 local varieties of cotton growing in non-saline and salt stress areas (200 mM NaCl). Changes in RAPD profiles are measured as genomic template stability (GST% = (1- a / n) x 100, where (a) is the average number of changes in the DNA profile and (n) the number of total bands in the control). As expected, the greatest changes occurred in two salt-sensitive, and the lowest - in 2 tolerant varieties.

The work of Olorunfemi et al. is an example of the use of molecular markers in eco-genotoxicology (Olorunfemi et al., 2015). In this study, RAPD analysis was used to assess the level of DNA damage in the cells of the root meristem of Allium cepa L. grown in contaminated drinking water. It turned out that in the DNA samples of plants that were watered with polluted waters, there were big changes in RAPD patterns. The authors suggest that the determined DNA polymorphism is caused by the genotoxic action of substances present in contaminated drinking water, which in turn can cause health problems. The results of such ecotoxicological studies are of great importance in genotoxicology

Altwaty and others studied the genotoxicity of one plant - D.glaucum, which is widely grown in Saudi Arabia - on another plant (Altwaty et al., 2016). The authors studied DNA polymorphism in a V. faba plant exposed to 3 types of Dipterygium glaucum extract (water, ethanol and ethyl acetate extracts) using RAPD markers and found the polymorphic effect.

RAPD markers in combination with STR markers were useful in detecting polymorphism in plants chronically irradiated with low doses of ionizing radiation (Khudaverdieva et al, 2005).

The sensitivity of lichens to air pollution is a well-known fact. But a detailed analysis of DNA with RAPD markers (Cansaran-Duman et al., 2015) made it possible to uniquely identify the most sensitive bioindicator of pollution (sulfur dioxide, nitrogen oxides, PM, generated by low-quality lignite used as fuel for household heating, and also released into the atmosphere by motor transport) of atmospheric air. P.praetextata proved to be the most sensitive species among the 4 investigated and the authors suggested using this species as a bioindicator of the environmental quality. It is proved that in order to obtain a reliable estimatation of the effects (Abdelhaliem and Al-Huqail, 2016), various methods (isozyme analysis and RAPD-PCR, as well as molecular cytogenetic assays such as single-cell gel electrophoresis-comet assay) for the analysis of genotoxicity of environmental pollutants should be applied in a comprehensive manner. RAPD-PCR and comet assay are the two

qualitative and quantitative methods that have been used to detect DNA damage and mutation. The authors studied the genetic effects of elevated levels of CO_2 and O_3 (alone or in combination) on wheat proteins and DNA (*Triticum aestivum* L.) under irrigated and non-irrigated conditions.

AFLP markers. Was developed by Vos P. et al. (Vos et al., 1995).

The sequence of procedures in this PCR technology is as follows:

1) restriction of genome DNA by two restriction enzymes (EcoRI and MseI) with the formation of fragments with protruding 3 '- ends; 2) restriction fragments of DNA are ligated with oligonucleotide adapter containing "sticky" ends for these restriction sites; 3) two consecutive PCRs are then carried out: in the first PCR, there are used primers that are fully complementary to the EcoRI and MseI adapters, therefore, a large number of amplicons are created between the EcoRI and MseI adapters, while EF separation is impossible. In the second PCR, primers are used, at the 3 ' – end of which there are bases that are non-complementary to adapters (1 to 3) and due to them selective amplification takes place. The Electrophoretic separation of DNA fragments is carried out in a denaturing PAAG gel.

AFLP are dominant markers, their polymorphism is higher than that of RAPD and ISSR. In this, as well as in an unlimited number of samples in each analysis, AFLP is superior to RAPD and ISSR. The method allows the specific co-amplification of high numbers of restriction fragments (typically 50-100 fragments). Expensive equipment, software and also expensive consumables are its drawback.

SRAP (sequence related amplified polymorphism). SRAP was developed by Li and Quiros, mainly to amplify open reading frames in 2001 (Li G and Quiros, 2001). This marker system is based on the use of 2 primers (Li G. et al., 2013). The initial idea of the authors was to simplify AFLP and improve productivity, as well as to improve reproductive performance compared to RAPD. To obtain a simplified detection procedure, the authors in the AFLP - detection protocol omitted the stage of restriction digestion and DNA ligation of target fragments. SRAP primers were created in size close to the primers AFLP, but instead of 2 cycles in AFLP, 1 cycle of PCR was applied. To detect multiple loci with a pair of SRAP primers, the authors invented a special PCR procedure program (94°C for 1 minute, 35°C for 1 minute, 72°C for 1 minute / first 5 cycles) and the next 30 cycles at an annealing temperature of 50°C. Relatively low annealing temperature (35°C) at the beginning of PCR allows the SRAP primers to be annealed to multiple loci in the target DNA, thereby multiple loci were amplified and profiles similar to those in the AFLP were obtained. Similar to AFLP, most SRAP markers are dominant. Unlike RAPD, SRAP uses a pair of primers of 16 and 22 nucleotides, instead of 10-nucleotide short primers. This is a great advantage of SRAP technology compared to the RAPD system, since 1 SRAP primer can be combined with an unlimited number of other primers. Although SRAP PCR starts with 35°C t^{o}-annealing in the first 5 cycles, larger sizes of SRAP primers allow increasing the annealing temperature up to 50°C in the following cycles, which greatly improves reproductive performance in SRAP. In addition, SRAP primers can be labeled with fluorescent labels and combined with untagged SRAP primers, so SRAP PCR products can be separated in capillary instruments such as ABI genetic analyzers.

It is known that there are differences in the GC content between the coding and non-coding sequences in the plant genome. These differences can be used in the development of two sets of SRAP primers. The forward primers containing the GGCC cassette closing the 3' end of SRAP primers that might preferentially anneal to the GC-rich regions while the reverse SRAP primer set was incorporated with an AATT cassette that would preferentially anneal SRAP could preferentially amplify gene-rich regions in a genome. After sequencing the SRAP fragments and constructing the SRAP of the B. oleracea genetic map, it was found that SRAP did indeed amplify the sequences from the genes, and a larger number of SRAP markers fell in the region of the chromosome arms, and less in the centromeric regions, which normally filled the AFLP markers.

Depending on the goal of the researcher, it is possible to design various SRAP primers, this is possible due to the wide flexibility of SRAP primers in development. To increase the capacity and effectiveness of SRAP technology, SRAP Illumina's Solexa sequencing can be combined to directly integrate genetic loci into the genetic map of *B.rapa*, based on paired-end Solexa sequences (W.Li et al., 2011).

Like multi-locus, SRAP markers have many advantages and it is an effective tool for conducting comparative genomics (Guo et al., 2012). In addition, SRAP markers do not require a complex of restriction enzymes and for pre-amplification, what is needed for AFLP, which makes SRAP a more effective molecular marker system for genome research.

SRAP - technology has several advantages for gene tagging, and for this feature it is superior to other molecular markers. The authors show that

they used SRAP technology for cloning and the characteristics of a gene that controls features such as color of the seed coat and hairiness in *B.rapa* (Long, Y. et al., 2010).

SRAP - technology is an effective molecular marker system also for qualitative and quantitative analysis of resistance to phytopathogens. Usually qualitative resistance is an oligogenic feature, and quantitative resistance is multigenic. Both the use of SRAP and other markers, as well as the correlation of results, have made it possible to identify the multi-gene character of resistance to many diseases in many plants (Chen et al., 2012; Li et al., 2012; Li et al., 2008; Mutlu et al., 2008; Tanhuanpaa et al., 2007; Yi et al., 2008).

Some authors have done an enormous job for the development of molecular markers associated with resistance genes to various factors (in this case, resistance to nematodes of cucumber root nodes (Meloidogyne spp) (Devran et al. 2011). They tested 100 AFLP markers and 112 SRAP (sequence related amplified polymorphism) markers. They used them to screen for resistant and susceptible parents for detecting polymorphism. Out of the 100 AFLP primers, 92 bands were synthesized, 2 of which were selected as putative candidate markers. These 2 strips of gel were transferred (eluted) to the solution, cloned and sequenced. The primers synthesized from these sequences did not give polymorphic bands between the parents. In these sections of DNA, there were no restriction sites to convert them to CARS or SCAR markers. Therefore, in the future SRAP primers and fragments developed from these primers AFLP became to be used to detect polymorphism between parents. Of the 112 primer combinations, 11 polymorphs were detected.

Molecular markers associated with resistance to root nematodes were also used in the other work (Ali et al., 2014). The authors investigated the inheritance, as well as the mapping of the mj-2 gene in carrots, the crop of which is severely affected by this pest (*M.javanica*), resulting in deformations of the carrot roots. And this reduces the productivity of agricultural products, especially in warm climates, which is very important for our region as well. Using the appropriate molecular markers associated with the mj gene, an arrangement was found for the so-called mj-2 gene on the same chromosome as mj-1 - previously described resistance gene to nematodes, but in another locus. It was found that the sign of resistance to root nematodes is controlled not by a single gene i.e. it is not formed under the control of one gene.

Genetic diversity was first studied among 13 species of Silene genus, growing in Iran, using SRAP markers (Bargish and Rahmani, 2016). This genus (Silene) is one of the most complex regarding taxonomy. With the use of 15 combinations of SRAP primers, 62 fragments were obtained, 71.9% out of which were polymorphic. The polymorphism varied in the range of 50-100%.

Consequently, SRAP markers are highly reproductive and optimal markers for evaluating genetic variation in clove (Silene). This and many other works show that molecular markers can be successfully applied in the analysis of genetic diversity. And the study of genetic relationships among plant taxa at the species and generic levels is of great importance, since this analysis gives information about the direction and the successive stages of plant evolution (Savolainen and Chase, 2003).

The bacterial wilt caused by Ralstonia Solanacearum is one of the diseases that causes huge damage to the potato yield. SRAP technology was used to detect loci that determine resistance to this pathogen (Yanping et al., 2014). In this work there were used easily cultivated, highly resistant species to produce F1 to carry out bulked segregant analysis (BSA) for screening and identifying SRAP markers associated with potato resistance to the bacterial wilt. A clutch map was developed for 23 DNA markers distributed over 3 clutch groups. The results of this work show that this marker system can be used in marker assisted selection (MAS) of plants.

SRAP markers are mainly used for agronomic purposes, developing in modern hybrids loci of quantitative traits and for assessing the genetic diversity of large germplasm collections. But some authors believe that SRAP markers should also be used to solve hypotheses in systematics, biogeography, conservation, plant ecology and others (Robarts and Wolfe, 2014).

The paper (Soleimani et al., 2012) gives the results of assessing genetic diversity using SRAP markers of 63-cultural, wild and decorative garnet genotypes, selected from 5 different geographical regions of Iran. The authors confirm with their results that SRAP markers can be powerful tools and an effective marker system for determining the genetic diversity and genetic structure of the pomegranate population.

TRAP (targed region amplification polymorphism). A rapid and efficient PCR-based target region amplification polymorphism (TRAP) technique was developed by Hu and Vick (Hu and Vick, 2003). TRAP markers are a PCR-based method by which a large number of loci can be determined (Barakat et al., 2013).This marker system is characterized by simplicity, high throughput, with numerous co-dominant markers, with high reproducibility (Alwala et al., 2006).

TRAP uses bioinformatics tools and the EST database information to generate polymorphic markers around targeted putative candidate gene sequences. Thus, it should be useful in plant genomics research in genetic mapping and marker-trait association (Liu et al., 2005). Previously, TRAP was also used to estimate the genetic diversity in the genetic stocks of wheat (Xu, Hu and Faris, 2003; Al-Doss et al., 2011).

In TRAP technology, 2 types of 18 nucleotides are used to create markers. One of the primers is stable, developed on the basis of EST bank data, and another primer is designed for AT- or GC-rich regions. Unlike the SRAP method, TRAP requires information about the cDNA or EST sequence to design a specific primer. Like SRAP markers, TRAP markers are used in the analysis of the genetic diversity of germplasm, in the construction of genetic maps (including the construction of transcriptome maps), genetic tagging of important features and in the cloning of genes in many crop plants (Hu et al., 2005; Chen et al., 2006).

In TRAP technology, almost the same number of markers as AFLP is created. But, it does not require extensive pre-PCR processing of templates, as does the AFLP technique. An evaluation of 60 clones of the economically important species, *Paullinia cupana* var. *sorbilis*, preserved in Active Germplasm Bank (BGA), was conducted using TRAP and SRAP markers (Elizangela Farias da Silva et al., 2016). The level of polymorphism was 79% when using TRAP - and 74.5% using SRAP-markers. Thus, the combination of 2 markers can expand the genetic characteristic and facilitate the selection of parental forms in breeding, what is also confirmed in other works (Menzo et al., 2013).

ISSR (inter simple sequence repeat) markers. This marker system was developed by Zietkiewicz et al. (Zietkiewicz et al., 1994). ISSRs makes it possible to determine polymorphism in intermicrosatellite loci (using primers of 2 or 3 nucleotide repeats). This method does not require information about the sequence of the genome and a high level of polymorphism can be realized (Chatterjee et al., 2004). ISSR is based on the amplification of DNA segments located between two identical but oppositely directed microsatellite repeats. The distance between these microsatellites should allow the flow of amplification. The primers used in this method-microsatellites can be di-, tri-, tetra- and penta-nucleotide repeats. In this method, high annealing t^{o} (40-60°C) can be used, while amplicons have a length of 200-2000 bp (Fang and Roose, 1997, Moreno et al., 1998). Although ISSR markers are characterized as dominant, they can also be used in the development of co-dominant markers (Zietkiewicz et al., 1994; Tsumura et al., 1996; Ng and Tan, 2015). Relative low reproducibility is a disadvantage of these markers (Semagn et al., 2006).

The authors used several marker systems, in particular ISSR markers, to evaluate the effects of lead on DNA (Manfouz and Rayan, 2016). The main facts observed in the ISSR patterns of Hordeum vulgare, in the study of the effects of lead exposure, were the following: loss of bands that are present in the control, and the appearance of new ones that were not present in the control. Of the 9 used primers, almost all had more than one change and the maximum number of changes in the ISSR bands corresponded to a high concentration of lead (150 g / l). The authors explain the differences in the patterns by the presence of photoproducts (e.g. pyrimidine dimers) in the DNA, which could block or reduce polymerization of DNA in PCR. It is suggested that the DNA damage may be serious in the majority of the barley seedlings exposed to toxic chemicals. Apparently, at high lead concentrations, DNA damage is so great that DNA polymerase is more often blocked and as a result, the differences in PCR profiles are greater compared to other variants. The authors note that ISSRs are simpler among the molecular markers and this system is more adaptable, since their use does not require preliminary information on target sequences, they are effective and reproducible (Bornet and Branchard, 2001; Fang and Roose, 1997; Pradeep-Reddy et al., 2002).

Various calluses of the same parent cauliflower were analyzed by ISSR markers to characterize genetic instability (Xavier et al., 2000). Various ISSR markers exhibited a different degree of polymorphism. After sequencing, one sequence showed homology with the A.thaliana gene, closely associated with the mammalian genes involved in the regulation of cell proliferation. This marker is characterized by 3 microsatellites with palindromic sequence. Possible causes of mutations in this marker are discussed. ISSR amplification appears to be a reliable method for determining genetic instability at the early stages of in vitro culture. ISSR markers in combination with other markers are also used for genetic mapping (Chen et al., 2011).

With the help of ISSR technology (in combination with other molecular markers - ITS1 and ITS4, PCR-RFLP), it was possible to detect changes in the DNA methylation profile of maize under fractionated UV-C irradiation, and also to establish the relationship of these changes with the release of chromosomal aberrations (Kravets et al., 2013). This relationship was also previously reported (Hauser et al., 2011).

MSAP (Methylation Sensitive Amplified Polymorphism) markers. This method is designed

to assess the degree of methylation of cytosine and has been successfully applied to the genomes of many species (Arabidopsis, grape, maize, tomato, and pepper species). The method is based on the use of isoschizomers, which differ in their sensitivity to methylation. MSAP approach is used also to assess the extent of cytosine methylation under salinity stress (which is a major ecological and agronomical problem and this problem is more acute in regions where salt water is used for irrigation) in salinity-tolerant and salinity-sensitive rapeseed cultivars (Marconi et al., 2013). Data of this study show that salinity affected the level of DNA methylation (an increase of 16.8%). In particular, methylation decreased in salinity-tolerant and increased in salinity-sensitive cultivars. Nineteen DNA fragments showing polymorphisms related to differences in methylation were sequenced. In particular, two of these were highly similar to genes involved in stress responses and were chosen to further characterization. The authors concluded that plants can employ regulatory strategies, such as DNA methylation, to enable relatively rapid adaptation to new conditions.

Another example of the use of MSAP markers is a study conducted by Guangyuan et al., (Guangyuan et al., 2007). In this work, genetic damage and DNA methylation induced by salt stress in *B. napus L.* were evaluated by AFLP, SRAP and MSAP markers. In the MSAP analysis, 3 strips with significant deviations from other PCR bands were identified. Analysis of the results showed that after saline treatment, methylated CCGG sites increase by 0.2 -17.6%. Nine methylation sites were sequenced and 1 site identified with a high degree of homology with the ethylene responsive element binding factor (ERF) sequence. These results demonstrate clear genetic and epigenetic changes in plants in response to salt stress and these changes underlie the mechanism of plant adaptation to salt stress.

Two forms of sequence based marker, Simple Sequence Repeats (SSRs), also known as microsatellites, and Single Nucleotide Polymorphisms (SNPs) now predominate in modern genetic analysis. Reducing the cost of DNA sequencing has led to the availability of large sequence data sets derived from sequencing of whole genomes and to the detection of large scale EST (Expressed sequence Tag), which allow the development of SSRs and SNPs, and the latter in turn can be applied to diversity analysis, genetic trait mapping, association studies, and marker assisted selection. These markers are inexpensive and require minimal laboratory work, for the development.

SSRs (Simple Sequence Repeats). These markers are short tandem repeats and simple sequence length polymorphisms. SSRs are tandemly repeating 20 bp motifs from 1 to 6 nucleotides. SSRs are present in a large number in the genomes of various taxa (including those in prokaryotes) (Tautz, 1989; Schlotteröer et al., 1991). Microsatellites can be mono - (A), di - (GT), tri - (ATT), tetra - (ATCG), penta - (TAATC) and hexa - nucleotides (TGTGCA) (Weber, 1990). They occur in the nuclear and cytoplasmic (in mitochondria and chloroplasts) genomes (Rajendrakumar et al., 2007; Melotto-Passarin et al., 2011). There is evidence that SSRs exist within protein-coding genes, as well as in ESTs (Morgante et al., 2002; Ding et al., 2015). SSRs are codominant markers, have high reproducibility, provide a high level of polymorphism, can be used in studies on plant gene mapping (Kashi and King, 2006). This system requires a very small amount of DNA and there is the possibility of automating procedures. SSRs were initially considered evolutionarily neutral markers, but later there was obtained an evidence of their important role in the genome evolution (Moxon and Wills, 1999). It is believed that SSRs are involved in the expression, regulation and functioning of genes, they are of functional importance even in noncoding regions of the gene (Kashi et al., 1997; Li et al., 2002; Mortimer et al., 2005).

cpSSRs (chloroplast microsatellites). The chloroplast genome is characterized by a low mutation rate and therefore it is difficult to detect enough sequence variations. Conversely, cpSSRs provide a high degree of polymorphism, due to which they are very useful in studies on population genetics (Provan et al., 2001; Melotto-Passarin et al., 2011). cpSSRs are mononucleotide motifs that repeat 8 to 15 times. The level of polymorphism in cpSSRs is quite variable within the species and locus. cpSSRs differ from nuclear microsatellites by such properties as single-parent inheritance and non-recombinantity of the molecule. They have found wide application in both fundamental and practical fields of plant science (Park et al., 2016; Gregory et al., 2014).

Mitochondrial microsatellites (mtSSRs). mtSSRs of plants are highly dynamic, the greatest and smallest density of genes are present namely in mtDNA. Their sizes range from 200 to 2500 bp and contain various repeating elements and introns (Liu et al., 2011). mtDNA - markers are characterized by a low rate of evolution. The use of these markers is mainly limited to population genetics (Duran et al., 2009).

SNPs (single nucleotide polymorphism). SNPs markers are the most abundant source of genetic polymorphism, represent single nucleotide

differences in a specific DNA location of two individuals. There are 3 different categories of SNPs: transitions - C / T or G / A, transversions (C / G, A / T, C / A or T / G) and small inserts / deletes (InDels). SNPs in certain places can be bi-, tri-, or tetra-allelic, although tri- and tetra-allelic SNPs are rare, but most SNPs are biallelic (Doveri et al., 2008). This disadvantage of SNPs as compared to multiallelic SSRs markers is compensated to some extent by the relatively large abundance of SNPs. The abundance of SNPs in the genome provides a high density of markers near the interesting locus researcher. SNPs are evolutionarily stable markers and this is their advantage. They do not change from generation to generation and this low variability makes SNPs excellent markers for studying complex genetic features and a tool for understanding genomic evolution, as well as for identifying parasites (Rogers et al., 2012; Gujaria-Verma et al., 2016; Li X. et al., 2015). SNPs are also the dominant markers in biomedical applications due to the availability of data on the sequence of the human genome (due to what is known from the HapMap Project) and the knowledge of allelic variability (Altshuler et al., 2005).

The ability to screen a large number of individuals for SNP options allows predicting sensitivity to many diseases and creates conditions for personal medicine (Khatkar et al., 2007; Pavlovic S. et al., 2014). Thus, it is suggested that SNPs will still co-exist with other marker systems (Rafalski, 2002; Gupta et al., 2001). However, probably new technologies that reduce the cost of developing SNPs will find a wider application.

CAPS (cleaved amplified polymorphic sequences). They are also called as PCR-RFLP markers (due to being the combination of PCR and RFLP) (Maeda et al., 1990; Pouryasin et al., 2017; Bielikova et al., 2010). In this method, the DNA under study is amplified in PCR and then digested by restriction enzymes. If such changes as SNP and InDel occur in DNA, restriction enzyme recognition sites also change, resulting in DNA fragments having different lengths, thus polymorphism is clearly demonstrated.

The codominance and locus-specificity of CAPS-markers make it easy to distinguish homo- and heterozygous alleles. The need for a very low amount of DNA, the ability to differentiate codominant alleles, simplicity and cheapness - are the advantages of CAPS technology. With the use of CAPS markers, changes in the DNA sequence associated with the mutations create limitations and therefore the level of polymorphism is not as high as that of the SSR and AFLP markers. However, CAPS is widely used in mapping studies, on molecular tagging of genes (Filiz ve Koç, 2011). The primers used in this technology are developed based on the sequence information available in the genomics data banks or the cloned RAPD bands and DNA sequences. CAPS markers are universal and the possibility of finding polymorphism can be improved by combining CAPS with SCAR, AFLP, RAPD or SNPs markers (Agarwal et al., 2008; Shavrukov, 2016).

CAPS which are closely related to target genes are particularly useful in marker-assisted selection. Such CAPS markers are widely used in the selection of wheat, barley, soybean, potato, tomato and other plants to create disease-tolerant forms. Like many others, CAPS markers are also used to improve the plant's such important features as development, grain quality and cereal tolerance to pathogens, as well as the shape of tomato fruit (Shavrukov, 2016). CAPS markers are used in genotyping, in map-based cloning, and in gene identification studies (Spaniolas et al., 2006; Hou et al., 2010; Sandal et al., 2005)

SCAR (sequence characterized amplified regions). SCAR markers were first developed in 1993 for the genes for resistance to downy mildew of lettuce leaves (Paran and Michelmore, 1993). And later they started to be widely used for research of other plants (Busconi et al., 2006; Hernandez et al., 1999; Arnedo-Andres et al., 2002; Yuskianti and Shiraishi, 2010). Compared to RAPD markers, their reproducibility and specificity are higher. These markers are co-dominant and monospecific and they are mainly used for physical mapping. To develop SCAR primers, the procedures are performed as follows: after carrying out PCR, polymorphic bands are selected, the DNA sequence of the selected bands is sequenced. Sequence analysis of these polymorphic DNAs is performed through comparison with known DNA sequences present in the NCBI database (National Center for Biotechnology Information). This nucleotide sequence of polymorphic DNA is then used to synthesize specific SCAR primers.

ISTR (Inverse sequence-tagged repeat). ISTR markers are referred to as Retrotransposon-based molecular markers, like IRAP (Inter-Retrotransposon Amplified Polymorphism), REMAP (Retrotransposon-Microsatellite Amplified Polymorphism), iPBS (Inter Primer Binding Site amplification), S-SAP (Sequence-Specific Amplification Polymorphism), RBIP (Retrotransposon Based Insertion Polymorphism), ISAP (Inter Sine Amplified Polymorphism), etc.

Retrotransposons, especially LTR retrotransposons, form a significant part of the plant genome. Using the RNA mediator (intermediate) they are inserted into different parts of the genome

and cause mutations. Like all transposing elements they are present in the DNA of various parts of the chromosomes (in the telomeric, pericentromeric, centromeric parts, in introns and exons, in regulatory regions). Their number positively correlates with the size of the genome. For example, in the genome of A.thaliana they are much less than in the genome of Hordeum vulgare (Schulman and Kalendar, 2005). These properties and dynamism of LTR retrotransposons in plant genomes have made them excellent sources of molecular markers (Kalendar and Schulman, 2006; Gozukirmizi et al., 2015). The ISTR method is a multi-locus marker system based on the selective PCR amplification. The first ISTR primers were designed from coconut copia-like retrotransposon (Rhode, 1996). ISTR analyses of various plant species with the identical primers developed from coconut sequences have been discovered that these primers are universally applicable (Aga and Bryngelsson 2006).

Sequence-based markers. Molecular markers, which are based on identifying the sequence of individual DNA regions in a common pool of unknown DNA, are called Sequence-based markers. The development of this technology is due to the fact that markers based on hybridization are less reliable and less polymorphic. The advent of sequencing technologies such as next-generation sequencing (NGS) and genotyping by sequencing (GBS) caused dramatic changes in plant breeding by developing SNP, leading to high polymorphism (Davey et al., 2011, Jiang et al., 2016). Sequence-based markers are also used to study the evolution of primates (Bergey et al., 2013)

Due to high productivity, sequencing of the complete genome is achieved for economically important species such as rice, corn, soybean, sorghum, tomatoes, potatoes and Chinese cabbage, etc. While it is still difficult to use NGS technologies to mate the entire complex genome of plants such as barley and wheat. Within NGS technologies there have been identified thousands of SNPs that can be used to develop molecular markers in species with a complex genome. In addition, NGS is directly used in the detection of SNP, and several dozen genotypes can be progressively sequenced to collect ultra-dense genetic maps. In addition, various strategies are used to create partial genomes that can be used to directly monitor SNP using NGS technologies.

EST technology. For expression, each gene must be converted (transcribed) into mRNA. The mRNA formed during transcription serves as a template for protein synthesis during translation. However, there is a particular problem: mRNA is very unstable outside the cell. Therefore, a so-called enzyme, reverse transcriptase, is used to convert mRNA into cDNA (i.e., a reverse transcription process). cDNA which is much more stable than mRNA is an experimental DNA sequence because it is formed from mRNA. All mRNA in some tissue is a transcript that serves as a template for the synthesis of the cDNA library. These cDNAs refer to genes that are expressed in this tissue. Short segments (<100 bases) at one or both ends are sequenced on these cDNAs. These sequences - tags - carry within themselves sufficient information to identify the expression gene - they are called ESTs (Saccone and Pesole, 2003).

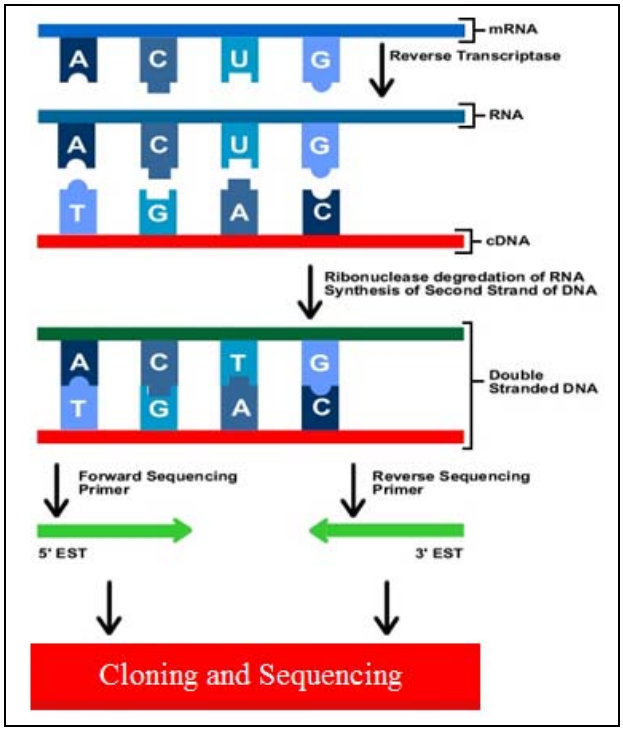

Fig. 1. Scheme of obtaining EST-tags of an expression gene.

EST sequences are used as a tool for gene identification, gene discovery, sequencing and the development of EST-based important molecular marker systems such as RFLP, SSR, SNP, CAPS. In addition, EST sequences can be used as probes for the determination of gene expression, in DNA microarrays, genetic linkage maps and physical maps (Davis et al., 1999). ESTs are a useful source for SSR markers, in various plant species the total share of SSR of 20 bp or more is 1 to 5% (Kantety et al., 2002).

SSR-regions are found in transcriptional loci, which are more conservative (which is the reason for their low polymorphism) than non-transcribed regions and their transfer to close species seems logical. Using primers obtained from EST sequences, amplification and sequencing of the relevant region can reveal many SNPs. More than

45 million ESTs have been created from 1,400 different species of eukaryotes. ESTs are used in areas such as phylogenetics, transcript profiling (or so called "expression profiling" is a quantitative study of the expression of the gene of many genes at the transcriptional level - at the RNA level) (Varshney et al., 2007).

Interdisciplinary approaches in the development of molecular markers. In recent years, new interdisciplinary approaches have been used to develop molecular markers. The mechanisms of response to radiation are conservative in plants and animals. The mechanisms of response to DNA damage (DDR) are the prevailing molecular mechanisms that are activated by radiation in plants and animals. This conservative nature of DDR in plants and animals can contribute to interdisciplinary researches which cross the traditional boundaries between plant biology and animal biology, which can expand the collection of markers currently used in REM (radiation exposure monitoring) for environmental and biomedical purposes.

Genes involved in trans-kingdom conservatively stored DDR networks often work with IR and UV-irradiation, deposited in biological databases. The authors adopted an innovative approach which uses data from available banks related to plants and humans to develop a "plant radiation dosimeter", i.e. based on DDR and plant genes, a platform that could serve as a marker for detecting the DNA damage and for assessing radiation-related risks for both environment and health (Nikitaki et al., 2017).

Quantification of DNA damage can be carried out on the basis of the analytical method of substances that induce DNA damage (LLC 4/16 2024APrinted in USA in 2016. Patents: www.moleculardevices.com/productpatents). This method is based on immunofluorescence detection of phosphorylated histone H2AX and 53BP1 protein. Therefore, the histone H2AX phosphorylation on serine 139 as well as the tumor suppressor protein 53BP1 can serve as a kind of marker for spontaneous or induced DNA damage. These molecular markers can be used to identify and characterize the mechanisms of action of genotoxicity of these agents. Since, with double-stranded DNA breaks, rapid phosphorylation occurs, this change can be used as an early and sensitive marker of double-stranded DNA breaks. In turn, 53BP1 protein is associated with repair processes, its phosphorylation and the formation of nuclear foci in response to DNA damage, as well as phosphorylation of H2AX, is an indirect but very sensitive method for determining double-stranded DNA breaks.

Using the specific - UV-B induced molecular markers, it is possible to study the mechanisms of the reaction of organisms to the impact. Some authors suggest that plants have at least 2 signal transduction mechanisms that regulate gene expression after UV-B absorption (Kalbina et al., 2008). Based on this approach, the nature of the dependence of fluence-response for mRNA transcripts of 4 molecular markers induced in A.thaliana by UV-B at 4 wavelengths in the range of 280-360 nm is found:

1) CHS (encoding chalcone synthase)

2) PDX1.3 (the coding enzyme involved in the formation of pyridoxine)

3) MEB5.2 (encoding a protein with unknown function, but very strongly regulated by UV-B)

4) LHCB1*3 (encoding xl a/b binding protein)

After analyzing the results, the authors concluded that there are 2 different UV-B responses: a) sensitive to rays in the range 300-310 nm: molecular markers CHS and PDX1.3 are regulated by a chromophore absorbing at a wavelength of 300 nm; b) sensitive to rays in the range 280-290 nm: molecular markers MEB5.2 and LHCB1* 3 are regulated by a chromophore absorbing at a wavelength of 280-290 nm.

Consequently, unlike many stress factors, the effect of UV radiation is highly specific and responses also differ in specific mechanisms and markers, what should be taken into account in the relevant studies.

CONCLUSION

The development of new molecular methods of research expands the possibilities of the human mind in understanding the finest regulatory mechanisms present in the functioning of the genome of all living organisms. The knowledge gained thereby allows us to manage these mechanisms, to introduce useful ones into genomes, where they were previously absent (both hybridologically and genetically engineered). Therefore, molecular-marker technologies are considered as one of the most promising approaches in genetic analysis, in selection, in diagnostics, etc.

A multidisciplinary, inter-kingdom approach in the development of molecular markers is promising, as it promotes the creation of universal markers, which is economically most effective.

The existing close relationship between sequencing and molecular marker technologies promotes a more complete detection of single nucleotide polymorphism in the population, which is especially important in the personalization of

medicine.

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Molekulyar Marker Texnologiyası: Müasir Vəziyyət Və Perspektiv İstiqamətlər (Icmal)

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Məqalədə elmin müxtəlif fundamental və tətbiqi sahələrində geniş tətbiq olunan molekulyar marker texnologiyaları haqqında müfəssəl informasiya təqdim edilmişdir. Məqalədə həmçinin mövcud molekulyar marker texnologiyalarının əksəriyyətinin xarakterik xüsusiyyətləri, onların üstün və çatışmayan cəhətləri göstərilmişdir. Genomun bir hissəsi olmaqla, molekulyar markerlər onun filogenez və ontogenezin gedişində baş verən dəyişiklikləri haqqında böyük informasiyanı özlərində daşıyırlar. Bu məqam molekulyar markerləri təkamülün, sistematikanın, adaptasiyanın genetik və epigenetik mexanizmlərinin öyrənilməsində, stres amillərinə qarşı yüksək davamlı formaların yaradılmasında, genetik xəritələrin yaradılmasında, genlərin identifikasiyasında, "genomun korreksiyasında" (genome editing), ətraf mühitin keyfiyyətinin müəyyənləşdirilməsində, eko – və genotoksikoloji problemlərin həllində, diaqnostikada, tibbi-məhkəmə ekspertizası praktikasında və s. sahələrdə mükəmməl alətə çevirir. Məqalədə həmçinin Hibridizasiya – və PCR - əsaslı molekulyar markerlərin yuxarıda xatırladılan müxtəlif məqsədlərlə tətbiq olunduğu çoxsaylı tədqiqatların nəticələri müzakirə edilir.

Açar sözlər: *Molekulyar marker, restriksiya fraqmenti, polimorfizm, amplifikasiya, cpSSRs, ESTs*

Молекулярно-Маркерные Технологии: Современное Состояние И Перспективные Направления (Обзор)

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В статье представлена подробная информация о молекулярно-маркерных технологиях, широко применяемых в фундаментальных и прикладных областях науки. Также изложены характерные особенности большинства существующих молекулярно-маркерных технологий, показаны их преимущества и недостатки. Будучи частью генома, молекулярные маркеры несут в себе огромную информацию об изменениях, происходящих в ходе онтогенеза и филогенеза. Это обстоятельство превращает молекулярные маркеры в совершеннейший инструмент в изучении эволюции, систематики, генетических и эпигенетических механизмов адаптации, в создании генотипов живых организмов, обладающих высокой устойчивостью к действию стрессовых факторов, в составлении генетических карт, идентификации генов, “коррекции генома” (genome editing), определении качества окружающей среды, диагностике, практике судебно-медицинской экспертизы, решении проблем эко - и генотоксикологии и др. В статье также обсуждаются результаты многочисленных исследований, в которых в вышеупомянутых целях были применены молекулярные маркеры, основанные на гибридизации и ПЦР.

Ключевые слова: *молекулярный маркер, фрагмент рестрикции, полиморфизм, амплификация, cpSSRs, ESTs*

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Effect of Salt Stress on the Productivity Indices of Wheat (*Triticum aestivum* L. and *Triticum durum* Desf.) Genotypes

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The effect of salt stress on productivity indices (weight of grain per 1m^2 area, 1000 grain weight, spike weight, weight and the number of spikelets in a spike) of local perspective bread (Giymatli-2/17, Nurlu-99, Azamatli-95) and durum (Garagilchig-2, Barakatli-95) wheat genotypes, regionalized in Azerbaijan has been studied. The research was carried out under field conditions. Salt stress was found to negatively affect productivity of varieties and structural elements of productivity. According to the tolerance indices (productivity under normal conditions, productivity under stress, mean productivity, geometric mean productivity, stress sensitivity index, stress tolerance index) Giymatli-2/17 is productive and medium tolerant, Nurlu-99 and Barakatli-95 are productive and tolerant, Azamatli-95 is medium productive and medium tolerant, and the Garagilchig-2 genotype is sensitive.

Keywords*: Wheat genotypes, elements of productivity, stress tolerance index*

INTRODUCTION

Azerbaijan is one of the countries that suffer from severe salinity problems. Approximately 1.144 million ha of cultivated land is already salinized (Isgandarov, 2015). Wheat is the most important and widely adapted crop that contributes more calories and protein than any other crop. It is necessary to increase wheat production in Azerbaijan by raising wheat grain. The most efficient way to increase wheat yield is to improve salt tolerance of wheat genotypes, because increasing salt tolerance is much less expensive for farmers than using other techiques.

Salt tolerance of crops may vary depending on the growth stage (Maas and Grieve, 1994). In general, cereal plants are the most sensitive to salinity during the vegetative and early reproductive stages and less sensitive during flowering and grain filling stage (Maas and Poss, 1989). Yield formation of wheat is a complex process that depends on various stress factors during ontogenesis. Salinity caused a decrease in plant height, dry biomass, assimlation organs such as, leaf, stem, ear and parametres of productivity (ear lenght and mass, the number of ears, the grain number) (Francois, 1994). All of these limit plant productivity. The agronomic parametres of each genotype are the result of all physiological activities of plant during onthogenesis (Flowers, 2004). Therefore, analysis of productivity parametres is important for evaluating salt tolerance in wheat.

MATERIALS AND METHODS

In order to evaluate the salinity impact on the grain quantity of wheat, the field experiment was conducted. The seeds were obtained from the Research Instute of Crop Husbandry and harvested in the field, in two variants: control and stress. Soil salinity of stress variants was 0.9%, which is considered as a saline soil for glycophytes, including wheat. As a research material, local perspective *Triticum aestivum* L. (Giymatli 2/17, Nurlu 99, Azamatli 95) and *Triticum Durum* Desf. (Garagilchig 2, Barakatli 95) genotypes were taken. Measurements were carried out at grain maturity stage. Main spikes were separated from the others, the straw biomass and the weight of grain per1 m^2, the number of spikelets per spike, weight and lenght of spike, thousand kernel weight (TKW) were determined. Tolerance indicies were evaluted based on agronomic parametres:

Salt tolerance indices:

Stress Tolerance $TOL=y_p-y_s$ (Rosielle and Hamblin, 1981);

Mean Productivity $MP = (y_p + y_s) / 2$; (Rosielle and Hamblin, 1981);

Geometric Mean Productivity $GMP=(y_p*y_s)^{0.5}$; (Fernandez, 1992);

Stress Susceptibility Index $SSI=[1-\bar{y}_s / \bar{y}_p]/ [1-\bar{y}_s / \bar{y}_p]$; (Fisher and Maurer, 1978);

Stress Tolerance Index

$STI = \left(y_p \cdot y_s\right) / \left(\bar{y_p}\right)^2$ (Fernandez,1992)

Where, y_s and y_p are average yield of all genotypes under stress and optimal conditions, respectively. y_s and y_p are the mean yields of all genotypes evaluated under stress and non-stress conditions.

RESULTS AND DISCUSSION

The final harvest of total biomass can be divided in two components: the grain yield and the straw yield. The grain yield and straw yield are the principal criteria used by farmers to choose the salt tolerant accessions under salt stress. The major part of the straw production takes place during the early stage of growth cycle and it is related to the vegetative growth as the tiller number, the leaves number, the height of plants. In our study, the total biomass of straw ranged from 1526 to 1903 g/m^2 in Giymatli 2/17, 1572-1866 g/m^2 in Nurlu 99, 1464-1824 g/m^2 in Azamatli 95, 1300-1751 g/m^2 in Garagilchig 2 and 1838-2107 g/m^2 in Barakatli 95 (Table 1). The huge reduction in straw biomass was obsereved in salt sensitive Garagilchig 2 (25.7 %). The grain yield per 1 m^2 was reduced by an average 33% for the tolerant Barakatli 95 whereas it was reduced by 57% for the least tolerant genotype (Garagilchig 2).

On average, spike length, spike weight and TGW were reduced by 29%, 33%, 14% in Giymatli 2/17, 19%, 24%, 7% in Nurlu 99, 7%, 36.4%,6.3% in Azamatli 95, 8%, 21%, 6% in Barakatli 95 and 38%, 56%, 18% in Garagilchig 2. Obviously, a significant decrease occurred in Giymatli 2/17 and Garagilchig 2.

Improving the grain yield of wheat is the main target in plant breeding. Therefore, the evaluation of final grain yield and growth parametres is a critical aspect of breeding programs. The effect of salinity on tiller number and spikelet number during early growth stages has a greater influence on final grain yield (Maas et al., 1983). The negative effect of salinity on spikelet number and on tiller number indicates that they are sensitive at the vegetative stage. The number of spikes is highly correllated with the number of tillers. Whereas, the grain yield takes place mainly during the productive phase and it is essentially influneced by the spike number,the spike fertility during the grain-filling period. The reduction in plant productivity under salinity is caused by the osmotic effect, water deficiency and by toxic effects of ions such as Na and Cl leading to inability of plants to acquire water (Aldesuquy et al., 2012). The various yield components showed different responses to salinity. The TGW was the least sensitive to salinity, whereas spike weight was the most sensitive yield component, which is in agreement with observation in rice (Zeng and Shannon, 2000; Mahmood et al., 2009).

To evaluate response of plant genotypes to salt stress some indices based on a mathematical relation between stress and optimum condition such as, productivity under normal conditions (Y_p), productivity under stress (Y_s), mean productivity (MP), geometric mean productivity (GMP), stress sensitivity index (SSI), stress tolerance index (STI) have been used. Fernandez divided the reaction of genotypes, on the basis of their yields, into 4 categories under stressed and non-stressed conditions: group A are genotypes which have high yield under both of conditions; group B are genotypes which have a high yield under non-stressed conditions; group C including genotypes 2 which have a good yield under stressed conditions and finally group D are genotypes which have a low yield under both conditions (Fernandez, 1992). In our study, salt tolerance of plants was evaluated based on the indices given in Table.

Table 1. Agronomic parametres in wheat genotypes under non-stress and stress condition

Genotypes	Variants	Total biomass g/m²	Grain weight g/m²	TKW g	The lenght Of spike (sm)	The number of spikelet per spike	The weight of spike, g
Giymatli-2/17	I	1903±133	740±44	54.0±2.72	10.0±0.38	21.4±1.24	3.84±0.17
	II	1526±122	482±24	46.4±3.25	7.1±0.18	17.0±0.61	2.56±0.09
Nurlu-99	I	1866±93	684±47	48.0±1.63	10.0±0.32	17.0±1.02	2.64±0.13
	II	1572±94	430±21	44.6±1.56	8.1±0.28	15.0±0.43	2.00±0.07
Azamatli-95	I	1824±145	658±52	47.0±2.67	12.3±0.41	20.0±0.96	3.32±0.23
	II	1464±87	399±24	44.0±1.49	9.0±0.19	18.0±0.71	2.11±0.05
Garagilchig-2	I	1751±140	600±42	47.3±2.36	9.8±0.44	16.2±0.93	2.44±0.06
	II	1300±117	258±13	39.0±1.13	6.0±0.15	12.0±0.61	1.08±0.03
Barakatli-95	I	2107±168	654±39	51.0±2.12	8.7±0.41	20.0±0.58	3.02±0.19
	II	1838±128	435±22	48.0±1.29	8.0±0.21	19.0±1.33	2.38±0.13

I – control; II-stress

Table 2. Estimation of sensitivity rate of wheat genotypes under normal and stressed conditions.

Genotypes	Y_p	Y_s	MP	GMP	Tol	SSI	STI
Giymatli-2/17	740±44	482±34	611±37	597±30	258±15	0.88±0.03	0.80±0.04
Nurlu-99	684±34	430±22	557±39	542±33	254±17	0.95±0.04	0.66±0.03
Azamatli-95	658±32	399±24	528±42	512±31	259±13	0.98±0.05	0.59±0.03
Garagilchig-2	600±36	258±15	429±21	393±24	342±17	1.42±0.06	0.35±0.01
Barakatli-95	654±45	435±26	544±33	533±32	219±13	0.82±0.04	0.64±0.03

Mean comparison showed that Giymatli-2/17 with 740 g/m^2 under non-stress condition(Yp) and with 482 g/m^2 under stress condition (Ys) had the highest grain yield. Also Barakatli 95 and Nurlu 99 had the high grain yields under stress condition, whereas Qaragilchig 2 had the lowest grain yield under stress condition. According to TOL and SSI indices Garagilchig 2 is relatively sensitive to stress. According to studies, the genotypes with SSI more than 1 are considered the most sensitive to salinity (Clarke,1992; Golabadi et al.,2006; Mardeh et al., 2006). Moreover, the STI indice was lower in Garagilchig 2 (0.35).

Based on tolerance indicies, the accessions were divided in three groups: the lowest, the highest and the moderate salinity. Giymatli 2/17 was productive and medium tolerant, Nurlu 99 and Barakatli 95 were productive and tolerant, Azamatli 95 was medium productive and medium tolerant, and the Garagilchig 2 genotype was sensitive.

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Duz Stresinin Buğda Genotiplərinin (*Triticum aestivum* L. və *Triticum Durum* Desf.) Məhsuldarlıq Göstəricilərinə Təsiri

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Duz stresinin Azərbaycanda rayonlaşdırılmış perspektivli yerli yumşaq - *Triticum aestivum* L. (Qiymətli-2/17, Nurlu-99, Əzəmətli) və bərk-*Triticum durum* Desf.(Qaraqılçıq-2, Bərəkətli-95) buğda genotiplərinin məhsuldarlıq göstəricilərinə (1 m^2-də dənin kütləsi, 1000 dənin kütləsi, sünbülün kütləsi, 1 sünbüldə olan sünbülcüklərin sayı və kütləsi) təsiri öyrənilmişdir. Tədqiqatlar tarla şəraitində aparılmışdır. Müəyyən edilmişdir ki, duz stresi sortların məhsuldarlığına və məhsulun struktur elementlərinə mənfi təsir göstərir. Davamlılıq indeksləri (normal şəraitdə məhsuldarlıq, stres şəraitində məhsuldarlıq ,orta məhsuldarlıq, orta həndəsi məhsuldarlıq, stresə həssaslıq indeksi, stresə tolerantlıq indeksi) əsasında Qiymətli-2/17 genotipinin məhsuldar, orta davamlı, Nurlu-99 və Bərəkətli-95 genotiplərinin məhsuldar, davamlı, Əzəmətli-95 genotipinin orta məhsuldar, orta davamlı, Qaraqılçıq-2 genotipinin isə həssas olduğu müəyyən olunmuşdur.

***Açar sözlər**: Buğda genotipləri, məhsuldarlıq elementləri, stresə tolerantlıq göstəriciləri*

Влияние Солевого Стресса на Показатели Продуктивности Генотипов Пшеницы (*Triticum aestivum* L. и *Triticum Durum* Desf.)

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Изучено влияние солевого стресса на показатели продуктивности (масса зерна, масса 1000 зерен, масса колоса, количество колосков в колосе, количество и масса зерен в колосе) мягкой (*Triticum aestivum* L.) и твердой (*Triticum durum* Desf.) пшеницы, районированной в Азербайджане. Исследования проводились в полевых условиях. Обнаружено, что солевой стресс влияет на продуктивность сортов и на структурные элементы продукта. На основание индексов толерантности (продуктивность в нормальных условиях, продуктивность в условиях стресса, средняя геометрическая продуктивность, индекс чувствительности к стрессу, индекс толерантности к стрессу) установлено, что генотип Гийметли 2/17 является продуктивным, средне-толерантным, Нурлу 99 и Баракатли 95 являются продуктивными и толерантными, Азаматли 95 - средне-продуктивным и средне-толерантным, а Гарагылчыг 2 - чувствительным генотипом.

***Ключевые слова:** Генотипы пшеницы, элементы продуктивности, показатели толерантности к стрессу*

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Abiotic Stress and Morphogenic Potential of Wheat *in vitro* in Conditions of Elevated Temperatures

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The influence of the stress effect of an elevated temperature on the morphogenic callus of Tr. durum and Tr, aestivum wheat varieties was studied. The dependence of the intensity of growth processes on the duration of the applied thermal effects schemes is shown. It was established that the presence of endophytic bacterial infection also cardinally changes morphogenetic processes *in vitro*.

Keywords: *Wheat, in vitro, temperature stress, resistance, regenerative potential, endophytic infection*

INTRODUCTION

In recent decades, in crop production, due to significant climate changes and amid unfavorable environmental conditions, there are many problems associated with inadequate plant resistance to abiotic stresses. As a result, obtaining high and stable crop yields is systematically one of the most urgent and important breeding and genetic tasks.

Research on the development of adaptive breeding methods based on modern achievements of genetics and biotechnology, allowing in a short time to improve the effectiveness of traditional breeding approaches based on the creation of new genotypes - donors of resistance to stress factors, are gaining special relevance in realization of aforementioned tasks. Cell selection is a promising method of cell engineering that allows *in vitro* direct selection of genotypes with predetermined properties.

Somaclonal variability, based on genetic mechanisms (chromosomal aberrations, point mutations of DNA (Larkin, 1981)) and epigenetic variability of plants in vitro, which do not affect changes in the nucleotide sequence of DNA and are associated with gene activation or silencing (Kaepleretal, 2000), is the basis of *in vitro* cell selection technologies.

Despite the obvious progress in the cell selection of different cultures, in particular wheat, the development of the theoretical foundations of callus formation, embryogenesis and morphogenesis in cereal culture, the establishment of factors determining the success of non-traditional approaches for increasing useful, inherited *in vitro* variations in selective selection, there are still unsolved problems , associated with a sharp decrease in the proliferation of cell cultures after the stressful effects of abiotic and biotic factors and loss of ability to regenerate (Nikitina et al., 2015; Miguel et al., 2011; Rakoczy-Trojanowska, 2002; Hussain et al., 2001; Abouzied, 2011; Akhtar et al., 2012).

MATERIALS AND METHODS

The purpose of this research was to study the individual schemes for selecting heat-resistant wheat cell lines *in vitro*. The starting material was immature embryos of 3 varieties of *Tr. aestivum* (Gobustan, Azamatli-95, Apsheron) and 3 varieties of *Tr. durum* wheat (Barakatli-95, Garabagh, Saray) of local selection.

Callus tissue was obtained from isolated embryos on days 13-17 after pollination.The embryos were planted scute up on MS medium (Murashiqe et al., 1962) with addition of 2 mg / l of 2,4D.Callus was cultivated at a temperature of 25-260°C.The transplant was performed every 4 weeks, subcultured only with morphogenic callus.Cell selection was carried out after the 2nd passage according to the following schemes:

Scheme I - Callus strains at the end of each passage were subjected to a single exposure at a temperature of 45°C for a different time (20-60 minutes).

Scheme II - the cells were exposed to temperature in the middle of the passage (after 2 weeks of cultivation) and at the end of the passage.

Scheme III - cultivation was carried out at 36-37°C throughout the passage.

The total length of the stress effect was at least 2 passages for all schemes. After stressful action, the induction of morphogenesis was carried out by placing the callus on a medium containing IAA (0.5 mg/l) under illumination conditions and 16/8 photoperiod.

RESULTS AND DISCUSSION

In this paper the results of 60-minute warming of callus masses are discussed (schemes I, II). Visual assessment of the morphogenic potential of the tissue was made on a five-point scale (Nguyen Thi Li an, 1995). Stress effects in all cases reduced the intensity of growth processes, which correlated with the duration of heat exposure, depending on the schemes used (Table 1).

Table 1. The effect of high temperature on the growth of morphogenic callus in different genotypes *Triticum durum* Desf. and *Tríticum aestívum* L. (in % of control)

Variety	Passage	Variants of exposure to high temperature		
		scheme I	schemeII	scheme III
Barakatli-95	1	20.4	19.3	17.2
	2	18.2	17.0	16.1
Garabagh	1	20.6	19.4	40.4
	2	19.1	18.3	43.2
Saray	1	21.3	21.0	35.5
	2	20.1	19.2	35.7
Gobustan	1	30.0	29.2	44.1
	2	28.7	27.1	42.2
Azamatli-95	1	40.0	38.0	30.1
	2	38.4	35.0	32.2
Absheron	1	50.3	46.3	60.7
	2	44.2	41.0	65.6

As follows from the data provided, a single exposure to elevated temperatures had little effect on the growth of calli in solid wheat varieties. Higher temperatures affected growth rates in Tr. aestivum varieties to a greater degree. With the increase in the number of passages, the inhibition of growth activity is slightly reduced under the stresses of schemes I and II. According to the scheme III of exposure, a similar pattern was observed only in varieties Barakatli-95, Gobustan and did not differ in Saray variety.

To a certain extent, the growth of culture under the influence of stress factors can be an integral criterion of its stability. The change in the intensity of growth of callus tissues, depending on the amount of temperature effects, may also indicate the nature of adaptability associated with the selection of cells with increased resistance. Multistage selection was aimed to select cell lines that are resistant to heat stress and a primary evaluation of the genetically determined resistance and adaptive capacity of isolated cells.

Previous research with these genotypes of wheat which studied the effect of elevated temperatures on the induction of callus formation, proliferation, the regeneration capacity of the strains obtained, their ability to maintain the regeneration potential, depending on the contribution of different types of callus cells (individually and in mixed callus types), allowed to establish patterns for selection of genotypes with a certain degree of preservation and expression of signs of resistance, as well as to obtain cell strains possessing increased resistance to high temperatures in comparison with the original forms. However, recent studies with the same genotypes, but grown on a different agrophone,the results of which are presented in this paper, showed a completely different picture of the realization of the morphogenic potential, both in the control and in the experimental variants.

Transplantation of callus strains, subjected to stress on the medium for regeneration, showed that exposure to temperatures in schemes I and II reduced the morphogenic potential by 70-90%, except for Barakatli-95 and Azamatli-95, in which the morphogenic potential and regenerative capacity decreased by 21% and 58% respectively. In the case of varieties of *Tr. aestivum* wheat (Gobustan; Absheron) and Tr. durum wheat (Garabagh; Saray), cultivated on a constant background of high temperatures (Scheme III), there was an even more significant decrease in the morphogenic potential. There was a 30% mortality of strains. Such morphological changes as an increase in the share of rhizogenic callus were observed (Fig. 1). The Gobustan variety showed a change in the morphology and consistency of the callus, a change in its color.

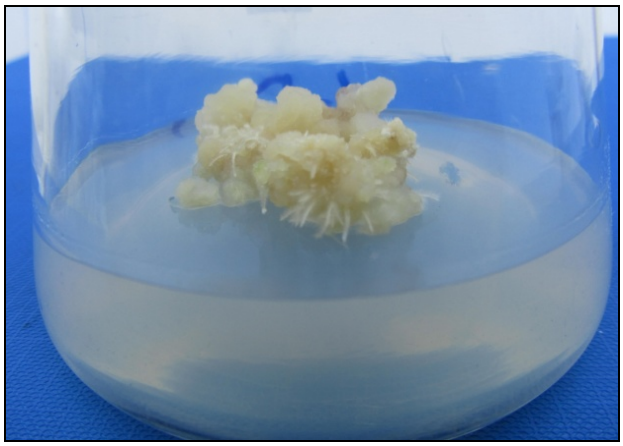

Fig. 1. Risogenesis in callus culture in Gobustan variety

Cellular masses of *Tr. durum* wheat also showed little difference in the degree of suppression of the morphogenic potential. The greatest suppression of the morphogenic potential took place in *Tr. aestivum* wheat varieties. Tr. aestivum wheat also had a varietal relationship with respect to the heat stressor, regardless of the treatment schemes used. With the increase in the cultivation time under the stress scheme II, the suppression of the morphogenic ability in all strains decreased slightly, which can be attributed to the

manifestation of adaptability or the acquisition of a certain resistance to a thermal stressor.

In callus cells, mainly *Tr. durum* wheat, subjected to double treatment with elevated temperatures according to Scheme II, no changes in morphology were observed visually in passage 1. During the 2nd passage the growth of biomass slowed down. In the case of surviving strains of Absheron, Gobustan, Garabagh, Saray, cultured according to Scheme III, in the 4th passage, after the termination of thermal exposure, under normal conditions, the manifestation of endophytic infection was observed. The manifestation of infection was sometimes accompanied by a darkening of the agar medium. Endophytic pathogens were mainly observed on the surface and in the thickness of callus cells (Fig. 2).

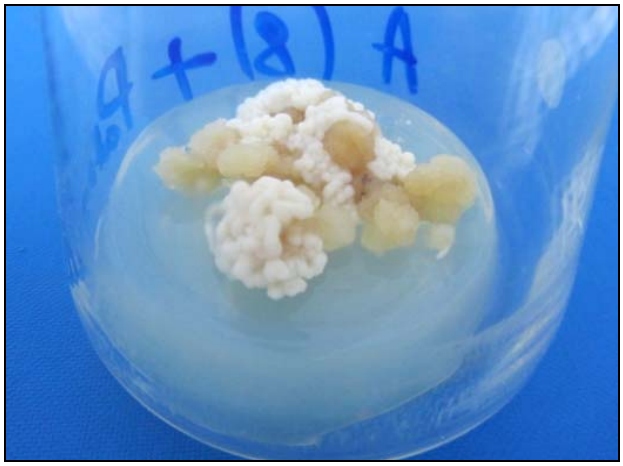

Fig. 2. The manifestation of bacterial infection in variety Absheron.

Transplantation to the hormone-free medium of infected callus cells changed the morphology of pathogens unidentified for this period. Pathogenic microorganisms associated with isolated plant tissues in culture *in vitro* negatively influenced the processes of morphogenesis and this fact should be taken into account in studies on cell selection.

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Abiotik Stress və Yüksək Temperatur Şəraitində Buğdaın *in vitro* Morfogen Potensialı

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Yüksək temperatur stressorının bərk və yumşaq buğda sortlarının morfogen kallusuna təsiri öyrənilmişdir. Böyümə proseslərinin intensivliyinin tətbiq olunan temperaturun təsiri müddəti sxemlərindən asılılığı göstərilmişdir. Müəyyən edilmişdir ki, endofit bakterial infeksiyanın mövcudluğu da *in vitro* morfogenetik prosesləri əsaslı surətdə dəyişdirir.

Açar sözlər: *Buğda, in vitro, temperatur stresi, rezistentlik, regenerasiya potensialı,, endofit infeksiya*

Абиотический Стресс и Морфогенный Потенциал Пшеницы *in vitro* в Условиях Повышенных Температур

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Изучено влияние стрессорного воздействия повышенной температуры на морфогенный каллус у твердых и мягких сортов пшеницы. Показана зависимость интенсивности ростовых процессов от длительности применяемых схем теплового воздействия. Установлено, что наличие эндофитной бактериальной инфекции кардинально изменяет также морфогенетические процессы *in vitro*.

Ключевые слова: *Пшеница, in vitro, температурный стресс, резистентность, регенерационный потенциал, эндофитная инфекция*

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Effect of Drought Stress on Physiological Traits, Grain Yield of Durum (*Triticum durum* Desf.) and Bread Wheat (*Triticum aestivum* L.) Genotypes

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We aimed to study the influence of soil water deficit on gas exchange parameters, relative water content, photosynthetic pigment contents of flag leaf, as well as on assimilation area and dry matter of leaves, stem, spike and grain yield of durum and bread wheat genotypes in the 2014-2015 growing season. Water stress caused reduction of photosynthesis rate, stomatal conductance, transpiration rate, an increase of intercellular CO_2 concentration. Water stress severely affected on relative water content, Chl *a, b* and Car (x+c) content, assimilation area formation and dry matter accumulation of sensitive wheat genotypes.

Keywords: *Drought stress, gas exchange, relative water content, chlorophyll, assimilation area, dry matter, grain yield*

INTRODUCTION

With the population growing rapidly and the limited water resources becoming scarcer, maintenance of sustainable productivity of cereal crops is of great importance. Wheat (*Triticum* L.) is one of the main cereal crops for food safety in the world. Global wheat production in 2017 amounted to 754 million tons, which is 2.7 percent more than in 2016 (FAO, 2017). Despite the fact that the conventional selection and the use of new biotechnological tools allow a significant increase in wheat production in recent years, unfavorable factors of the environment greatly affect the production and quality of wheat. Yield safety can only be improved if future breeding attempts will be based on the valuable new knowledge acquired on the processes of the determining plant development and its responses to stress (Barnabas et al., 2008). To accelerate yield improvement, physiological traits at all levels of integration need to be considered in breeding (Long et al., 2015). Physiological approaches have already demonstrated significant genetic gains in Australia and several developing countries of the International Wheat Improvement Network (Reynolds and Langridge, 2016). Drought is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world (Izanloo et al., 2008). Wheat is one of the widely cultivated (about 651.000 hectares) crops in Azerbaijan, where drought is the main limiting factor for its production (Aliyev, 2001). Azerbaijan is in the second place of the top 15 wheat-dependent countries (http://necsi.edu, 2011). Drought is a non-uniform phenomenon that influences plants differently depending on the development stage at the time of its occurrence, adversely affects physiology, morphology, growth and yield traits of wheat (Hossain and Da Silva, 2012). Drought stress reduces photosynthetic characteristics, shortens the duration of photosynthesis and promotes the senescence of leaves (Liu et al., 2016). A decrease in photosynthesis rate limits expansion of assimilation area of vegetative organs and the accumulation of dry mass. Drought induces a wide range of molecular, biochemical and physiological alterations in plants, including accumulation of osmolytes, reduction of photosynthesis, stomata closure and the induction of stress-responsive genes (Lata et al., 2015). Higher photosynthetic rates during drought and rapid recovery after re-watering produced less- pronounced yield declines in the tolerant cultivar than the sensitive cultivar (Abid et al., 2018). In wheat, greater genetic variability can be explored with germplasm from its centers of origin and diversity (Dvorak et al., 2011). Ceccareli stated that local varieties have 25-61% of advantage over modern varieties under stressful environments, while modern genotypes have 6-18% of advantage over local varieties under favorable conditions (Ceccareli, 1989).

Drought tolerance is a complex trait controlled by numerous genes, each with minor effects (Bernardo, 2008). Phenotypic, biochemical and genomics-assisted selection methodologies are discussed as leading research components used to exploit genetic variation for drought tolerance

(Mwadzingeni et al., 2016).

The purpose of the study. The purpose of this research was to study the influence of soil water deficit on some physiological traits, grain yield of durum and bread wheat genotypes.

MATERIALS AND METHODS

Field studies were carried out during the 2014/15 growing season at the experimental field of the Department of Plant Physiology and Biotechnology Research Institute of Crop Husbandry, located in the Apsheron peninsula, Baku. Durum wheat genotypes (Garagylchyg 2, Vugar, Shiraslan 23, Barakatli 95, Alinja 84, Tartar, Sharg, Gyrmyzy bugda) and bread wheat genotypes (Nurlu 99, Gobustan, Akinchi 84, Giymatli 2\17, Gyrmyzy gul 1, Azamatli 95, Tale 38, Ruzi 84, Pirshahin 1, 12ndFAWWON№97, 4thFEFWSN№50, Gunashli, Dagdash, Saratovskaya 29) were grown under two conditions: drought (non-irrigation) and irrigated (three irrigations: at seedlings, stem elongation and grain filling). The plot size was 1.05 m x10 m, with 15.0 cm row spacing. Each plot had three replications under drought and irrigation.

Measurements: Gas exchange parameters (photosynthesis rate- P_n, stomatal conductance- g_s, intercellular CO_2 concentration- C_i, transpiration rate- E) were measured using LI-COR 6400XT Portable Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA) at the anthesis growth stage. Measurements were carried out between 10:00 and 12:00 a.m. Data logging started after 45 seconds of the insertion of leaves into chamber. Leaf Chl a, b and Car (x+c) contents were determined following the method of Lichtenthaler (1987), with little modifications. The leaf, stem plus sheath and spike dry mass was measured after oven drying at 105 °C for 24 h. Leaf area per stem (LAS), also projected area of stem multiplied by 3.14 according to Květ and Marshall (1971), and spike multiplied by 2 according to Alvaro et al., (2008) were measured with an area meter (AAC-400, Hayashi Denkon Co, LTD, Japan). The flag leaf RWC was determined gravimetrically. RWC was calculated using the following formula: RWC (%) = (FW-DW)/(TW-DW)x100, where FW-fresh mass, DW-dry mass, TW-turgid mass.

Statistical analysis: Mean values were calculated by Excel program. Correlation among traits was calculated by SPSS 16 software.

RESULTS

Stomatal conductance, net photosynthesis rate and transpiration rate decreased significantly in flag leaf of genotypes in response to drought stress at anthesis (Table 1). A decrease of stomatal conductance, net photosynthesis rate and transpiration rate amounted to an average 45%, 44%, 37% in the sensitive genotypes Garagylchyg 2, Vugar, Shiraslan 23, Barakatli 95, Alinja 84, Tartar, Nurlu 99, Gobustan, Akinchi 84, Giymatli 2/17, Azamatli 95, Tale 38, Ruzi 84, Pirshahin 1, 12ndFAWWON№97 and Saratovskaya 29. A relatively less decrease of g_s, P_n and E was revealed in the genotypes Sharg, Gyrmyzy bugda, Gyrmyzy gul 1, 4thFEFWSN№50, Gunashli and Dagdash. The intercellular CO_2 concentration in flag leaf of most genotypes increased under drought condition.

Although the RWC in flag leaf was maintained at a relatively high level at the heading and postanthesis grain formation stages, genotypic variations in this trait was revealed at the early milky ripe stage (Fig. 1). The RWC of most genotypes was around 70% under normal water supply. The lowest RWC was detected in the genotypes Nurlu 99, Azamatli 95, Pirshahin 1 and Gunashli, with early heading time. Despite the fact that the RWC was maintained at a high level under normal water supply, there was a strong decline under water stress conditions in the genotypes Garagylchyg 2, Shiraslan 23, Akinchi 84, Tale 38, Ruzi 84, 12ndFAWWON№97 and 4thFEFWSN№50. A relatively higher RWC under normal water supply and slight decrease of this trait was revealed in the genotypes Vugar, Tartar, Sharg, Gyrmyzy bugda, Giymatli 2/17 and Dagdash. We consider these genotypes as drought tolerant.

A relatively high Chl a+b content was detected in flag leaf of the genotypes Garagylchyg 2, Tartar, Gyrmyzy bugda, Gobustan, Giymatli 2/17, Gyrmyzy gul 1, 4thFEFWSN№50 and Saratovskaya 29 under irrigation (Table 2). A relatively low Chl a+b content was detected in the genotypes Shiraslan 23, Sharg, Nurlu 99, Akinchi 84, Azamatli 95, Dagdash. Water stress caused reduction in Chl a, b and Car (x+c) content in all genotypes with exception Azamatli 95. A strong reduction of pigments under water stress was observed in the genotypes of durum wheat Garagylchyg 2, Tartar, Sharg, Gyrmyzy bugda, in the genotypes of bread wheat Gobustan, Akinchi 84, Giymatli 2/17 and Gunashli. Photosynthetic apparatus of some durum wheat genotypes (Vugar, Shiraslan 23, Barakatli 95), and bread wheat genotypes (Gyrmyzy gul 1, Azamatli 95, Tale 38, 12ndFAWWON№97, Dagdash, Pirshahin 1, Saratovskaya 29) were relatively tolerant to water stress. A decrease of Chl a/b ratio was observed in 11 genotypes, while an increase in 10 genotypes. A Chl a/b ratio remained unchanged in the genotype Ruzi 84. In comparison with Chl a and b, Car (x+c) were more resistant to water deficiency, as a result, the Chl (a+b)/Car (x+c) ratio is reduced in most

genotypes. An increase in Chl (a+b)/Car (x+c) ratio was detected in some genotypes.

Water stress limited the expansion of the assimilation area of leaves, stem and spike, as well as the accumulation of biomass in these organs (Table 3). At the milky ripe stage the assimilating area of the leaves decreases due to senescence of the leaves in the underlying layers, which is accelerated under condition of water deficiency. A strong reduction of the assimilation area of leaves, stem and spike was detected in the genotypes Garagylchyg 2, Shiraslan 23, Akinchi 84, Tale 38, 12ndFAWWON№97 and Dagdash. A deep limitation in biomass of leaves and stem in the condition of water deficiency was detected in the genotypes Tartar, Sharg, Gyrmyzy bugda, Nurlu 99, Gobustan, Azamatli 95, Pirshahin 1. A strict decrease in the biomass of leaves was not accompanied by a similar decrease in the biomass of the stem in some genotypes, such as, Akinchi 84, Giymatli 2/17, Ruzi 84, 12ndFAWWON№97, 4thFEFWSN№50 and Gunashli. A strong reduction of spike biomass was revealed in the genotypes Sharg, 4thFEFWSN№50, Gunashli, Dagdash and Saratovskaya 29.

Table 1. Effect of drought stress on gas exchange parameters (I - irrigated, D - drought).

Wheat genotypes	Growth condition	Gas exchange parameters			
		P_n, µmol CO_2 m^{-2} s^{-1}	g_s, mol H_2O m^{-2} s^{-1}	C_i, µmol CO_2 mol^{-1}	E, mmol H_2O m^{-2} s^{-1}
Triticum durum Desf.					
Garagylchyg 2	I	12.10	0.142	223.5	2.56
	D	6.55	0.087	250.4	1.79
Vugar	I	13.24	0.142	206.5	2.65
	D	6.68	0.072	219.5	1.45
Shiraslan 23	I	16.77	0.138	159.0	2.57
	D	7.36	0.086	225.6	1.81
Barakatli 95	I	13.16	0.158	223.0	2.89
	D	7.07	0.078	227.1	1.55
Alinja 84	I	11.03	0.128	223.0	2.43
	D	8.31	0.078	201.6	1.65
Tartar	I	16.31	0.177	235.0	3.13
	D	8.75	0.078	180.6	1.68
Sharg	I	14.23	0.132	182.0	2.54
	D	11.73	0.113	189.6	2.22
Gyrmyzy bugda	I	11.73	0.102	166.0	1.98
	D	8.13	0.075	190.4	1.54
Triticum aestivum L.					
Nurlu 99	I	15.64	0.164	202.0	3.13
	D	4.96	0.115	302.0	2.35
Gobustan	I	12.92	0.159	225.4	3.01
	D	5.86	0.083	256.8	1.87
Akinchi 84	I	13.63	0.186	235.2	3.48
	D	8.82	0.086	195.5	1.91
Giymatli 2/17	I	15.36	0.143	186.2	2.98
	D	7.24	0.098	245.6	2.18
Gyrmyz gul 1	I	8.43	0.087	204.2	1.83
	D	6.21	0.066	210.5	1.53
Azamatli 95	I	12.56	0.146	220.0	2.67
	D	6.89	0.065	176.4	1.41
Tale 38	I	14.97	0.167	206.0	2.76
	D	6.78	0.066	195.0	1.42
Ruzi 84	I	11.66	0.145	229.5	2.56
	D	8.09	0.074	193.0	1.52
Pirshahin 1	I	10.81	0.121	217.0	2.27
	D	5.94	0.089	260.3	1.84
12ndFAWWON№97	I	10.37	0.119	224.0	2.23
	D	6.03	0.072	234.0	1.46
4thFEFWSN№50	I	13.56	0.118	175.0	2.41
	D	9.74	0.101	213.0	2.01
Gunashli	I	9.20	0.131	242.0	2.53
	D	6.11	0.115	281.0	2.17
Dagdash	I	15.21	0.150	193.0	2.72
	D	12.70	0.098	146.0	2.04
Saratovskaya 29	I	11.11	0,121	206.0	2.34
	D	4.54	0,075	273.5	1.56

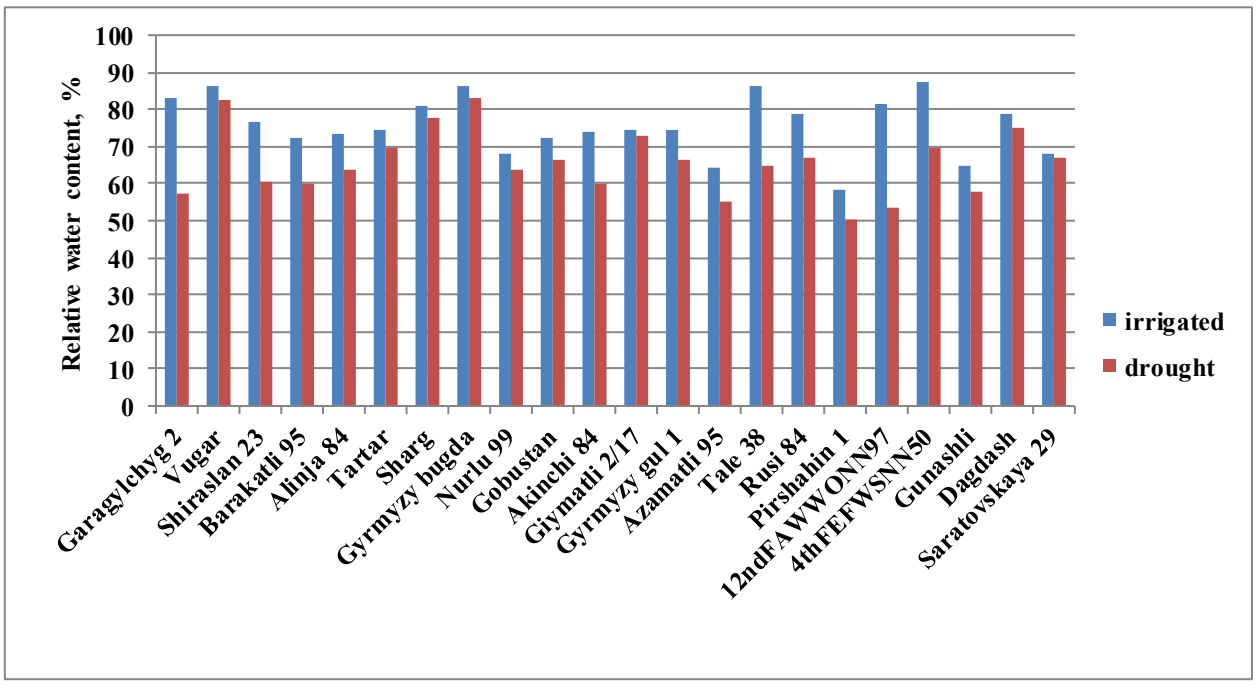

Fig. 1. Effect of drought stress on relative water content of flag leaf

Table 2. Effect of drought stress on Chl a, b and Car (x+c) content.

Wheat genotypes		Chl a	Chl b	Chl (a+b)	Car (x+c)	Chl a/b	Chl (a+b)/Car (x+c)
Triticum durum Desf.							
Garagylchyg 2	I	7.51	2.65	10.15	2.29	2.84	4.43
	D	4.89	1.88	6.77	1.73	2.60	3.92
Vugar	I	6.82	2.38	9.20	2.16	2.87	4.26
	D	5.87	2.04	7.91	1.71	2.88	4.63
Shiraslan 23	I	5.23	1.92	7.15	1.47	2.73	4.86
	D	5.15	1.77	6.92	1.65	2.91	4.19
Barakatli 95	I	7.16	2.65	9.81	2.02	2.70	4.85
	D	6.60	2.52	9.12	1.87	2.62	4.87
Alinja 84	I	6.59	2.47	9.06	1.87	2.66	4.85
	D	5.41	1.89	7.29	1.65	2.86	4.43
Tartar	I	7.48	2.93	10.41	2.16	2.55	4.83
	D	5.98	2.38	8.36	1.78	2.52	4.69
Sharg	I	6.52	2.34	8.86	1.90	2.78	4.66
	D	5.09	1.66	6.75	1.63	3.07	4.15
Gyrmyzy bygda	I	7.63	2.54	10.17	2.14	3.01	4.76
	D	6.06	2.05	8.11	1.77	2.96	4.57
Triticum aestivum L.							
Nurlu 99	I	4.32	1.62	5.94	1.27	2.67	4.69
	D	4.14	1.53	5.67	1.16	2.70	4.91
Gobustan	I	7.82	2.80	10.62	2.15	2.79	4.94
	D	4.85	1.61	6.45	1.41	3.01	4.58
Akinchi 84	I	6.51	2.43	8.95	1.95	2.68	4.60
	D	4.78	1.81	6.59	0.74	2.65	8.92
Giymatli 2/17	I	7.78	2.72	10.50	2.24	2.86	4.70
	D	6.18	2.19	8.37	1.72	2.82	4.87
Gyrmyzy gul 1	I	7.96	2.96	10.92	2.26	2.69	4.84
	D	7.71	2.84	10.55	2.25	2.72	4.67
Azamatli 95	I	4.87	2.09	6.95	1.34	2.33	5.20
	D	5.23	1.95	7.18	1.46	2.67	4.92
Tale 38	I	7.22	2.57	9.79	2.07	2.81	4.73
	D	6.56	2.48	9.03	1.90	2.65	4.75
Ruzi 84	I	6.76	2.36	9.12	1.93	2.86	4.73
	D	5.58	1.95	7.54	1.58	2.86	4.77
Pirshahin 1	I	7.01	2.54	9.55	2.01	2.76	4.76
	D	6.31	2.25	8.56	1.88	2.80	4.55

Continued table 2

12ndFAWWON№97	I	6.97	2.51	9.48	2.04	2.77	4.64
	D	6.12	2.43	8.55	1.72	2.52	4.97
4thFEFWSN№50	I	7.68	2.78	10.46	2.23	2.76	4.70
	D	6.17	2.29	8.45	1.88	2.70	4.50
Günəşli	I	7.26	2.72	9.98	1.92	2.67	5.20
	D	5.08	1.95	7.03	1.44	2.61	4.87
Dagdash	I	6.39	2.14	8.52	1.99	2.99	4.28
	D	5.66	2.00	7.66	1.93	2.83	3.97
Saratovskaya 29	I	7.80	2.97	10.78	2.06	2.62	5,24
	D	7.00	2.53	9.53	2.05	2.77	4.66

Table 3. Effect of drought stress on assimilation area and dry mass of leaves, stem and spike (milky ripe stage).

Wheat genotypes	**Growth condition**	**Assimilation area, cm²**			**Dry mass, g**		
		leaves	**stem**	**spike**	**leaves**	**stem**	**spike**
Triticum durum Desf.							
Garagylchyg 2	I	69.04	115.58	34.09	0.415	2.959	2.142
	D	48.81	86.68	29.22	0.304	2.628	2.515
Vugar	I	64.64	106.26	29.00	0.368	2.954	2.104
	D	58.00	101.80	30.18	0.360	2.934	2.492
Shiraslan 23	I	65.04	108.74	31.17	0.390	3.020	2.088
	D	46.11	84.83	26.11	0.301	2.489	2.094
Barakatli 95	I	53.80	103.87	36.02	0.394	3.454	1.794
	D	55.41	100.47	34.07	0.364	2.691	1.784
Alinja 84	I	64.57	99.91	28.41	0.342	2.642	1.913
	D	36.76	83.23	26.51	0.249	2.181	1.726
Tartar	I	71.51	108.64	36.78	0.488	3.007	2.415
	D	62.34	89.55	34.97	0.305	2.316	2.447
Sharg	I	80,49	158.48	43.16	0.552	4.322	2.523
	D	58.93	133.75	39.88	0.373	3.533	1.881
Gyrmyzy bugda	I	76.98	171.92	30.23	0.462	3.674	1.556
	D	48.39	129.31	29.48	0.338	2.781	1.914
Triticum aestivum L.							
Nurlu 99	I	38.24	68.33	21.16	0.228	1.834	2.047
	D	17.18	65.81	18.23	0.112	1.477	1.786
Gobustan	I	49.63	81.07	20.41	0.301	2.503	2.251
	D	31.63	67.68	18.64	0.192	1.964	2.181
Akinchi 84	I	45.23	115.38	27.06	0.275	2.924	1.925
	D	24.28	82.82	23.34	0.191	2.420	1.917
Giymatli 2/17	I	63.54	99.16	24.24	0.443	2.552	2.493
	D	38.08	90.37	20.80	0.262	2.368	2.141
Gyrmyzy gul 1	I	48.55	68.58	16.48	0.302	1.621	1.547
	D	41.56	35.17	12.12	0.271	1.341	1.423
Azamatli 95	I	39.16	93.62	24.30	0.274	2.180	2.282
	D	28.30	86.99	19.21	0.199	1.563	2.156
Tale 38	I	47.75	121.26	32.42	0.333	2.593	1.812
	D	38.08	83.59	23.10	0.292	1.895	1.651
Ruzi 84	I	41.71	89.77	27.94	0.341	2.580	2.399
	D	28.37	83.49	25.78	0.222	2.162	2.024
Pirshahin 1	I	40.63	96.73	24.62	0.357	2.914	2.390
	D	28.49	72.14	22.54	0.214	1.989	2.081
12ndFAWWON№97	I	21.47	73.29	17.50	0.133	1.235	1.105
	D	9.24	55.26	12.03	0.068	1.053	0.992
4thFEFWSN№50	I	74.71	132.18	42.71	0.400	2.233	2.215
	D	41.66	103.90	41.76	0.243	1.908	1.711
Gunashli	I	39.33	71.59	28.78	0.276	2.194	2.599
	D	23.71	61.79	27.49	0.166	1.823	1.922
Dagdash	I	67.19	163.97	28.95	0.431	3.184	1.857
	D	52.57	103.21	23.35	0.360	2.529	1.231
Saratovskaya 29	I	45.84	114.70	16.36	0.256	1.983	0.913
	D	30.59	100.94	14.43	0.183	1.663	0.708

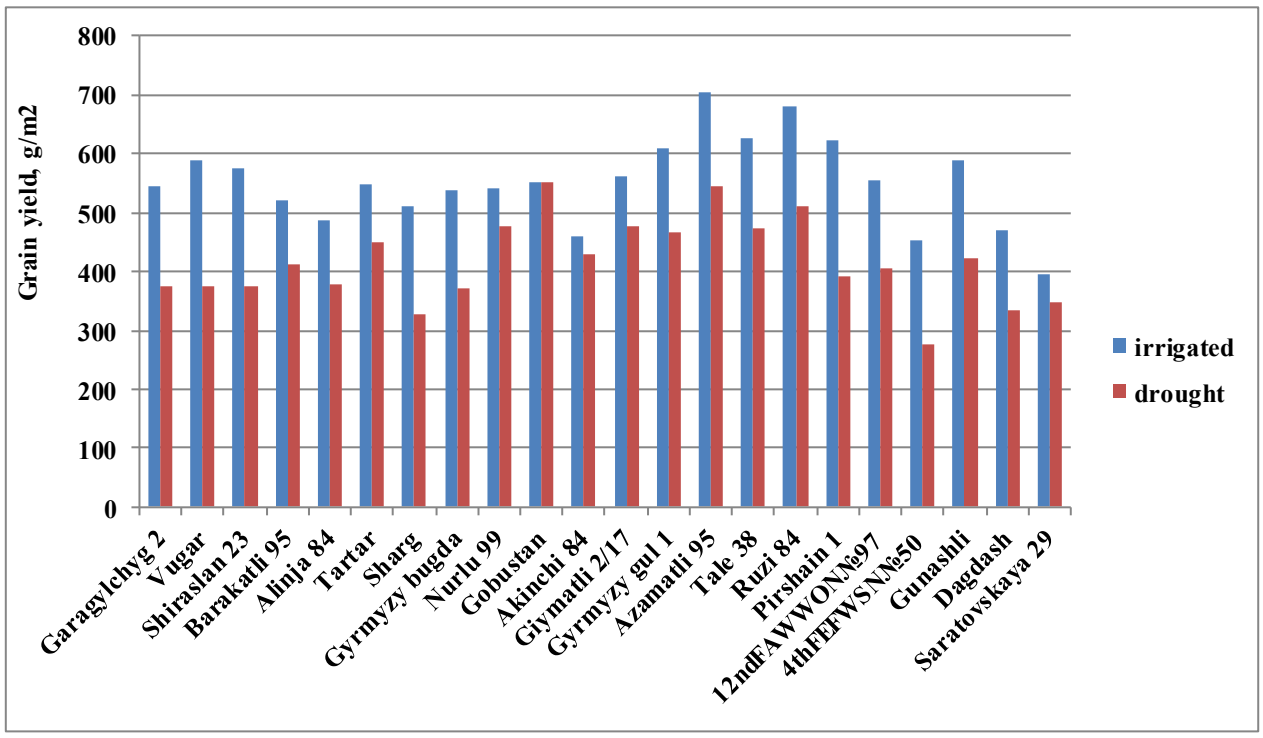

Fig. 2. Effect of drought stress on grain yield of wheat genotypes.

Reductions in grain yield under drought stress were strongly expressed in the genotypes Garagylchyg 2, Vugar, Shiraslan 23, Sharg, Gyrmyz bugda, Pirshahin 1, 4thFEFWSN№50, Gunashli and Dagdash (Fig.2). A slight decrease in grain yield was revealed in the genotypes Tartar, Nurlu 99, Gobustan, Akinchi 84, Giymatli 2/17 and Saratovskaya 29.

DISCUSSIONS

Photosynthetic responses to water stress have been the subject of studies and debates for decades, in particular, concerning which are the most limiting factors (stomatal or mesophyll limitations, photochemical and/or biochemical reactions) for photosynthesis under water stress (Flexas and Medrano, 2002; Lawlor and Cornic, 2002). Reduction of stomatal conductance in response to water deficiency is the main reason for the decrease in the rate of photosynthesis. However, during prolonged drought other non-stomatal factors play a dominant role in limiting the rate of photosynthesis. Our long-term gas exchange studies in wheat showed that relationship between photosynthesis rate and mesophyll conductance (calculated as the ratio of P_n to C_i) is more strong than relationship between photosynthesis rate and stomatal conductance (Allahverdiyev et al., 2015). An increase in C_i indicates non-stomatal limitation of photosynthesis. High gas exchange characteristics (P_n, g_s, E) of the genotypes Tartar, Sharg, Giymatli 2/17, Tale 38, Pirshahin 1, 4thFEFWSN№50, Dagdash positively associated with assimilation area formation and dry mass accumulation.

Despite the fact that gas exchange parameters of wheat genotypes are severely reduced at the booting, heading, flowering stages, a significant decrease in RWC and the Chl a, b and Car (x+c) contents was observed at grain ripening stages. In fact, although components of plant water relations are affected by reduced availability of water, stomatal opening and closing are more strongly affected (Anjum et al., 2011). In our opinion, a strong reduction of stomatal conductance allows keeping RWC at a relatively high level. There was positive and significant relationships between RWC and Car (x+c) under water stress (Allahverdiyev et al., 2018). The reduction of RWC and photosynthetic pigment contents were not significant in the genotypes Vugar, Gyrmyzy gul 1, Dagdash and Saratovskaya 29.

Flag and penultimate leaves, as well as spike and stem are the main assimilating surfaces at the heading, flowering and initial stages of kernel ripening. Our results showed that, an increase in the assimilation area of stem continued until watery ripe, while an increase in dry mass continued until milky ripe (Allahverdiyev and Huseynova, 2017). Translocation of photoassimilates from leaves to stem and further from leaves, stem and vegetative parts of spike into grains is accelerated under water deficiency. Spike dry mass decreases under drought

conditions due to insufficiency of sources.

The grain yield is the total output of all agronomical, morphological traits, physiological and biochemical processes. An average grain yield of durum and bread wheat genotypes was 539.3 and 558.4 g/m^2 under irrigated, 382.8 and 443.2 g/m^2 under water stress conditions, respectively. The reduction of grain yield constituted 29% for the durum wheat genotypes and 22% for the bread wheat genotypes.

On the basis of a decrease in gas exchange parameters, relative water content and Chl a, b and Car(x+c) contents of flag leaf, as well as a decrease in the assimilation area of leaves, stem and spike, we can conclude that some genotypes, such as Garagylchyg 2, Alinja 84, Tartar, Akinchi 84, Tale 38 and Gunashli, Pirshahin 1, 12ndFAWWON№97 are sensitive to drought stress. A deep decrease in grain yield of the genotypes Garagylchyg 2, Shiraslan 23, Sharg, Gyrmyzy bugda, Pirshahin 1, Gunashli, 4thFEFWSN№50 is more related with the limitation of biomass in leaves, stem and spike.

Despite weak correlations between physiological characteristics and grain yield, modern wheat genotypes, such as Tale 38, 4thFEFWSN№50 with high rate of photosynthesis, stomatal conductance and transpiration rate, as well as modern wheat genotypes, such as Vugar, Gyrmyzy gul 1, Dagdash with high indexes of relative water content and photosynthetic pigment contents under drought stress can be used in wheat breeding for improving productivity and drought tolerance. The classical tallest wheat genotypes, such as Sharg, Gyrmyzy bugda, Saratovskaya 29 show physiological tolerance to water stress. However, non-sufficient translocation of photoassimilates from leaves and stem into grains in the tallest genotypes, also less tillering capacity lead to low grain yield in a unit of area.

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Quraqlıq Stresinin Bərk Buğda (*Triticum durum* Desf.) və Yumşaq Buğda (*Triticum aestivum* L.) Genotiplərinin Fizioloji Əlamətlərinə və Dən Məhsuldarlığına Təsiri

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Tədqiqat işində torpaqda su çatışmazlığının müxtəlif bərk və yumşaq buğda genotiplərinin qaz mübadiləsinə, nisbi su tutumuna, fotosintetik piqmentlərin miqdarına, yarpaq, gövdə, sünbülün assimilyasiya sahəsi və quru biokütləsi, dən məhsuldarlığına təsiri öyrənilmişdir. Su stresi flaq yarpaqda fotosintezin sürətinin, ağızcıqların keçiriciliyinin, transpirasiya sürətinin azalmasına, hüceyrəarası sahələrdə CO_2 qatılığının artmasına səbəb olmuşdur. Su stresi bəzi həssas genotiplərin nisbi su tutumunun, xlorofil a, b və karotinoidlərin miqdarının, yarpaq, gövdə, sünbülün assimilyasiya sahəsi, quru biokütləsi və dən məhsuldarlığının kəskin azalmasına səbəb olmuşdur.

Açar sözlər: *Quraqlıq stresi, qaz mübadiləsi, nisbi su tutumu, assimilyasiya sahəsi, quru biokütlə, dən məhsuldarlığı*

Влияние Засухи На Физиологические Показатели и Урожайность Зерна Твердой (*Triticum durum* Desf.) и Мягкой (*Triticum aestivum* L.) Пшеницы

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Изучено влияние дефицита влаги на газообмен, относительное содержание воды, содержание фотосинтетических пигментов, площадь ассимиляции листьев, стеблей, колоса, сухую биомассу и урожайность зерна различных генотипов твердой и мягкой пшеницы. Водный стресс способствовал уменьшению скорости фотосинтеза, транспирации и проводимости устьиц флагового листа, а также увеличивал концентрацию CO_2 в межклеточном пространстве. У некоторых, чувствительных к дефициту влаги генотипов, под действием водного стресса наблюдалось резкое уменьшение относительного содержания воды в тканях, хлорофилла *a, b* и каротиноидов, площади ассимиляции листьев, стеблей, колоса, сухой биомассы и урожайности зерна.

Ключевые слова: *Засуха, газообмен, относительное содержание воды, площадь ассимиляции, сухая биомасса, урожайность зерна*

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Physiological Parameters of Maize *(Zea mays* L.*)* Varieties of Azerbaijan Breeding

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On the basis of the results obtained on Maize Breeding Program realized at Zagatala RES (Regional Experimental Station) of RICH, to compare the physiological parameters of C_4 plants *(Zea mays* L.) leaf assimilating surface area, leaf area index, dry matter accumulation, chlorophyll content in leaves, yield structural elements and productivity were analyzed and the relationship between the studied parameters were established.

Keywords: *Variety, maize, photosynthesis, chlorophyll content, leaf assimilating surface area, leaf index, yield structural elements, productivity*

INTRODUCTION

The most important biological features of maize are a broad genetic variability and high ecological plasticity, which ensure its adaptation in a wide range of environment (Chirkov, 1969, Kravchenko, 2010a, 2010b, Kravchenko, 2012). Due to the high biological adaptability, maize is able to grow and develop normally in various agro-climatic zones (Troneva, 2010). Therefore, the biological requirements of maize can fluctuate with large amplitude, due to the variation of the complex of interrelated biochemical, physiological, morphological and other characteristics (Francis, 1990). In addition, maize has a high productivity, which is due to the physiology of photosynthesis, a large leaf area, and high density of conducting system in them.

Maize refers to a small group of crops (mainly of tropical origin) that accumulate carbon dioxide in the process of photosynthesis according to the energy-efficient C_4 scheme (Shpaar, 1999). It gives a number of significant advantages in the formation of the yield. Maize has an increased coefficient of efficiency of photosynthetic active radiation (0.4-1.1% compared to 0.2-0.5% in wheat) and a biomass increase of 50-54 g /m^2 per day, while in plants of the C_3 group it is only 34 -39 g/m^2 (Gulyaev, 1989). The high energy absorption coefficient of solar radiation also ensured by the fact that leaves of maize plants contain a much higher amount of chlorophyll than other crops, which is the main pigment of green plants (Kayumov, 1989). The leaves of higher plants contain chlorophyll a and chlorophyll b, as well as carotenoids (carotin, xanthophyll, etc.).

The area of leaves in the sowing is expressed by the index of the leaf surface (ILS) - the ratio of the total surface of the leaves to the area of the soil covered with plants. Using this indicator, it is possible to evaluate the efficiency of the crop with respect to the accumulation of dry matter-the final result of photosynthetic activity.

The best is maintaining ILS, close to optimal. In this way, it is possible to maintain the optimum rate of accumulation of dry matter (Gelston, Devisc, Setter, 1983).

The purpose of the study. The purpose of this research was to study the effect of physiological parameters on the productivity of maize plants.

MATERIALS AND METHODS

Field experiments were conducted in the Parzivan experimental field of the Zagatala RES of the ARICH in 2014-2016. Phenological observations were carried out according to generally accepted methods. The area of the assimilation surface of leaves was measured using an automatic area meter (AAC-400, Hayashi Denkon Co., LTD, Japan).

The concentration of photosynthetic pigments (chlorophyll a, b and carotenoids) was determined in 96% ethanol. In the leaf extract, chlorophyll a, b and carotenoids were determined by spectrophotometer (Genesys 20, Thermo Scientific, USA) at wavelengths of 664, 648, 470 nm in 96% ethanol (Lichtenthaler, 1987). The yield was calculated by the grain output from cob. The subjects of the study were 10 varieties and variety samples of *Zea mays* L.

related to the species *Indendata Flavoruba, Indurata Vulqata* and *Indendata Leykodon*.

RESULTS AND DISCUSSION

During the vegetation period, phenological observations were carried out to ensure the establishments of stages of plant development, the duration of inter stage and vegetative periods, which, with respect to maize, are important feature that largely determines the level of productivity (Kravchenko, 2010a, 2010b).

For high yield, the leaves of maize varieties, which are considered to be the main photosynthetic apparatus, the assimilation surface area, their dynamics of growth and activity, are key indicators and their study is of great practical importance. The dynamics of the formation of the assimilating surface area of leaves is shown in Table 1. As can be seen in the table 1, at the beginning of ontogenesis in all studied varieties, the assimilating surface area of the leaves increased, after the tasseling the growth of the leaves somewhat weakened, and reached its maximum at the end of the flowering stage. This indicator in varieties Zagatala Yerli Yakhshylashdyrylmysh (Zagatala Local Improved), Gurur, Emil and Umud was respectively, 1.797, 1.403, 1.389 and 1.379 m^2, while in the other studied varieties it changed in the range of 1.082-1.373 m^2. Assimilating surface area of the variety Zagatala Yerli Yakhshylashdyrylmysh was more: 21.9-39.2% compared to other varieties. During the growing season, the assimilating surface area of the leaves of the lower part gradually decreased, and at the upper parts increased and the leaves located at the top had higher physiological activity. The difference in the photosynthetic activity of the leaves, as a result of the shading of the lower middle and upper parts, is explained by weak illumination.

The leaf area index of the varieties was also calculated and determined that at silking stage in varieties Zagatala Yerli Yakhshylashdyrylmysh (10.24 m^2/m^2), Gurur (7.999 m^2/m^2), Emil (7.918 m^2/m^2) and Umud (7.861 m^2/m^2) this indicator was higher in comparison to other varieties, while toward to the end of the vegetation it was decreased.

In varieties with a larger area of the assimilating surface and functional activity, the grain formation proceeds more intensively, this is reflected in the final yield. As a result of yellowing and biological aging of leaves, by the end of ontogenesis, the assimilating surface area decreased even more. The quantity and quality of the final maize yield during the growing season are closely related to the accumulation of dry biomass of the leaves. Therefore, a regular study of the accumulation of dry biomass of leaves and other parts of plant is of great practical importance. The accumulation of dry biomass during vegetation depends on not only developmental stages; it depends also on the genetic characteristics of the plant.

As can be seen in Fig. 1 the accumulation of dry biomass in one plant increases until the period of milk ripening, reaches a maximum and decreases to a period of complete maturity.

This parameter during the milk ripening in varieties Gurur, Zagatala 68 and Emil was 95.9, 91.3 and 90.8 g respectively, while in the other studied varieties - Fakhri and Umud varieties, 85.0 and 76.4 g, respectively. Accumulation of dry leaf biomass in the Variety Gurur was 4.54-19.4 g more in comparison with other varieties. And at the end of vegetation period this indicator changed in the range of 41.8-50.3 g.

Table 1. Assimilating surface area of leaves of one plant, m^2.

Name of variety	Development stages and leaf indices									
	15 leaves	leaf index	tasseling	leaf index	silking	leaf index	milk ripening	leaf index	wax ripening	leaf index
Zagatala 68	0.810±0.17	4.617	0.923±0.51	5.261	1.098±0.37	6.394	1.075±0.48	6.120	0.599±0.41	3.415
Zagatala 380	1.005±0.24	5.732	1.243±0.75	7.085	1.373±0.44	7.826	1.015±0.89	5.784	0.583±0.85	3.323
Zagatala 420	0.903±0.10	5.148	1.103±0.27	6.289	1.208±0.30	6.883	0.958±0.55	5.506	0.559±0.27	3.184
Zagatala 514	1.077±0.30	6.136	1.302±0.58	7.426	1.369±0.82	7.804	1.218±0.30	6.942	0.784±0.37	4.469
Zagatala Yerli Yakhsh.	1.305±0.27	7.439	1.565±0.27	8.921	1.797±0.68	10.24	1.309±0.37	7.458	0.889±0.92	5.068
Mirvari	0.847±0.55	4.825	1.022±0.79	5.826	1.189±0.48	6.775	0.870±0.92	4.960	0.463±0.68	2.638
Gurur	1.101±0.41	6.276	1.296±0.30	7.388	1.403±0.55	7.999	1.313±0.68	7.484	0.705±0.41	4.019
Umud	0.983±0.65	5.604	1.183±0.44	6.744	1.379±0.17	7.861	1.210±0.20	6.897	0.691±0.72	3.939
Fakhri	0.882±0.30	5.028	0.999±0.68	5.695	1.255±0.41	7.154	0.941±0.55	5.364	0.594±0.85	3.386
Emil	0.923±0.72	5.262	1.122±0.58	6.397	1.389±0.58	7.918	1.028±0.82	5.860	0.682±0.61	3.888
Populyasiya 2001 B	0.742±0.55	4.228	0.909±0.44	5.181	1.099±0.68	6.264	0.913±0.37	5.203	0.426±0.30	2.428
Populyasiya 2008 H	0.752±0.34	4.286	0.939±0.48	5.351	1.082±0.27	6.167	0.845±0.61	4.816	0.450±0.27	0.257

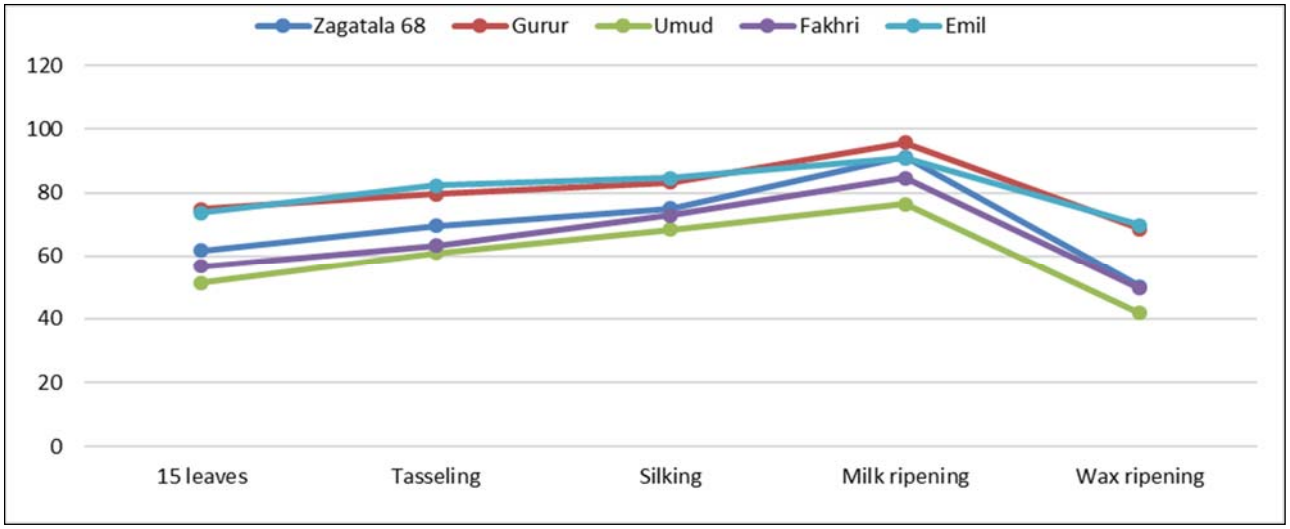

Fig. 1. Dynamics of leaf dry matter accumulation in one plant, g.

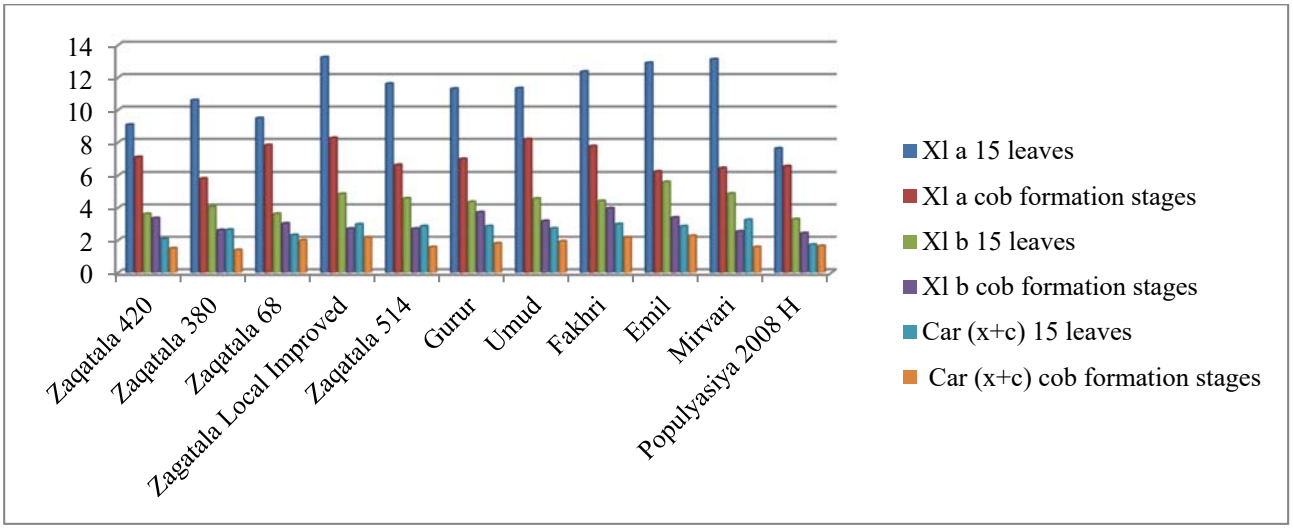

Fig. 2. Amount of chlorophyll a, b and carotenoid in maize leaves.

Thus, compared to other varieties in the varieties Zagatala Yerli Yakhshylashdyrylmysh, Gurur, and Umud the assimilating surface area and leaf index are high. Also the amount of dry biomass in the varieties Gurur, Zagatala 68 and Emil is high.

Also, the amount of photosynthetic pigments (chlorophyll a and b, carotenoids) in the studied varieties and perspective samples were determined in stages of 15 leaves and cob formation. The amount of chlorophyll creates an idea of the potential of plants such as CO_2 assimilations and yield formation, which enables them to evaluate the role of various plant organs in the formation of the yield (Andrianova, 1988, Andrianova, Tarchevskiy, 2000).

The higher amount of chlorophyll in the stage of 15 leaves was in the varieties Zagatala Yerli Yakhshylashdyrylmysh, Mirvari, Emil and Fakhri. Depending on the growing conditions, the amount of chlorophyll in the leaves varies and in sunny plants was constituted 0.68-1.30% and 1.12-1.18% of the dry weight of the leaves in shady plants. The amount of chlorophyll b was higher in Zagatala Yerli Yakhshylashdyrylmysh and Umud, while carotenoids were higher in Emil and Mirvari varieties. The amount of carotenoids is constituted 0.1-0.3% of dry weight on the leaves of higher plants, i.e. 3-6 times less than the amount of chlorophyll a and b. In the Cob formation stage, all three parameters decreased and a higher amount of chlorophyll was in Fakhri and Gurur, chlorophyll b in Fakhri and Emil and carotenoids were in the Emil, Fakhri and Zagatala Yerli Yakhshylashdyrylmysh varieties (Fig. 2).

During the harvesting period, maize varieties are characterized by productivity and structural elements of the cob. According to the duration of the growing season, with the exception of the variety Zagatala Yerli Yakhshylashdyrylmysh (120 days), the varieties under study were early- and mid-season varieties (96-110 days).

Table 2. Biometrical parameters, productivity and yield structural elements of maize varieties.

Name of variety	Vegetation period, day	Plant height, cm	Height of insertion of upper cob, cm	Number of leaves at complete maturity stage, pc	Length of the cob, cm	Number of rows per cob, pc	Number of grains per row, pc	Grain output per cob, %	1000 kernel weight, g	Productivity , c/ha
Zagatala 68	110	281	113	15.0	23.3	18.0	52.0	82.7	350	53.8
Zagatala 380	108	290	84.0	16.0	23.8	18.0	53.0	80.5	331	52.9
Zagatala 420	106	239	82.0	14.0	24.0	18.0	48.0	80.8	322	53.2
Zagatala 514	110	311	98.0	16.0	22.6	16.0	46.0	82.0	351	51.0
Zagatala Yerli Yakhs.	120	294	150	16.0	24.0	17.0	51.0	78.0	358	51.3
Mirvari	96,0	240	75.0	12.0	25.0	18.0	52.0	83.0	330	49.8
Gurur	105	247	93.0	14.0	23.3	16.0	49.0	81.2	348	50.6
Umud	105	232	92.0	13.0	22.0	16.0	48.0	83.1	339	53.7
Fakhri	105	229	83.0	13.0	22.7	17.0	46.0	83.8	343	56.9
Emil	104	260	96.0	13.0	28.0	16.0	50.0	84.0	368	58.5
Populyasiya 2008 H	106	239	82.0	14.0	24.0	18.0	45.0	80.8	352	53.2

Table 3. The correlation between biometrical parameters, productivity and yield structural elements of maize varieties.

Indicators	VP	PH	HUC	NL	LC	NGPC	NGPR	GOPC	1000KW	P
VP	1	-	-	-	-	-	-	-	-	-
PH	0.660*	1	-	-	-	-	-	-	-	-
HUC	0.861**	0.544	1	-	-	-	-	-	-	-
NL	0.822**	0.866**	0.543	1	-	-	-	-	-	-
LC	-0.236	-0.014	-0.010	0.284	1	-	-	-	-	-
NGPC	-0.089	-0.078	-0.155	0.062	0.021	1	-	-	-	-
NGPR	0.046	0.335	0.268	0.152	0.327	0.306	1	-	-	-
GOPC	-0.693*	-0.415	-0.603*	0.678*	0.162	-0.258	-0.149	1	-	-
1000KW	0.413	0.531	0.420	0.326	0.190	-0.600	-0.275	0.049	1	-
P	-0.076	-0.264	-0.152	-0.294	0.392	-0.164	-0.182	0.535	0.168	1

Note: Abbreviations are as follows: VP - Vegetation period, PH - Plant height, HUC - Height of uppermost cob, NL - Number of leaves, LC - Length of the cob, NRPC - Number of rows per cob, NGPR - Number of grains per row, GOPC - Grain output per cob, 1000KW – 1000 kernel weight, P– Productivity

The studied parameters such as the height of plants, the height of insertion of upper cob (height of the node bearing the uppermost cob), number of leaves in the complete maturity stage varied within, 229-311cm, 75.0-150 cm and 12.0-16.0 cm respectively.

Biometrical parameters, productivity parameters and results of structural analyzes of maize varieties are given in Table 2. In the samples after drying, the length of the cob was 22.0-28.0 cm, the number of rows per cob 16.0-18.0, the number of grains per row was 45.0-53.0, the grain output per cob at threshing was 78.0-84.0%. 1000 kernel weight varied in the range of 322-375 g and productivity was 49.8-58.5 cwt/ha.

The correlation between productivity and structural elements was studied (Table 3). Positive correlations between the height of plant and vegetation period, the height of insertion of upper cob (height of the node bearing the uppermost cob) and vegetation period, the number of leaves in maturity stage and vegetation period and height of plant, and the number of leaves in maturity stage and grain output were determined.

Thus, productivity of new maize varieties Gurur, Umud, Fakhri and Emil (*Indentata Flavorubra*) varied between 56.6-58.5 cwt/ha and was 5.00-8.04% higher than the standard variety Zagatala 68.

As a result of the carried out research the maize varieties Gurur and Umud were released, varieties Fakhri and Emil were submitted to the State Service for Plant Varieties Registration and Seed Control under the Ministry of Agriculture of the Republic of Azerbaijan.

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Azərbaycan Seleksiyasının Qarğıdalı *(Zea mays* L.*)* Sortlarının Fizioloji Parametrləri

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Əkinçilik Elmi Tədqiqat İnstitutu Qarğıdalı Proqramı üzrə Zaqatala Bölgə Təcrübə Stansiyasında əldə edilmiş nəticələr əsasında C4 *Zea mays* L. bitkisinin fizioloji parametrlərinin müqayisəsi üçün yaprağın assimilyasiya səthi sahəsi, yarpaq indeksi, quru maddənin toplanması, yarpaqlarda xlorofilin miqdarı, məhsulun struktur elementləri və məhsuldarlıq təhlil edilmiş və bu göstəricilərlə böyümə prosesləri arasında əlaqə öyrənilmişdir.

Açar sözlər: *Sort, qarğıdalı, fotosintez, xlorofilin miqdarı, yaprağın assimilyasiya səthi sahəsi, yarpaq indeksi, məhsulun struktur elementləri, məhsuldarlıq*

Физиологические Параметры Сортов Кукурузы *(Zea mays* L.*)* Азербайджанской Селекции

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В статье приведены результаты, проводимых по программе Селекции Кукурузы в Закатальской зонально-опытной станции НИИ земледелия исследований, по сравнению физиологических показателей C_4 растений (*Zea mays* L.). С этой целью проанализированы такие показатели, как: площадь ассимиляционной поверхности листьев, листовой индекс, накопление сухой биомассы, содержание хлорофилла в листьях, продуктивность, структурные элементы продуктивности и изучена взаимосвязь этих показателей с ростовыми процессами.

Ключевые слова: *Сорт, кукуруза, фотосинтез, содержание хлорофилла, ассимиляционная площадь поверхности листьев, листовой индекс, структурные элементы продуктивности, продуктивность*

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Results of the Primary Ecological Test of Nurseries Organized on the Basis of International Winter Wheat Improvement Program Performed in Absheron

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The information on the leading International research centers of wheat such as International Maize and Wheat Improvement Center (CIMMYT), International Center for Agricultural Research In the Dry Areas (ICARDA), and International Winter Wheat Improvement Program (IWWIP), the goals and results of the scientific collaboration of the Research Institute of Crop Husbandry with these centers, and the results of the primary ecological tests performed in the International nurseries (in 2015 and 2016) have been presented in the paper.

Keywords: *Wheat, variety, selection, introduction, international nurseries, earing, grain yield, yellow rust, brown rust, powdery mildew*

INTRODUCTION

Currently, due to widespread of natural anomalies such as various climate changes, drought, flood, degradation and desertification of lands, wheat having a strategical importance, play a crucial role in food security of every country, including Azerbaijan. Wheat is distinguished by its wide cultivation spectrum and adaptive properties in the ecological point of view (Briggle, Curtis, 1987). Wheat can be cultivated under a broad range of soil and climatic conditions except the tropics. According to the geographical conditions 2 main "wheat zones" exist in the world: between 30°C and 60°C of the northern latitude, and between 27°C and 40°C of the southern latitude (Nuttonson, 1955). Mean annual precipitation in the wheat cultivated dry areas varies between 375 mm and 875 mm. Wheat can be cultivated even in the territories with annual precipitation from 250 mm to 1750 mm (Leonard, Martin, 1963). Adverse environmental factors - frost, heat, humidity, drought have a serious impact on wheat productivity and quality. Therefore, along with the developing varieties tolerant against harmful biotic and abiotic factors, it is also very important to improve wheat cultivation in non-irrigated territories. International research centers of wheat such as International Maize and Wheat Improvement Center (CIMMYT), International Center for Agricultural Research In the Dry Areas (ICARDA) were created to solve such issues.

CIMMYT being a leading international research center that possesses the largest genbank of wheat, is committed to providing global food security, reducing poverty, improving wheat and maize productivity. ICARDA performing the same mission, manages the research on the cultivation of wheat, barley and leguminous plants in non-tropical, dry territories. Various programs were developed for the Central Asia, West Asia and North Africa (CWANA) by ICARDA and CIMMYT.

IWWIP was established in the mid-1980s jointly by Ministry of Food and Agriculture of Turkey, CIMMYT and ICARDA as an International Program to improve embryo plasma of winter bread wheat. The major goal of the program is to improve the genetic material of facultative and winter wheat for Central and Western Asia regions. IWWIP focuses on the exchange of wheat genetic materials related to the world breeding programs, creation of genetic materials for irrigated and dryland areas, conducting international tests of genetic materials belonging to national selection programs of the regional countries.

The Research Institute of Crop Husbandry created direct relationships with CIMMYT and ICARDA in 1996. Since then the institute has implemented the introduction and exchange of genetic materials for wheat breeding (Abdullayev, Musayev, Talai, 2008, Aliyev et al., 2013, Talai, 2005, Talai, 2013). The main purpose of this scientific collaboration is the choice of hybrid lines suitable to Azerbaijan regions with contrasting soil and climatic conditions and their use in breeding. The cultivation of the developed varieties have been organized in farms of the irrigated and dryland regions of Azerbaijan (Talai J.M. et al., 2017). As a result of the joint investigations performed with the International Centers for many years, 7 bread wheat

varieties (Azamatli 95, Nurlu 99, Gobustan, Tale 38, Gyzyl Bughda, Guneshli, Layagatli 80) have been developed, regionalized, included in the State Registry of the Selection Achievements and patented (Mahmudov, Talai, Morgunov A, 2002). The cultivation area of these varieties in Azerbaijan covers more than 180 thousand ha.

The aim of the research: The implementation of the primary ecological tests of durum and bread varieties of various purpose introduced from the international selection centers- CIMMYT and ICARDA, evaluation of these varieties according to their life conditions, morphological traits, productivity, resistance to diseases and the choice of samples for the future research and hybridization.

MATERIALS AND METHODS

Field experiments were performed in the experimental field of the Research Institute of Crop Husbandry situated on the Absheron peninsula. The objects of the study were wheat genotypes introduced from the International Selection Centers - CIMMYT and ICARDA in accordance with IWWIP. The phenological observations were performed from germination till the complete maturation phase according to Kuperman (Dospekhov, 1985, Kuperman, 1984). Exposure to diseases was conducted based on the methods of the International Selection Centers (Rust scoring guide, 1986). The field experiments were carried out according to the scheme recommended by the International Organization ICARDA (Instruc-tions rrigated twice during the vegetation period and ammonium nitrate fertilizer was applied in Spring.

RESULTS AND DISCUSSION

Life conditions, overwintering, earing period (number of days from the 1st of January to earing) of the studied varieties from the wheat genfund were determined, the heights of the plants were measured, and resistance to lodging and diseases was examined. Evaluation of the infection with yellow rust, brown rust and powdery mildew was performed according to the scales, meeting the international standards. Productivity indices of the planted samples were determined. Samples recommended for hybridization were chosen to develop new varieties suitable to various agro-ecological conditions of Azerbaijan.

Based on the results of the ecological tests, we could conclude that not the all studied materials were suitable to the local soil and climatic conditions. Samples chosen from the nurseries organized by the joint program of Turkey's government, CIMMYT and ICARDA- Facultative and Winter Wheat Observation and Yield Trial (FAWWON, IWWYT, WWON-IR, WWON-SA), Winter Wheat East European Regional Yield Trial (WWEERYT), Winter Wheat Elite Yield Trial (WWEERYT-IR and WWEERYT-SA) - appeared to have high overwintering ability, medium and late earing period (110-125 days from the 1st of January). They are resistant to rust diseases, and lodging, tall and medium height. These varieties are high productive (6-8 t/ha) and more suitable to the Azerbaijan ecological conditions compared with most spring varieties acquired by CIMMYT from Mexico, which have low overwintering ability, early earing period (85-100 days from the 1st of January). They are sensitive to rust diseases (20-90 MS), short, and their seeds drop.

Varieties acquired from CIMMYT are short, earing of these varieties occur early. In spring wheat varieties developmental phases proceed fast, tube formation occurs early during autumn sowing, forced earing under relatively cold conditions prevents normal insemination in the spikelets. However, due to the late earing (the 2nd decade of May) of typical winter samples, grain filling stage that proceeds during severe drought period (spring-summer) becomes shorter, which seriously affects productivity. In spite of some differences observed during many years of experiments, optimum earing period for bread winter wheat varieties introduced to Azerbaijan is considered to be the 3rd decade of April and the 1st decade of May. Because of the absence of severe cold and drought, this period is favorable for the physiological processes proceeding in the plant organism.

The results of the primary tests performed in the Absheron Experimental Base can provide comprehensive characterization of 4 nurseries of Facultative and Winter Wheat Observation (22nd-23rd FAWWON-SA and 22nd -23rd FAWWON-IR) and 4 nurseries of International Winter Wheat Yield Trial (17th-18th IWWYT-SA and 18th-19th IWWYT-IR). Some physiological and agronomical indices of the mentioned nurseries corresponding to the results of the research performed in 2015-2016 are presented in Table 1 a and b.

As seen in the Table in 2015 earing period covered the 1st and 2nd decades of May and in 2016 it covered the 2nd and 3rd decades of April and the 1st decade of May. In IWWIP nurseries the plant height ranged from 93cm to 139 cm in 2015 and from 75 to 130 cm in 2016. Shorter varieties were observed in the 19th IWWYT-IR nursery. Yellow and brown rust did not occur in 2015, but powdery mildew was widespread (from 2 to 9).

Table 1. IWWIP nurseries

(a) Absheron 2015				
Parameters	*22nd FAWWON-IR*	*22nd FAWWON-SA*	*18th IWWYT-IR*	*17th IWWYT-SA*
Cultivation territory	Absheron	Absheron	Absheron	Absheron
Number of samples	116	97	40 (2)	36 (2)
Earing period (from the 1st of January)	121-136 The I and II decades of May	119-141 The I and II decades of May	117-134 The I and II decades of May	119-136 The I and II decades of May
Height (cm)	95-137	112-139	95-131	93-133
Plant disease (powdery mildew)	2-9	3-9	3-9	4-9
Grain yield (g/m2)	360-900	200-800	320- 1050	280-790
Number of chosen samples (Total 69)	31	12	15	11
(b) Absheron 2016				
Parameters	*23rd FAWWON-IR*	*23rd FAWWON-SA*	*19th IWWYT-IR*	*18th IWWYT-SA*
Cultivation territory	Absheron	Absheron	Absheron	Absheron
Number of samples	160	100	40 (2)	36 (2)
Earing period (from the 1st of January days)	109-125 The II and III decades of April and the I decade of May	111-126 The II and III decades of April and the I decade of May	109-125 The II and III decades of April and the I decade of May	111-126 The II and III decades of April and the I decade of May
Height (cm)	85-115	80-130	75-110	90-124
Plant disease (powdery mildew, yellow rust)	*P.m.* 1-7 y.r.10-20 S	*P.m.* 2-7 y.r.5-10 S	*P.m.* 2-7 y.r.10-30 S	*P.m.* 2-8 y.r.10-20 S
Grain yield (g/m²)	300-800	250-650	350-700	350-700
Number of chosen samples (Total 38)	13	7	12	6

Table 2. Samples chosen in the IWWIP nurseries.

(a) Absheron 2015						
Parameters		*22nd FAWWON-IR*	*22ndFAWWON-SA*	*18th IWWYT-IR*	*17th IWWYT-SA*	*Control Tale-38*
Number of chosen samples		31	12	15	14	-
Earing period (number of days from the 1st of January to earing days)		122-130 The I-II decades of May	122-130	121-130	121-130	129
Height, cm (mean)		110	124	115	122	98
Plant disease	Y.r.	-	-	-	-	-
	B.r	-	-	-	-	-
	P.m	9	8	8	9	8
Grain yield (mean) g/m²		700	670	800	680	524
(b) Absheron 2016						
Parameters		*23nd FAWWON-IR*	*23ndFAWWON-SA*	*19th IWWYT-IR*	*18th IWWYT-SA*	*Control Tale-38*
Number of chosen samples		13	7	12	6	-
Earing period (number of days from the 1st of January to earing days)		11-127 The I-II decades of May	115-126 The III decade of April, The I decade of May	111-126 The III decade of April, The I decade of May	110-127 The III decade of April, The I decade of May	119 The III decade of April
Height,cm (mean)		98	110	94	111	96
Plant disease	Y.r.	-	-	-	-	-
	B.r	-	-	-	-	-
	P.m	1-4	2-7	2-6	3-6	7
Grain yield (mean) g/m²		730	628	663	691	553

Note: Y.r. – yellow rust, b.r. – brown rust, P.m. – powdery mildew

In 2016 powdery mildew spread ranged from 1 to 8 and yellow rust occurred (5-30 S) in all nurseries. The samples of the 19th IWWYT-IR nursery were more infected with yellow rust (10-30S). In the IWWIP nurseries grain yield ranged from 200 to 1050 g/m^2 in 2015, and from 250 to 800 g/m^2 in 2016. The number of high productive samples increased. At the end of the vegetation year, 107 perspective varieties were chosen for cultivation the following year in a broader areas.As seen in the Tables, during 2015 and 2016 earing of the samples, chosen in the observation and yield trial nurseries of facultative and winter wheat was early or late compared with the Tale-38 variety taken as a control variant.

But in both cases the optimum earing period ranged from 110 to 130 days. Their height changed between 94 cm and 124 cm and they were resistant to lodging. The chosen samples were not infected with rust diseases, but powdery mildew was widespread among these varieties.

Average grain yield was found to range from

600 to 800 g/m^2 and far exceeded the control variant. The chosen samples were planted in 5 and 10m^2 beds for more comprehensive morphological investigations.

Thus, in 2015-2016, from the IWWIP nurseries 107 perspective, hybrid lines with contrasting productivity, resistance to diseases were chosen for using in the selection process under irrigated and drought conditions. These chosen winter and facultative hybrid lines have high overwintering ability and optimum earing period. They are tall and medium-height, high productive (600-800 g/m^2), resistant to rust diseases and lodging.

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Payızlıq Buğdanın Yaxşılaşdırılması Üçün Beynəlxalq Proqram (IWWIP) Əsasında Tərtib Olunmuş Pitomniklərin Abşeronda İlkin Ekoloji Sınağının Yekunları

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Məqalədə qarğıdalı və buğdanın yaxşılaşdırılması üzrə Beynəlxalq mərkəz (CIMMYT) və Quraq ərazilərdə kənd təsərrüfatı tədqiqatları üzrə Beynəlxalq mərkəz (ICARDA) kimi aparıcı Beynəlxalq buğda tədqiqat mərkəzləri və payızlıq buğdanın yaxşılaşdırılması üçün Beynəlxalq Proqram (IWWIP) haqqında məlumat verilmiş, Əkinçilik Elmi Tədqiqat İnstitutu ilə bu mərkəzlər arasındakı elmi əməkdaşlığın məqsəd və nəticələri, həmçinin son iki ildə (2015 və 2016-cı illər) Beynəlxalq pitomniklərdə aparılmış ilkin ekoloji sınağın yekunları barədə danışılmışdır.

Açar sözlər: *Buğda, sort, seleksiya, introduksiya, beynəlxalq pitomniklər, sünbülləmə, dən çıxımı, sarı pas, qonur pas, unlu şeh*

Результаты Первичного Экологического Теста, Проведенного в Питомниках Абшерона по Международной Программе Улучшения Озимой Пшеницы

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В статье представлены данные о ведущих международных исследовательских центрах пшеницы, таких как Международный центр по улучшению кукурузы и пшеницы (CIMMYT), Международный центр сельскохозяйственных исследований в засушливых районах (ICARDA) и Международная программа улучшения озимой пшеницы (IWWIP), цели и результаты научного сотрудничества Научно-исследовательского института земледелия с этими центрами, а также результаты первичных экологических испытаний, проведенных в Международных питомниках в 2015 и 2016 годах.

Ключевые слова: *Пшеница, сорт, селекция, интродукция, международные питомники, колошение, выход зерна, желтая ржавчина, бурая ржавчина, мучнистая роса*

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Determination of Drought Tolerance of Chickpea and Lentil Plants, and Structural Elements of their Varieties

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Water retention capability of leaves in chickpea and lentil varieties, temperature of the sowing surface and structural elements of the production have been studied. A direct relationship was found among productivity, the number of beans and the grain number per plant in chickpea varieties. A positive correlation was also observed between productivity and the number of grains per plant in lentil varieties. As a result of the research, varieties relatively tolerant to drought were chosen.

Keywords: *Chickpea, lentil, leaf water retention capability, production index, productivity*

INTRODUCTION

Plants are exposed to stress factors worldwide due to the global climate change and shortage of irrigation water. The enhancement of the ambient temperature and deficiency in irrigation water are main ecological factors limiting plant productivity in arid regions (Bedenko, 1980). Drought having a significant impact on plant development and growth, causes chlorophyll distruction, lipid peroxidation, accumulation of hydrogen peroxide, which leads to the cell membrane damage, increases in ascorbic acid and proline contents (Mukherjee et al., 1983; Aetinkut et al., 2001), stomatal closure, reducing rates of transpiration and photosynthesis and lowered water potential in plant tissues (Yordonov et al., 2003; Gunes et al. 2008).

Generally, plants with high leaf water retention capability are more tolerant to drought (Gunes et al., 2008). Drought tolerance is stipulated by functional stability of the cell structure, high water potential of tissues and adaptive properties of the stem, leaves and generative organs, which allows plants to maintain their growth, development and reduce production loss.

A little-time consuming, simple devices and express-methods have been used in the recent studies of plant drought tolerance. Leaf water retention capability was determined using "Turgomer-1" (Kushnirenko, 1991), temperature changes at the sowing level at the expense of leaf transpiration were measured using the infrared thermometer.

Using these methods, leaf drought tolerance was studied in wheat (Abdulbagiyeva et al., 2007) and field bindweed (Aetinkut et al., 2001) and drought tolerant varieties were chosen.

MATERIALS AND METHODS

To determine plant drought tolerance, measurements were conducted on 12 chickpea and 15 lentil varieties under field conditions. Experiments were performed at the subsidiary experimental field of the Research Institute of Crop Husbandry.

Turgometric measurements were conducted on leaves of intact plants for establishing leaf water retention capability. Then the leaves were excised and turgometric measurements were conducted after 4 hours in four replicates.

Over the past 4 hours, a lot of water exited the leaves via transpiration. Leaf water retention capability was calculated as the ratio T_2/T_1, where T_1 is the result of the first and T_2 the second measurement.

RESULTS AND DISCUSSION

The results of the measurements carried out on chickpea varieties are shown in Table 1. Higher values of the ratio T_2/T_1 corresponds to better water retention capability.

As seen in Table 1, the T_2/T_1 ratio ranged from 0.56 to 0.71. The minimum leaf water retention capability was observed in the variety F.08-116. Higher leaf water retention capability was detected in the F.08-89, Sultan and Sechma L. varieties. The highest value of this paramter -0.71- was found in F.08-89. When temperature of the soil surface was 28.7°C, temperature of the sowing surface changed from 18.2°C to 22.5°C due to transpiration. In the F.08-89 variety having the highest water retention capability and in Sechma L. this parameter was found to be 22.5°C and 22.3°C, respectively.

The leaves of these varieties are also distinguished by more water use efficiency. Moreover, Sechma L. was found to be high productive.

The results of the measurements conducted on lentil varieties are presented in Table 2. In the lentil varieties the T_2/T_1 ratio was found to range from 0.52 to 0.68. The lower values of the T_2/T_1 ratios were observed in the lentil varieties, confirming higher leaf water retention capability of the chickpea varieties. The higher values of the ratio in the chickpea varieties prove that they are more tolerant to drought compared with the lentil varieties. Another distinguishing feature of the chickpea varieties is hairs covering leaves and stems. These hairs preventing transpiration of water, participate also in the reflection of solar radiation and thereby create conditions for drought tolerance.

The lowest T_2/T_1 ratio was observed for the F.2013-4 variety (0.52) and the highest for F.2014-006 (0.68). Considering the T_2/T_1 ratio and temperature indices, the lentil varieties F.2013-18 and F.2014-006 are more tolerant to drought than other varieties.

To determine structural elements of the production and productivity, samples were taken from the same field. 10 chickpea and 10 lentil varieties were used as the study objects.

Table 1. Turgometric and temperature indices of chickpea varieties.

No	Variety	T_1	T_2	T_2/T_1	Temperature, °C
1	F.07-289	18.3 ± 0.89	11.9 ± 0.93	0.65	21.8 ± 0.94
2	Sanford	22.9 ± 0.71	14.1 ± 0.75	0.62	21.1 ± 0.34
3	F.07-274	21.6 ± 0.48	13.3 ± 0.26	0.62	20.7 ± 0.66
4	Jamila	24.3 ± 0.23	15.4 ± 0.80	0.63	18.2 ± 0.10
5	F.08-89	19.5 ± 0.58	13.9 ± 0.76	0.71	22.3 ± 0.77
6	F.08-196	21.2 ± 0.37	13.3 ± 0.19	0.63	21.7 ± 0.37
7	F.08-116	19.7 ±0.74	11.0 ± 0.22	0.56	22.2 ± 0.50
8	Nazrin	19.3 ± 0.59	12.4 ± 0.83	0.64	21.6 ± 0.25
9	Sultan 2	15.9 ± 0.85	9.4 ± 0.6	0.59	21.3 ± 1.01
10	Sultan	22.6 ± 0.76	15.4 ± 0.14	0.68	21.2 ± 0.43
11	Narmin	24.6 ± 0.21	16.0 ± 0.76	0.65	20.5 ± 0.38
12	Sechma L.	23.6 ± 0.39	16.1 ± 0.05	0.68	22.3 ± 0.10

Table 2. Turgometric and temperature indices of lentil varieties.

No	Varieties	T_1	T_2	T_2/T_1	Temperature, °C
1	F.86-16 L.	17.7 ± 0.44	10.5 ± 0.61	0.59	20.5 ± 0.07
2	LC00600296	15.4 ± 0.25	8.1 ± 0.31	0.53	20.1 ± 0.60
3	F.2013-22	16.8 ± 0.65	10.3 ± 0.50	0.61	21.5 ± 0.17
4	F.2014-026	13.4 ± 0.49	7.3 ± 0.39	0.54	19.9 ± 0.34
5	F.2013-18	12.6 ± 0.37	8.5 ± 0.57	0.67	21.4 ± 0.34
6	F.2013-4	17.6 ± 0.60	9.2 ± 0.86	0.52	20.9 ± 0.70
7	F.2012-8	16.8 ± 0.19	10.3 ± 0.16	0.61	20.9 ± 0.65
8	F.2013-26	16.6 ± 0.71	10.5 ± 0.64	0.63	20.2 ± 0.34
9	Surian Loc.L.	16.8 ± 0.35	11.2 ± 0.91	0.67	19.8 ± 0.41
10	Arzu	18.4 ± 0.13	12.3 ± 0.56	0.67	19.4 ± 0.37
11	F.2014-006	16.6 ± 0.96	12.7 ± 0.83	0.68	21.2 ± 0.35
12	F.2012-1 L.	17.4 ± 0.77	10.6 ± 0.84	0.61	22.3 ± 0.27
13	F.2013-29	16.1 ± 0.47	9.1 ± 0.37	0.56	23.0 ± 0.69
14	F.2012-18	16.5 ± 0.28	8.1 ± 0.06	0.49	20.0 ± 0.28
15	F.2014-009	19.2 ± 0.78	11.1 ± 0.41	0.58	19.5 ± 0.52

Table 3. Structural elements of the production in chickpea varieties.

No	Varieties	Plant height, (cm)	Height of the first bean above the ground, (cm)	Number of beans	Number of grains per plant	100-grain mass, (gr)	Production index	Productivity, (cwt/ha)
1	Sechma L.	65.5±5.2	34.9±4.4	47.4±6.6	55.5±5.3	35.6	0.46	28.5
2	Narmin	69.8±4.2	41.3±4.2	48.6±3.3	49.9±3.43	32.5	0.41	24.7
3	Sultan-2	72.5±0.2	42.9±1.7	28.3±0.7	29.0±0.7	44.0	0.37	23.2
4	Nazrin	68.4±5.3	44.6±3.2	18.9±1.31	19.4±1.1	41.2	0.38	22.7
5	Jamila	64.6±3.5	34.1±1.7	41.7±3.8	44.2±4.6	44.6	0.42	15.7
6	F-08-116	67.8±5.0	38.1±1.6	30.1±1.8	32.0±3.4	43.7	0.41	21.2
7	F-08-196	58.5±0.6	36.6±1.0	17.6±1.3	18.2±1.4	44.9	0.42	19.4
8	F-08-89	48.6±1.3	31.3±2.3	15.3±0.8	15.6±0.1	42.3	0.35	19.0
9	Sanford	50.9±4.2	33.5±1.0	15.7±0.4	21.4±0.9	38.4	0.37	13.9
10	Sultan (st)	57.5±4.0	34.9±3.5	16.0±1.8	16.8±0.9	35.4	0.39	23.3

One of the main economically important indices of chickpea is plant height. As tall plants grow, the distance between rows becomes smaller, preventing weed development and at the same time the shade maintains moisture of the soil. The height of the Sultan-2 variety was 72.5 cm. The shortest among the studied varieties was Flip – 08-89. The height of the Sultan variety accepted as a standard was 57.5 cm. In general, erect forms of chickpea varieties should be preferred. The forms intended for the mechanical harvesting should not be prone to lodging.

One of the main parameters for cereal plants (especially chickpea and lentil) is the height of the first bean above the ground. Usually huge crop losses occur during harvesting due to a short distance between beans and the ground. The height of the first bean above the ground in the studied varieties ranged from 31.3 to 44.6. This parameter was the smallest (31.3 cm) in the short variety Flip-08-89. The largest value of this parameter (44.6 cm) was observed in the Nazrin variety, whereas for the tallest variety it was equal to 42.9. A significant positive correlation – $r=0.813^{**}$ was observed between plant height and the height of the first bean above the ground. The correlation among structural elements of the production was determined using the SPSS 16.1 program (Table 4).

The number of beans per plant in the studied varieties ranged from 15.3 to 48.6. The largest number of beans was observed in the Narmin variety-48.6, the smallest number was found in the Flip-08-89 variety-15.3. In the high productive variety Sechma L. the number of beans per plant was 47.4 and in the tall Sultan-2 variety-28.3.

One of the main purposes of the modern selectionists is increasing the number of beans and grains per plant. The number of grains per plant changed from 15.6 to 55.5 in the studied varieties. The number of grains per plant for the high productive Sechma L. variety was found to be 55.5 and for the low productive Sanford variety -21.4. It should be noted that a significant positive correlation was established between the number of grains and beans per plant ($r=0.986^{**}$). Moreover, a positive correlation was also established among the number of grains per plant, the number of beans per plant and productivity.

100-grain mass of the studied chickpea genotypes ranged from 32.5 to 44.9 grams. The Flip-08-196 variety is distinguished by grain size, 100-grain mass was found to be 44.9 grams. Tiny grains were observed in the high productive Narmin variety. In the Sultan variety accepted as a standard 100-grain mass was equal to 35.4 grams. A negative correlation was found between 100-grain mass and the number of beans (r=-0.354).

Production index (index of economic suitability) characterizes the distribution of assimilates formed during photosynthesis between generative and vegetative organs. Productivity of agricultural plants can be significantly increased by enhancing productivity index, which is determined as the ratio of grain dry biomass to total dry biomass (Bezmenova M.F., 2010). Production index ranges from 0.1 to 0.8 depending on the cultivation conditions of various plants (Yordonov et al., 2003). The increase in the productivity of modern varieties occurs at the expense of enhanced production index as a result of redistribution of assimilates using genetic-selection methods (Kursanov A.L. 1976). Optimum distribution of photosynthetic active radiation increases total biomass leading to a significant decrease in production index (Nichiporovich A.A. 1956). But application of mineral fertilizers creates favorable conditions to increase production index. Therefore, the enhancement of production index is considered to be one of the important directions in the modern selection for developing intensive type varieties.

Production index of the studied chickpea varieties ranged from 0.35 to 0.46. Production index of the high productive and low productive Sechma L. and Flip-08-89 varieties were 0.46 and 0.35, respectively. It suggests that in the Sechma L. variety more photosynthetic assimilates are transferred to the generative organs compared with other varieties. Productivity of the studied varieties ranged from 13.9 to 28.5 cwt/ha.

Table 4. Correlation among structural elements of the production in chickpea varieties.

	PH	B1	BN	GN	HGM	PI	GY
PH	1						
B1	0.813^{**}	1					
BN	0.618	0.174	1				
GN	0.556	0.093	0.986^{**}	1			
HGN	0.008	0.042	-0.354	-0.394	1		
PI	0.398	-0.044	0.703^{*}	0.744^{*}	-0.250	1	
GY	0.547	0.378	0.434	0.412	-0.456	0.410	1

** - correlation is significant at the 0.01 level.

* - correlation is significant at the 0.05 level.

Note: PH – plant height, B1 – height of the first bean above the ground, BN – bean number per plant, GN– grain number per plant, HGM – 100-grain mass, PI – production index, GY – grain yield

Table 5. Structual elements of the production in lentil varieties.

No	Variety	Plant height, (cm)	Height of the first bean above the ground, (cm)	Number of beans	Number of grains per plant	100-grain mass (g)	Production index	Productivity, (cwt/ha)
1	F-2014-009	32.9±3.5	18.3±1.5	39.4±2.1	53.9±4.0	7.4	0.41	16.0
2	F-2012-18	34.4±1.0	18.7±1.2	38.4±3.4	42.4±2.4	7.5	0.44	15.0
3	F-2013-29	37.5±2.7	20.4±2.3	26.3±2.0	28.5±2.1	7.6	0.32	14.0
4	F-2012-1L.	37.7±2.4	20.8±1.4	30.8±3.3	31.2±2.1	6.7	0.29	13.5
5	F-2014-006	36.9±2.2	20.9±2.6	27.3±2.1	27.8±1.3	8.0	0.36	13.1
6	Surian Local L.	37.8±2.0	21.3±1.0	47.1±3.3	50.3±2.7	6.8	0.26	12.5
7	F-2013-26	35.5±1.2	19.9±1.7	39.2±1.7	39.8±2.6	7.7	0.40	11.8
8	F-2012-8	41.9±2.4	25.0±0.9	37.1±4.1	52.8±3.8	6.7	0.27	11.3
9	F-2013-4	37.9±2.3	20.7±2.3	36.1±2.0	38.7±2.2	8.4	0.27	11.0
10	Arzu (st)	38.1±2.7	21.2±0.1	19.3±2.8	21.8±1.8	7.5	0.33	12.6

Table 6. Correlation among structural elements of the production in lentil varieties.

	PH	B1	BN	GN	HGM	PI	GY
PH	1						
B1	0.931**	1					
BN	-0.249	-0.066	1				
GN	-0.156	0.128	0.887**	1			
HGN	-0.349	-0.439	-0.317	-0.414	1		
PI	-0.788**	-0.715*	0.004	0.005	0.337	1	
GY	-0.715*	-0.625	-0.026	0.104	0.069	0.616	1

** - correlation is significant at the 0.01 level.
* - correlation is significant at the 0.05 level.
Note: designations as in Table 4

The highest variety among the studied ones appeared to be Filip-2012-8 (41.9 cm).

The height of the first bean above the ground ranged from 18.3 cm to 25.0 cm. The largest value of this parameter was observed in Filip-2012-8 (25.0 cm). As seen in Table 6 a significant positive correlation was detected between the height of the first bean above the ground and plant height ($r=0.931^{**}$) in the lentil varieties. The number of beans per plant ranged from 19.3 to 47.1. The largest number of beans (47.1) was found in the Surian Local L. variety.The lentil varieties differ very much in the number of grains per plant. In the high productive Flip-2014-009 variety the number of grains per plant was found to be 53.9, whereas in the Arzu variety accepted as a standard this parameter was 21.8. As seen in Table 6 a significant positive correlation ($r=0.887^{**}$) exists between the number of grains per plant and the number of beans.

In the studied lentil varieties 100-grain mass ranged from 6.7 to 8.4 grams. The largest parameter was observed in the Flip-2013-4 variety and in the Arzu variety accepted as a standard this value was equal to 7.5 grams.

Another main parameter of plant productivity is production index. In the lentil varieties this parameter varied over a wide range (0.26-0.44). Varieties with high production index were more productive.

Positive correlations were observed between production index and productivity in both chickpea and lentil varieties (r=0.410 for chickpea and r=0.616 for lentil varieties).

Thus, it is recommended to use the chickpea varieties F-08-89, Sechma L. and lentil varieties F-2013-18, F-2014-006 for developing drought tolerant forms. Parameters, such as the height of the first bean above the ground and productivity index can also be used in developing drought tolerant varieties.

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Noxud və Mərciməyin Quraqlığa Davamlılığının Təyin Edilməsi və Nümunələrin Məhsulunun Struktur Elementləri

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Məqalədə noxud və mərcimək nümunlərinin sahə şəraitində yarpaqlarının su saxlama qabiliyyəti, əkin səthinin temperaturu və məhsulun struktur elementləri haqqında məlumatlar əks olunmuşdur. Məlum olmuşdur ki, noxud nümunələrində məhsuldarlıqla paxlalıların sayı və bir bitkidə olan dənlərin sayları arasında birbaşa müsbət əlaqə mövcuddur. Mərcimək bitkisi üçün isə bir bitkidə olan dənlərin sayı ilə məhsuldarlıq arasında müsbət əlaqə aşkar olunmuşdur. Tədqiqat nəticəsində nisbətən quraqlığa davamlı nümunələr seçilmişdir.

Açar sözlər: *Noxud, mərcimək, yarpağın su saxlama qabiliyyəti, məhsul indeksi, məhsuldarlıq*

Определение Засухоустойчивости и Элементов Структуры Урожая у Сортов Нута и Чечевицы

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В статье приведены сведения о водоудерживающей способности листьев и температуре поверхности посева в полевых условиях у образцов нута и чечевицы, а также определены элементы структуры урожая. У образцов нута выявлена положительная связь между продуктивностью и числом зерен в бобах и числом зерен с одного растения. Обнаружена также положительная корреляция между продуктивностью и числом зерен с одного растения у образцов чечевицы. В исследованиях выявлены относительно засухоустойчивые образцы.

Ключевые слова: *Нут, чечевица, водоудерживающая способность листа, индекс урожайности, урожайность.*

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Relations Between Protein Content and Technological Quality Indices in Grains of Bread Wheat Varieties Under Different Watering Regimes

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Protein content and technological quality indices in grains of bread wheat varieties have been compared. Protein content in grains of bread wheat varieties was found to change sharply depending on watering regime. In order to evaluate the nutritional value of bread wheat varieties, sedimentation, gluten amount, gluten deformation index and grain vitreousity were determined. Vitreousity and gluten amount ranged from 22.0% to 90.0%, and from 19.2% to 28.8%, respectively, under optimal watering. Whereas, the same parameters changed between 25.0-100.0%, and 21.2-34.8%, respectively, in unwatered variants.

Keywords: *Bread wheat, productivity, protein, vitreousity, gluten, sedimentation, protein yield per hectare*

INTRODUCTION

The development of agriculture and achievement of production abundance, which is possible by increasing grain production at the expense of wheat, other cereals and grain legumes, are the main issues facing our country in the modern market economy system. Consideration of soil types is very important due to complex climatic conditions and soil properties in wheat cultivation regions. Nowadays, to obtain high-quality and high-yielding crops, organic and mineral fertilizers should be applied with consideration of ecological factors of these regions (Konovalov, 1981). Lately, diseases and pests have been widely spread in the wheat cultivation regions of Azerbaijan. Absence of measures against diseases and pests leads to loss of the production and a decline in its quality (Huseynov, 2009; Huseynov et al., 2005).

The main issue in developing a new, high-productive bread wheat variety having high quality, resistant to diseases and pests, tolerant to frost, drought and extreme environmental conditions is the construction of the complete model of wheat. Academic J.A. Aliyev laid the foundation for the new high-quality, high-yielding wheat variety model (Aliyev, 1982). The study of grain quality and technological indices related to watering regime is of great importance. Because these indices play an important role in the baking industry (Trufanov, 1994; Tutukova et al., 2011). We aimed to improve the selection methods and prepare recommendations for selectionists by studying biological and molecular properties of high-quality, high-yielding wheat varieties, tolerant to biotic and abiotic factors. Bread wheat varieties with contrasting productivity, quality, drought tolerance, resistance to diseases and pests from the local wheat genefund were planted in the Absheron Experimental Station of the Research Institute of Crop Husbandry.

Physiological and biochemical properties of high-quality grains have been studied in wheat varieites cultivated in different soil types, under contrasting climatic conditions. The possibility of application of these parameters in the selection process for developing new, high yielding, high-quality and drought-tolerant varieties has been elucidated.

MATERIALS AND METHODS

The experimens were performed in 4 replicates, in 2 variants, with 14 perspective local bread wheat varieties planted in 50m^2 beds. The nitrogen content in grains was determined in the "Keltek 1003 (LKB)" device, using the modified Kjeldahl micromethod. Coefficient N x 5.7 was used for the estimation of the protein content (Pleshkov, 1976). Grain vitreousity was measured with DZC-2 diophonoscope. For the determination of the gluten amount starch was washed out of the flour and gluten was dried and weighed. Gluten deformation index was measured with the IDK-1 device. Sedimentation was estimated after the precipitation of high-molecular protein particles in 2.0% acetic acid (QOST, 1986).

RESULTS AND DISCUSSION

To assess the nutritional capability, baking quality and quality of flour depending on water regime, grain vitreousity, gluten deformation index, sedimentation and gluten amount were determined

in bread wheat varieties. Gluten amount ranged from 19.2% to 28.8 %, gluten deformation index from 77.7 units to 97.0 units under optimal watering regime. Whereas, the same parameters changed between 21.2%-34.8%, and 84.7 units - 106.7 units, respectively, in unwatered variants.

The highest amounts of gluten was found in the bread wheat varieties Tale 38 (28.8%) and Azamatli 95 (26.8%), the smallest amounts of gluten were detected in the varieties Nurlu 99 (19.2%) and Pirshahin 1(21.2%) under optimal watering conditions. Under unwatered conditions the highest amounts of gluten were detected in the Sartovskaya 29 (34.8%) and Guneshli (28.8%) varieties, the smallest amounts in the 2 nd FAWWON N97 (21.2%) and Nurlu 99 (21.6%) varieties.Vitreousity ranged from 35.0% to 96.0% in bread wheat varieties under optimal watering regime and from 25.0% to 100.0% under unwatered conditions. Sedimentation changed from 18.0 ml to 27.0 ml under optimal watering regime, while in the unwatered variants this parameter changed between 24.0 ml and 37.5 ml.

Economic suitability index ranged from 0.26 to 0.36 and from 0.26 to 0.34 in optimally watered and unwatered variants, respectively.

Protein compounds of wheat grain were determined to elucidate the processes under the effect of biotic and abiotic factors and develop new high-yielding, high-quality varieties, tolerant to frost and drought, resistant to diseases and pests, using the purposeful selection process.

Protein content and protein yield per hectare in the bread wheat varieties cultivated under optimal watering were found to be 11.8% -13.2 % and 442.9 kg/ha-727.1 kg/ha, respectively. Whereas, these parameters ranged from 12.2 % to 15.4 % and from 381.9 kg/ha to 667.2 kg/ha, respectively, under unwatered conditions (Fig. 1).

The largest protein amounts were detected in the varieties Tale 38 (13.2%), Sartovskaya 29 (13.0%) and the smallest protein amounts were found in the varieties Giymatli 2/17 (11.8%), Akinchi 84 (11.9%), Pirshahin 1 (11.9%) under optimal watering. While under unwatered conditions, the highest values of this parameter were found in the varieties Sartovskaya 29 (14.4%), Tale 38 (13.9%), Guneshli (13.5%) and the smallest values in the varieties Ruzi 84 (12.2%), 2 nd FAWWON N97 (12.2%), Akinchi 84 (12.2%).

According to previous reports, nitrogen fertilizers in soils are insufficient for the formation of numerous protein compounds in grains of the high-productive wheat varieties (Huseynov, 2009; Marushev, 1967; Urazaliyev et al., 2003; Strelnikova, 1971; Wrigley, 1994).

Table 1. Technological quality indices of grains in regionalized and perspective bread wheat varieties under different watering regimes.

Variety	Variant	1,000-grain mass, g	Vitreousity, %	Gluten, %	GDI, units	Sedimentation, ml	Economic suitability index
Giymatli 2/17	I	53.0	31.0	23.6	95.1	19.5	0.30
	II	49.3	48.0	24.4	86.1	25.5	0.29
Akinchi 84	I	47.7	42.0	23.6	97.0	24.0	0.33
	II	47.0	76.0	26.4	100.9	31.5	0.33
Azamatli 95	I	47.1	30.0	26.8	84.7	25.5	0.31
	II	41.4	58.0	26.4	96.0	31.5	0.31
Nurlu 99	I	40.5	38.0	19.2	79.9	18.0	0.27
	II	37.0	68.0	21.6	84.7	25.5	0.29
Gobustan	I	47.1	24.0	25.5	96.4	22.5	0.34
	II	44.2	35.0	25.2	96.6	27.0	0.34
Ruzi 84	I	48.8	34.0	22.4	84.3	27.0	0.33
	II	47.8	53.0	25.2	106.7	33.0	0.32
Gyrmyzy gul 1	I	39.6	22.0	24.8	80.7	27.0	0.36
	II	39.4	25.0	22.0	85.0	25.5	0.35
Tale 38	I	46.1	40.0	28.8	96.0	27.0	0.26
	II	43.6	73.0	25.6	98.7	33.0	0.28
Pirshahin 1	I	46.8	61.0	21.2	84.5	19.5	0.32
	II	46.5	84.0	25.2	95.1	28.5	0.33
2 nd FAWWON N97	I	38.3	24.0	22.8	88.4	24.0	0.34
	II	36.1	33.0	21.2	95.2	22.5	0.32
4 th FEWSN N50	I	44.2	45.0	26.0	98.3	24.0	0.30
	II	38.7	45.0	22.8	93.3	24.0	0.30
Guneshli	I	50.6	90.0	23.2	84.4	24.0	0.26
	II	46.3	100.0	28.8	95.2	31.5	0.26
Daghdash	I	38.8	81.0	23.2	77.7	22.5	0.27
	II	36.6	81.0	26.0	92.6	24.0	0.27
Sartovskaya-29	I	47.2	89.0	25.2	86.9	25.5	0.28
	II	44.5	91.0	34.8	92.0	37.5	0.28

I - optimal watering regime; **II** – unwatered variant

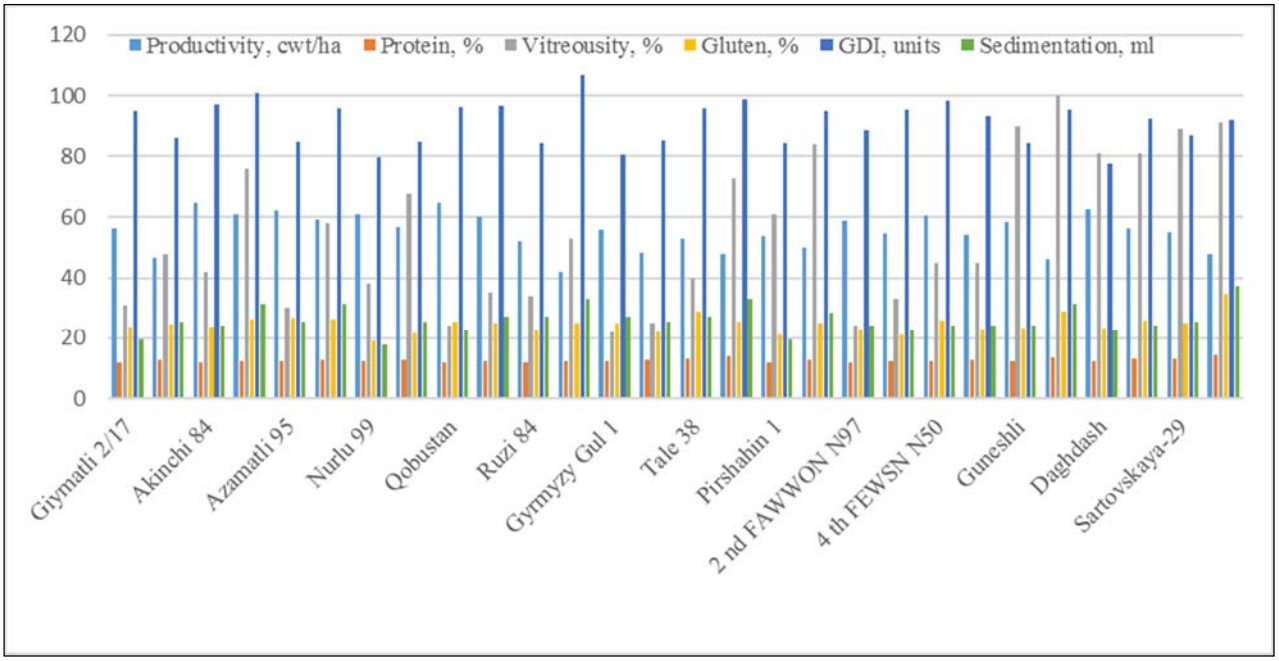

Fig. 1. Comparison of grain quality indices of regionalized and perspective bread wheat varieties under different watering regimes.

Table 2. Comparison of grain quality indices of regionalized and perspective bread wheat varieties under different watering regimes.

	Productivity, cwt/ha	Protein,%	Protein yield, cwt/ha	1000 grain mass,g	Vitreousity, %	Gluten, %	GDI, units	Sedimen-tation, ml
Protein,%	-0.509**							
Protein yield, cwt/ha	0.908**	-0.104						
1000 grain mass, g	-0.122	-0.170	-0.232					
Vitreousity, %	-0.274	0.553**	-0.041	0.100				
Gluten, %	-0.246	0.694**	0.044	0.242	0.389*			
GDI, units	-0.197	0.178	-0.146	0.237	0.060	0.403*		
Sedimentation, ml	-0.516**	0.682**	-0.268	0.099	0.422	0.741	0.461*	
Economic suitability index	0.146	-0.477*	-0.072	-0.056	-0.619**	-0.238	0.113	-0.023

*0.01 accuracy, ** 0.05 accuracy

Considering the lack of protein compounds in flour and flour products, and the increasing demand of population for plant proteins, correlations between technological quality indices of different bread wheat varieties have been studied.

A negative correlation (-0.509**) was found between productivity and portein amount in grains of the studied bread wheat varieties. A positive correlation (0.908**) exists between productivity and protein yield per hectare and a weak negative correlation (-0.104) was detected between protein yield per hectare and protein amount in grains. Positive correlations were also observed between protein amount in grains and vitreousity (0.553**), gluten amount (0.694**), sedimentation (0.682**) and a negative correlation between protein amount and Economic suitability index (-0.477*) (Table 2).

Considering the positive correlations between protein amount, productivity and protein yield per hectare, valuable recommendations can be given to selectionists for developing new, high-productive and high-quality bead wheat varieties.

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Suvarma Rejimindən Asılı Olaraq Yumşaq Buğda Sortlarının Dənində Zülalın Miqdarı ilə Texneloji Keyfiyyət Göstəricilərinin Əlaqəsi

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Məqalədə yumşaq buğda sortlarının dənində zülalın miqdarının texnoloji keyfiyyət göstəriciləri ilə müqayisəli təhlili verilmişdir. Aparılmış tədqiqatlar nəticəsində məlum olmuşdur ki, suvarma rejimindən asılı olaraq yumşaq buğda sortlarının dənində zülalın miqdarı kəskin surətdə dəyişir. Suvarma rejimində asılı olaraq yumşaq buğda sortlarının qidalılıq dəyərini öyrənmək üçün dəndə sedimentasiyanın, keykovinanın miqdarı, İDK və şüşəvarilik öyrənilmişdir. Tədqiq olunan yumşaq buğda sortlarının dənində optimal suvarma variantında şüşəvarilik (22,0-90,0%), kleykovinanın miqdarı isə (19,2-28,8 %), Suvarılmayan variantda isə bu göstərici uyğun olaraq (25,0-100,0%), (21,2-34,8 %) arasında dəyişmişdir.

Açar sözlər: *Yumşaq buğda, məhsuldarlıq, zülal, Şüşəvarilik, kleykovina, sedimentasiya, hektardan zülal çıxımı.*

Отношение Между Содержанием Белка и Показателями Технологического Качества в Зернах Сортов Мягкой Пшеницы при Различных Режимах Полива

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Проведено сравнение содержания белка и показателей технологического качества зерна сортов мягкой пшеницы. Обнаружено, что содержание белка резко меняется в зависимости от режима полива. Для оценки пищевой ценности сортов мягкой пшеницы были определены седиментация, содержание клейковины, индекс деформации клейковины и стекловидность зерна. При оптимальном поливе изменения стекловидности и количества клейковины составляли 22.0% - 90.0% и 19.2%-28.8% соответственно. Однако, в вариантах без полива эти параметры менялись в пределах 25.0-100.0% и 21.2-34.8%, соответственно.

Ключевые слова: *Мягкая пшеница, продуктивность, белок, стекловидность, клейковина, седиментация, выход белка с 1 га*

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Morpho-physiological Study of Wheat Genotypes With Contrasting Growth Periods, Cultivated Under Various Climatic and Soil Conditions

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The effect of drought on the surface area of various assimilating organs of durum and bread wheat genotypes with contrasting growth periods, cultivated under various climatic and soil conditions has been studied. To establish the accuracy of the obtained data, statistical analysis of some productivity indices was performed.

Keywords: *Drought, wheat genotypes, assimilating parts, plant metabolism, early maturing wheat genotypes*

INTRODUCTION

Recent global climatic changes have caused destructions in the ecological balance, and development in stress factors such as drought and salinity, which can lead to serious issues in satisfying the population demands for food.

It is known that biotic (pathogen, competition with other organisms etc.) and abiotic (drought, salinity, radiation, high and low temperature etc.) stresses cause drastic changes in physiological activity of plants, attenuate biosynthesis in cells, disturb normal vital functions and eventually can completely destroy the plant (Arora et al., 2002; Peet et al., 1977; Wang et al., 2003).

From this point of view, one of the main issues facing breeders is the creation of new, highly productive and stress-tolerant varieties using plant genotypes grown under unfavorable climatic conditions, tolerant to various stresses, including drought and salt-tolerant plant genotypes (Anjum et al., 2011; Blum, 1986; Witcombe et al., 2008).

To enhance plant tolerance to stress factors, physiological studies should be preferred for the revelation of mechanisms of tolerance and determination of physiological and genetic changes occurred in plants under stress (Moghaddam et al., 2012). Physiological investigations can facilitate the establishment of the stress tolerance associated genes in wheat genotypes. It should be noted that the establishment of genes tolerant to stress factors as well as the use of them in the hybridization process as donors is one of the most important issues facing modern plant breeding (Anjum et al., 2011; Tamrazov, 2016). The role of assimilating organs (leaf, stem, spike) is crucial in the proceeding photosynthesis and the formation of the product. Therefore, we measured assimilating surface area during vegetation and analyzed the obtained data.

MATERIALS AND METHODS

Durum and bread wheat genotypes with contrasting maturation periods were chosen as the study objects. Measurements were conducted with 4 samples of each group. However, the article presents data only on the early maturing durum and bread wheat genotypes. Although the study was performed in 3 regions and throughout 3 groups, only the results of the study in 1 group have been discussed.

The experiments were carried out on wheat genotypes with contrasting maturation periods (early, medium and late) at the Absheron, Jalilabad and Gobustan Experimental Stations of the Research Institute of Crop Husbandry during 2017-2018 year vegetation years. Regular phenological observations were performed on early maturing varieties. Assimilating surface area was measured using an automatic device AAS-400. Correlation was established between spike structural elements, characterizing productivity and statistical indicies were calculated using Excel and SPSS-19 statistical package programs.

As the studied varieties have various maturation periods, they divided into 3 groups: early, medium and late maturing varieties.

RESULTS AND DISCUSSION

The aim of the work was to determine drought effects on assimilating surface areas and productivity elements of bread and durum wheat genotypes differing in their maturation periods and grown under various climatic and soil conditions with following comparison of other physiological indices.

Maturing duration is known to be the most

important factor in the plant growth. Because of differences in climatic and soil conditions in the regions of our country, the study of this factor is needed. In this point of view, early and late maturing varieties should be preferred. In general, early maturing wheat genotypes are more characteristic of regions, where spring-summer drought onsets early. On the other hand, the main part of the genotypes are medium maturing that should be considered in the wheat cultivation in most regions.

Genotypes tolerant to soil and air water deficiency and able to provide high production are considered to be drought tolerant.

The results of the study (assimilating surface area) performed in Absheron are presented in Fig. 1. As seen in the figure assimilating surface area of various organs were comparatively studied in 2 durum (Garagylchyg 2 and Alinja 84) and 2 bread wheat (Alinja 84 and Gobustan) varieties, under normal water supply and drought conditions. The assimilating surface area in leaves of Garagylchyg-2 was measured since the 3rd decade of March. The maximum value was obtained in the middle of vegetation and then began to decline until the end of vegetation, which is attributed to the increase in the surface of other assimilating organs - stem and spike as a result of leaf senescence. The maximum values in the leaves are usually observed during the earing-flowering phase. The assimilating surface area in the Garagylchyg 2 leaf was 66.8 and 51.8 thous. m^2/ha, in watered and drought-exposed variants, respectively. The variants differ by 22.4%.

On the other hand, the maximum values in leaves of the bread wheat genotype- Gobustan were observed in the 1st decade of May, during the earing-flowering phase. Thus, in the watered and drought-exposed genotypes these values were found to be 64.2 and 61.2 thous. m^2/ha, respectively, with 4.6% difference between variants. In the Garagilchyg-2 variety the assimilating surface area of stem reached maximum values in the second decade of May and in watered and drought-exposed genotypes, was equal to 66.8 and 56.7 thous. m^2/ha, respectively, with 15.1% difference between the variants.

The similar situation was detected in the bread wheat genotype Gobustan. The stem assimilation surface area reached maximum values in the 1st and 2nd decades of May and in watered and drought-exposed genotypes, was equal to 69.7 and 61.2 thous. m^2/ha, respectively, with 12% difference between the variants.

Dynamics of the increase in the spike surface of the Garagylchyg-2 variety was as follows: during the maturation phase – at the end of May and at the beginning of June-31.2 and 20.9 thous.m^2/ha, in the watered and drought-exposed plants, respectively, with 33% difference between variants. Such a sharp variation is attributed to the intensification of drought to the end of vegetation.

In the Gobustan variety this index was equal to 28.6, and 23.4 thous. m^2/ha, in watered and drought-exposed variants, respectively, and the difference between the variants was 18%.

The dynamics of the changes in productivity indices were determined in 2 genotypes of each group (durum and bread genotypes). However, one genotype of each group was analyzed.

The same indices were also determined in the wheat varieties cultivated in the Jalilabad region of Azerbaijan.

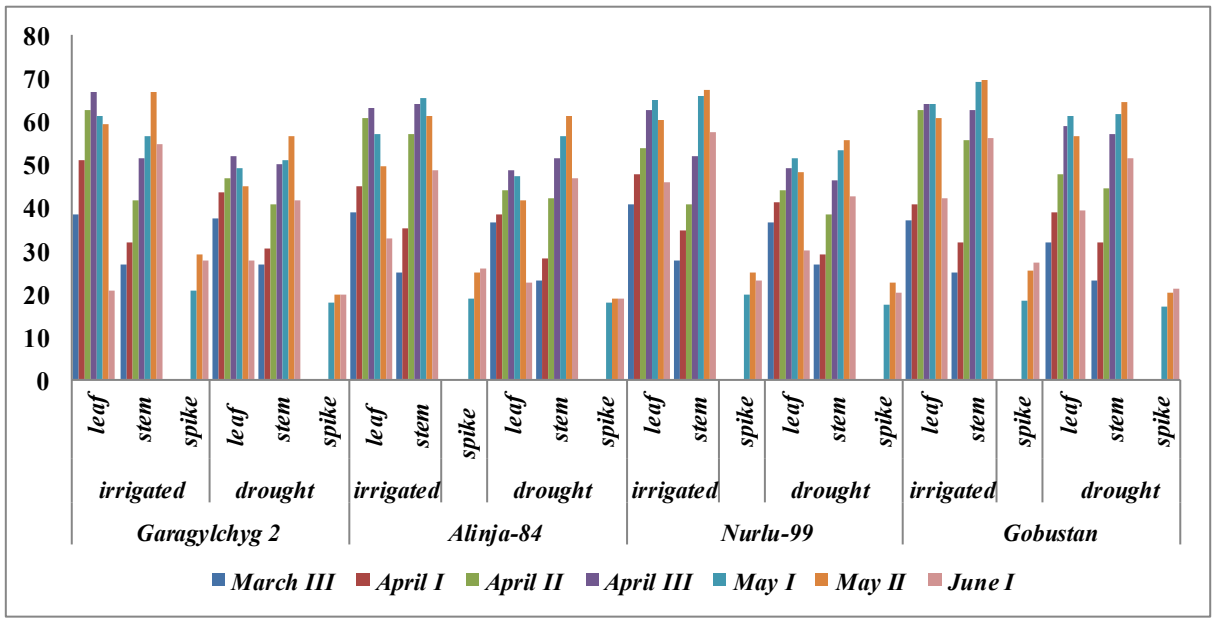

Fig. 1. The dynamics of the assimilating surface area of early maturing wheat genotypes-thous. m^2/ha (Absheron).

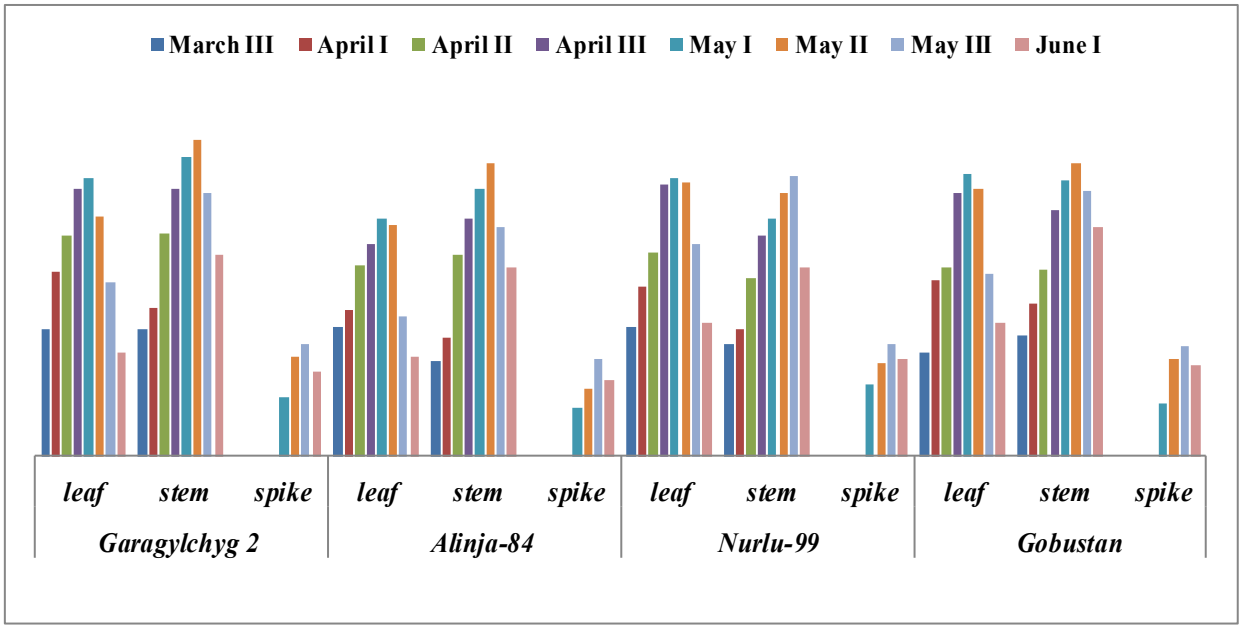

Fig. 2. The dynamics of the assimilating surface area of early maturing wheat genotypes-thous. m^2/ha (Jalilabad).

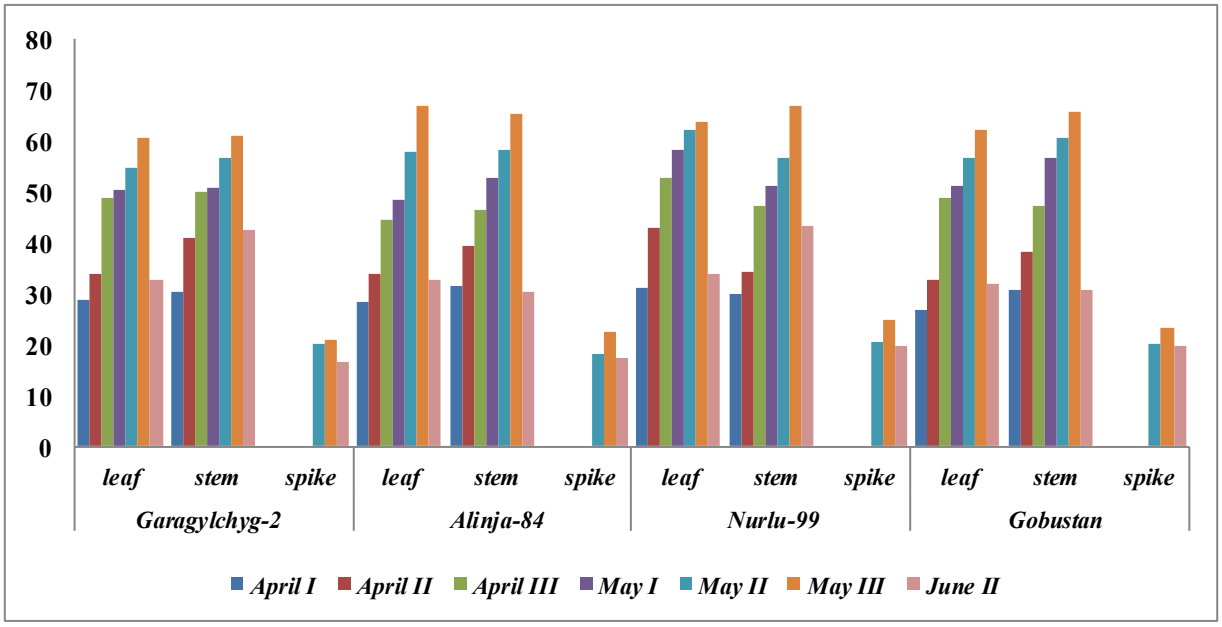

Fig. 3. The dynamics of the assimilating surface area of early maturing wheat genotypes-thousand m^2/ha (Gobustan).

As seen in the figure 2, the studied wheat genotypes did not change. However, contrary to Absheron, Jalilabab is a rainfed region and the measurements were conducted only with the drought-exposed plants. In the Garagylchyg 2 variety the maximum assimilating surface area of leaf - 61.3 thous.m^2/ha - was observed during the 1st decade of May. The maximum value of this index for stem- 69.7 thous. m^2/ha- was observed in the 2nd decade of May and for spike the maximum value detected in the 3rd decade of May was equal to 24.9 thous. m^2/ha.

Similar to Garaglchyg 2, in the Gobustan variety maximal values for the assimilating surface area in leaf (62.2 thous. m^2/ha), stem (64.4 thous.m^2/ha) and spike (24.4 thous. m^2/ha) were found in the 1st, 2nd and 3rd decades of May, respectively.

Thus, drought conditions characteristic of the Jalilabad region of Azerbaijan, earlier onset of drought and its intensity lead to the acceleration of the vegetation process (Tamrazov, 2016).

The next investigations were carried out in the Gobustan region, which climatic conditions are very different from those of Absheron and Jalilabad. Early onset of winter and late spring-summer drought affect the duration of vegetation. The results of the study are presented in Fig. 3.

Contrary to the first 2 regions, in Gobustan the measurements began in April. In the durum wheat variety Garagylchyg-2 the maximal value for the assimilating surface area in leaf was determined

(60.5 thous.m^2/ha) in the third decade of May - during the earing-flowering phase. However, in both stem (68.5 thous.m^2/ha) and spike (22.8 thous. m^2/ha) this index reached the maximum in June.

In the Gobustan variety the maximal values for the assimilating surface area in leaf (62.2 thous.m^2/ha) and stem (65.6 thous.m^2/ha) were detected in the third decade of May, in the same phase. However, in spike this index was maximum (27.9 thous. m^2/ha) in the 1st decade of June.

According to the obtained results, for the all assimilating organs of the early maturing genotypes the earing phase is considered to be the most favorable. Thus, the most active metabolism occurs during the mentioned growth phase of the plant (Peet et al., 1977; Witcombe et al, 2008).

The performed analysis showed that physiological processes proceeding in the plant changed due to the environmental factors. According to the obtained data drought-tolerant genotypes are more productive. Because, all physiological processes proceed to the end in these plants.

To determine productivity indices, sheaves were taken from 1m^2 area in 3 replicates prior to harvesting and 10 characteristic spikes were taken from each sample for the structural analysis.

From the development stage, there is a relative decline in the leaves and an increase in the body and spike.

Grain yield of the same varieties were determined in various regions. As seen in the table 1 in the durum wheat genotype-Garagylchyg 2 grown in Absheron the highest productivity index was found to be 617 g/m^2 and 457 g/m^2 in watered and drought-exposed plants, respectively, with 25.9% difference between the variants. In other durum wheat variety Alinja 84 this index was equal to 473 and 380 g/m^2 in watered and drought-exposed plants, respectively, and the difference between these values was 19.6%. The same parameter was determined in the bread wheat varieties Alinja 84 and Gobustan. The productivity index was found to be 518 and 493, respectively, for watered and drought-exposed plants of the Alinja 84 variety. However, for the Gobustan variety 423 and 405 were found, respectively, for watered and drought-exposed plants. The difference between varieties was as follows: 4.8% in Alinja 84 and 4.3% in Gobustan.

Considering the general dynamics, it can be concluded that productivity index and difference between variants were higher in Garagylchyg-2 compared with Alinja 84. The difference between variants in bread wheat varieties was almost the same, though productivity index was higher in Alinja 84.

Comparison of the productivity of durum and bread wheat genotypes showed that under Absheron conditions the durum wheat variety Garagylchyg 2 and the bread wheat variety Alinja 84 were more productive. In general, among early maturing wheat genotypes these genotypes were more efficient for the Absheron peninsula.

Maximal indices were also found for the durum wheat variety Garagylchyg 2 and the bread wheat variety Alinja 84 as a result of the analysis of productivity of wheat varieties cultivated in the Jalilabad region. Thus, similar results were obtained almost for all the studied genotypes. Regarding to the tolerance, Garagylchyg 2 and Alinja 84 were more tolerant as well.

The results of the experiments performed in Gobustan were completely different. Because, contrary to previous regions, early maturing genotypes are not considered as characteristic for the Gobustan region. Generally, bread wheat varieties were more productive compared with durum wheat varieties. Thus, maximum productivity for Gobustan and Garagylchyg 2 was found to be 550 g/m^2 and 400 g/m^2, respectively.

It should be noted that the evaluation of the studied early maturing wheat genotypes was conducted based on their tolerance to diseases, height, productivity and architectonics.

To examine the accuracy of the obtained data, some indices were analyzed statistically. First of all, according to the structural analysis, the correlation between structural elements was established and regression model was constructed.

As seen in the table there is a positive correlation between result trait and factor trait. So when factor trait (x) increases, result trait (y) also increases.

The results of the spike structural analysis are shown in table 2.

Table 1. Productivity indices of wheat genotypes.

Genotypes	Absheron Experimental Base Station		Jalilabad Regional Experimental Station	Gobustan Regional Experimental Station
	Irrigated, g/m^2	Drought, g/m^2		
Garagylchyg 2	617	457	515	400
Alinja 84	473	380	485	340
Alinja 84	518	493	585	450
Gobustan	423	405	455	550

Table 2. Characteristics of productivity and its structural elements in the studied wheat genotypes (Absheron-2017).

Wheat genotypes	Variant	Spike weight, g	Width of spike, cm	Length of spike, cm	Number of spikelets, number	Number of grains per spike	Weight of grain per spike, g
Garagylchyg 2	I	4.74	1.16	9.88	22.2	50	2.07
	II	3.3	1.14	7.25	19	48	1.02
Alinja 84	I	3.99	1.4	9.5	21	45.7	1.85
	II	2.91	1.16	8.4	17.8	40.2	1.02
Alinja 84	I	2.59	1.26	9.64	16.2	38.6	1.8
	II	2.5	1.44	9.46	16.2	37.2	1.05
Gobustan	I	2.73	1.22	11.7	17.8	42.5	2
	II	2.69	1.3	10.6	17.4	40.2	1.6

Table 3. Correlation analysis of the spike elements.

	Spike weight (y)	Width of spike (x_1)	Length of spike (x_2)	Number of spikelets (x_3)	Number of grains per spike (x_4)	Weight of grain per spike (x_5)
Spike weight (y)	1					
Width of spike (x_1)	-0.244	1				
Length of spike, (x_2)	-0.130	0.275	1			
Number of spikelets (x_3)	0.979	-0.256	-0.094	1		
Number of grains per spike (x_4)	0.877	-0.485	-0.231	0.910	1	
Weight of grain per spike (x_5)	0.435	0.002	0.725	0.434	0.321	1

As can be seen from the table, the spike elements of the studied wheat genotypes are analyzed and the results are presented in two variants. Given the difference in options, it is possible to note that in all the indicators of the spike there is a decrease in drought versus other variant. According to the research, the difference between the varieties of some wheat genotypes in comparison to other genotypes is characterized by drought resistance.

In the next table, there is a correlation between the indicators that accurately characterize the spike.

First of all correlation between the spike weight and other indices was analyzed. As can be seen in table 3, there is a closer connection between the number of spikelets and the number of grains per spike in the spike elements.

The correlation is weak, if the relation between these indices is expressed with a value less than 0.3, and a negative value shows that these indices do not depend on each other or even a negative correlation exists. On the other hand, if the values ranged from 0.5 to 0.7, the correlation is strong and above 0.9 corresponds to the formation of the functional correlation.

The smallest average index was observed in the spike width, the largest in the number of grains. This is attributed to the fact that average values were calculated on the basis of the spike width, which is the smallest index.

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Müxtəlif Torpaq İqlim Şəraitində Becərilən və Yetişmə Müddətinə Görə Fərqlənən Buğda Genotiplərinin Morfofizioloji Tədqiqi

T.H. Təmrazov

Azərbaycan Respublikası KTN Əkinçilik Elmi Tədqiqat İnstitutunun
Bitki fiziologiyası və biotexnologiya şöbəsi

Məqalədə, əsasən müxtəlif torpaq-iqlim şəraitində becərilən yetişmə müddətlərinə görə fərqlənən bərk və yumşaq buğda genotiplərində müxtəlif assimiləedici orqanların assimliyasiya səthi sahəsinə quraqlığın təsiri müəyyənləşdirilmişdir. Ölçmələr zamanı əldə olunan nəticələrin dəqiqliyini yoxlamaq üçün bir sıra məhsuldarlıq göstəriciləri arasında statistık tədqiqatlar aparılmışdır.

Açar sözlər: *Quraqlıq, buğda genotipləri, assimiləedici orqanlar, bitki metabolizmi, tez yetişən buğda genotipləri*

Морфо-физиологические Исследования Генотипов Пшеницы с Различным Периодом Созревания, Выращенных в Различных Почвенно-Климатических Условиях

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Изучено влияние засухи на площадь поверхности различных ассимилирующих органов генотипов твердой и мягкой пшеницы с различным периодом созревания, выращенных в различных почвенно-климатических условиях. Для установления точности данных, был проведен статистический анализ некоторых показателей производительности.

Ключевые слова: *Засуха, генотипы пшеницы, ассимилирующие части, метаболизм растений, рано созревающие генотипы пшеницы*

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Selective Evaluation of Winter Bread Wheat Advanced Lines for Adaptability in Garabagh Lowland Conditions

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The study was conducted to determine the adaptive promising lines of winter bread wheat. Adaptability was studied in bread wheat variety samples obtained from intraspecific crossings between 21 parental forms. Long-term observations of promising variety samples of winter bread wheat in the Garabagh Lowland conditions showed that not all varieties had the same behavior under the same growing conditions and differed in the potential productivity. Meanwhile, a few variety samples maintained their productivity over the years. The instability of productivity for many years indicated a lack of high adaptability of most of the variety samples studied. Some promising variety samples were distinguished by a neutral reaction to growing conditions of various years.

Keywords: *Intraspecific crossings, adaptability, perspective lines, variety samples, bread wheat, yield, selection*

INTRODUCTION

Being adapted to a wide range of moisture conditions, wheat is grown on more land area worldwide than any other crop and is an important food grain source for humans. To create qualitatively new varieties of crops in breeding practice, various methods are used to evaluate the initial material, crossing and selection of elite plants, methods for assessing disease resistance, etc., depending on the goals and regions for which the variety is created.

The same genotype grown in different environments often shows significant variation regarding productive performance (Condé et al., 2010; De Vita et al., 2010).

Varieties derived for certain local regions as a rule differ in high yield, but when cultivated in other regions that differ sharply from soil-climatic conditions, they quickly lose their advantages. In many cases, such varieties are inferior even to varieties with an average yield value, but a wide range of plasticity. In addition, it is known that the increase in grain yield as its share increases in the total biological yield leads to a reduction in the quality of the grain-content of protein and gluten (Pinheiro et al., 2013).

Obviously, the issues of increasing the sustainability of wheat production and stabilizing its quality should be addressed in an integrated manner, and above all, by use of varieties well adapted to local conditions. Orientation to varieties with high biological potential of any of the economically valuable traits, to a certain extent, contributes to reducing their resistance to adverse environmental effects (Ivannikov et al., 1998).

In this regard, an important role is assigned to the use of adaptive forms that have a wide range of plasticity to changing environmental conditions that can stably realize their potential. Therefore, along with the creation of new high-yielding and high-quality varieties of crops, the issue of creating varieties with different plasticity, the ability to produce a stable yield under different ecological conditions of cultivation and resistance to biotic and abiotic stresses, always remains an urgent task of breeding (Boyer, 1982). Recently, throughout the world, the trend of rational use of genetic resources, using local and introduced varieties in the breeding process, is aimed at creating, with a genetic basis, resistant to unfavorable environmental conditions varieties. This is primarily due to the global change in climatic conditions, the increase in stressful situations, the increase in temperature, the increase in radiation, salinization of soils, etc., as a result of natural disasters, as well as the emergence of new races of various diseases of crops (Naylor, 1993, Talebi et al., 2009).

In connection with the above-mentioned, based on the achievements of the world practice on wheat breeding, the research is being carried out at the Research Institute of Crop Husbandry to attract introduced and local accessions to create new varieties of wheat with the properties of optimal use of the bioclimatic potential of the growing area.

Azerbaijan among the neighboring countries

stands out with an exceptional variety of soil and climatic conditions and relief. Often, there is a sharp variation in weather conditions over the years, which is a limiting factor in yield, as a result of which, a decrease in the yield of wheat in particular years reaches a perceptible limit in some years. The presence of such a wide range of variation in climatic conditions determines the conduct of breeding activities aimed at adaptability and plasticity. It is known that the reaction rates of varieties of agricultural crops in particular wheat, to various ecological situations and changes in climatic conditions determine their adaptive value and plasticity. Even in extremely identical growing conditions, wheat varieties, depending on their biological characteristics and the genetic mechanism that respond to changes in environment, are differentiated in different degrees by yield over the years.

The purpose of the study. The purpose of this research was to determine the adaptive promising lines of winter bread wheat.

MATERIALS AND METHODS

The research was carried out under irrigation conditions at the Terter Regional Experimental Station (RES) of the Research Institute of Crop Husbandry and 21 varieties of bread wheat were used as parental forms. Parental genotypes were taken from the working collection, whose high yield was identified as a result of advance experiments. Intraspecific crosses of these varieties were carried out at the institute. During 2010-2012, 7 constant hybrids - TT 09214/3-1-2 (lutescens), TT 0887/2-1-1-1 (lutescens), TT 09706/2-4 (lutescens), TT 09704/2-4-1 (lutescens), TT 09704/5-2 (erythrospermum), TT 09704/2-4-1-1 (albidum), TT 09224/3-2-1 (lutescens) which are obtained as a result of crossing of landraces and variety samples introduced from international centers were sown on the experimental field of Terter RES in four replicates on plots each with an area of 50 m^2. Before sowing the seeds were treated with Vitavax (fungicide). During the growing season, the crops were treated with the herbicide (Topic 0.80). During the vegetation period, observations were made on the growth and development of plants in various stages of development.

Observations of resistance to diseases were made 3-4 times during the growing season. At the end of the milk ripeness, the plants were removed with roots and measurements were made on the test bundle according to the following characteristics: plant height, number of productive tillers, length of spike, number of spikelet, weight of grain per spike and plant, 1000 kernel weight per four replicates for each variety sample. At complete maturity, the harvesting was carried out and the average yield was determined. The LCD_{05} of experiment was 2.5 - 3.5 cwt/ha (Dospexov, 1985).

RESULTS AND DISCUSSION

Long-term observations of promising variety samples of winter bread wheat in the Garabagh lowland conditions showed that most varieties differ in the ways of the realization of potential productivity. At the same time, the number of varieties that preserved the stability of yields was relatively less by year. The instability of crops over the years indicated a lack of high adaptability of most of the studied varieties. Nevertheless, some promising varieties were distinguished by a neutral reaction to different years of cultivation.

It should be noted that the formation of the yield was largely influenced by the weather conditions at the time of flowering-fertilization and grain filling. This is evidenced by differences in the years, in the same varieties, in the number of seeds per spike, the weight of seeds per spike and1000 kernel weight.

Comparison of the analysis of the spike productivity elements and yield data as a whole showed that the lowest value was obtained in 2010, when the flowering-fertilization stage was accompanied by heavy rains that lasted almost to the beginning of the wax ripeness, which were followed by a sharp increase in the temperature, and led to mechanical maturity of varieties.

The maximum productivity of spike and yield of the studied variety samples was observed in 2012, when the weather favored the development of winter crops, the flowering-fertilization and grain filling stages lasted in mildly hot weather and good solar illumination.

It should be noted that the adaptability of varieties was judged by the difference in the productivity of the spike, by 1000 kernel weight and the yields of the varieties under study by year.

Thus, at the variety sample TT 09214/3 -1-2 lutescens in 2010, the number of grains per spike, the weight of grains per spike, 1000 kernel weight and yield were 42.9; 1.6 g; 30.0 g. and 38.9 cwt/ha respectively. Comparison of these data with the data obtained in 2012 showed that the number of grains per spike is -85.6% of the maximum value of this feature, the weight of grains per spikeis - 72.7%, and the 1000 kernel weight is -75.5% respectively. A big difference (42.6%) over the years is observed for the yield of the variety, which is 57.4% of the maximum value. In absolute terms,

it is expressed in 18.9 cwt/ha.

Similar results were observed in 75.7% of the varieties, which are classified as non-adapted varieties. Nevertheless, 24.3% of prospective varieties, out of 51, during the study years retained a neutral response to changes in meteorological conditions or less subjected to changes. TT 0887/2-1-1-1 lutescens, TT 09706/2-4 lutescens, TT 09704/2-4-1 lutescens, TT 09704/5-2 erythrospermum, TT 09704/2-4-1 lutescens, TT 09704/2-4-1-1 albidum, TT 09224/3-2-1 lutescens, etc. belonged to such variety samples, including TT 09704/2-4-1 lutescens named "Shafag 2" (in 2010 year) and TT 09706/2-4 lutescens named "Parvin" (in 2012 year) were transferred to the State Service for Registration of Plant Varieties and Seed Control under the Ministry of Agriculture of the Republic of Azerbaijan.

In varieties with a comparatively greater adaptive capacity, the average yield reached up to 70.0 cwt/ha (Fig. 1, 2).

As can be seen in Fig. 1, in the adaptive varieties, the curve of the average yield is approximately in a level position in the interval of yield curves of varieties for individual years. In varieties, of which the yield varies considerably over the years, the curve of the average crop yields as close as possible to the yield curves of the last two years, where the weather conditions were relatively stable and similar (Fig. 2).

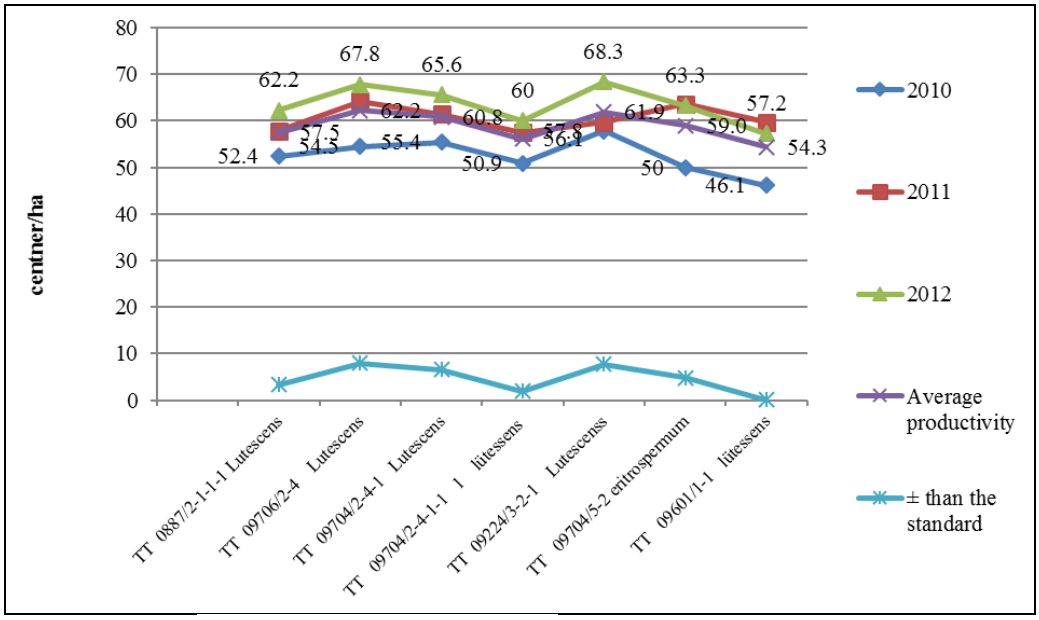

Fig. 1. The yield of comparatively adaptive varieties, cwt/ha.

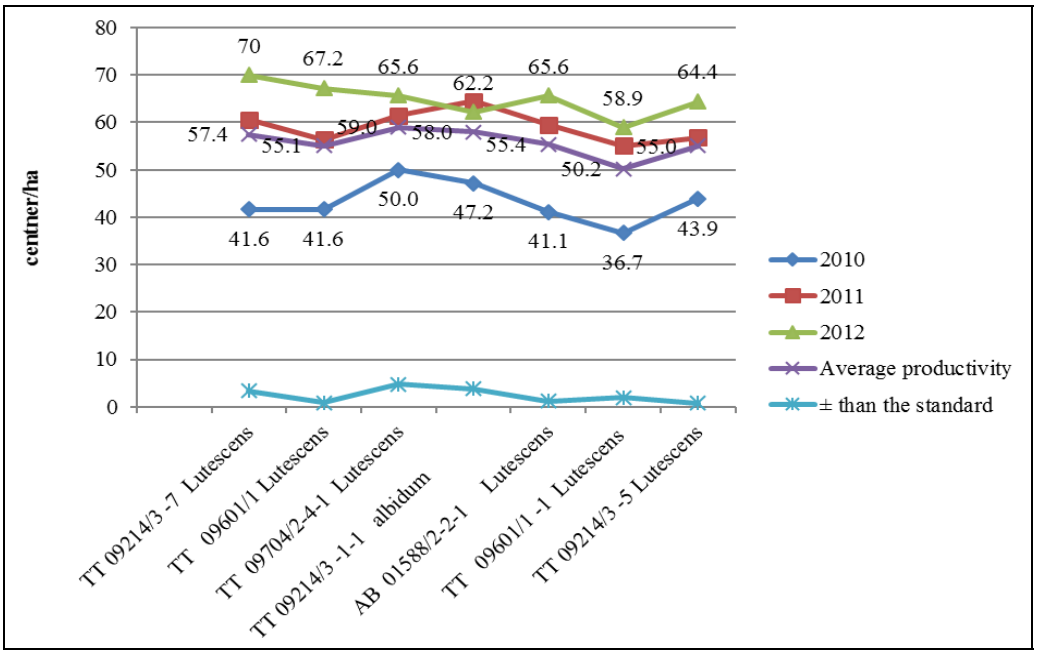

Fig. 2. Productivity of non-adaptive varieties, cwt/ha

It should be noted that to the number of varieties with an adaptive value were both high-yielding varieties, and varieties with an average value of this feature. In some cases, less productive varieties proved to be more stable to changes of the environment than high-yielding ones.

Of no less importance has the definition of the adaptive values of promising varieties for the quality of grain, of which is already impossible to consider the completion of breeding works. It is known that grain quality indicators are genetically determined, but highly dependent on growing conditions, levels of agricultural technology, fertilization and etc. (Dias and Lidon, 2010).

According to the results, the gluten content of adaptive varieties excluding TT 09704/2-4-1 lutescens and TT 09704/5-2 erythrospermum slightly changed by year and the average value of gluten indicators was between the mean value of this characteristic by years (Fig. 3).

Similar results were obtained with respect to the gluten quality index (GQI) of adaptive varieties, which indicates independence from environmental conditions over the years of cultivation (Fig. 4).

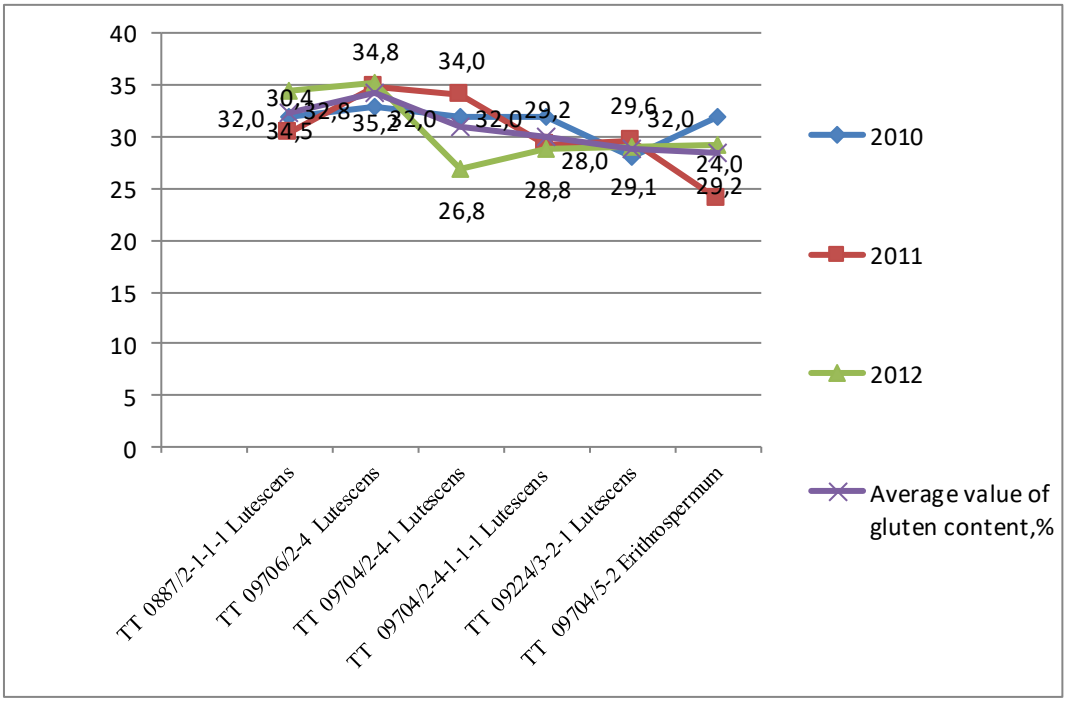

Fig. 3. Gluten content of adaptive varieties, %.

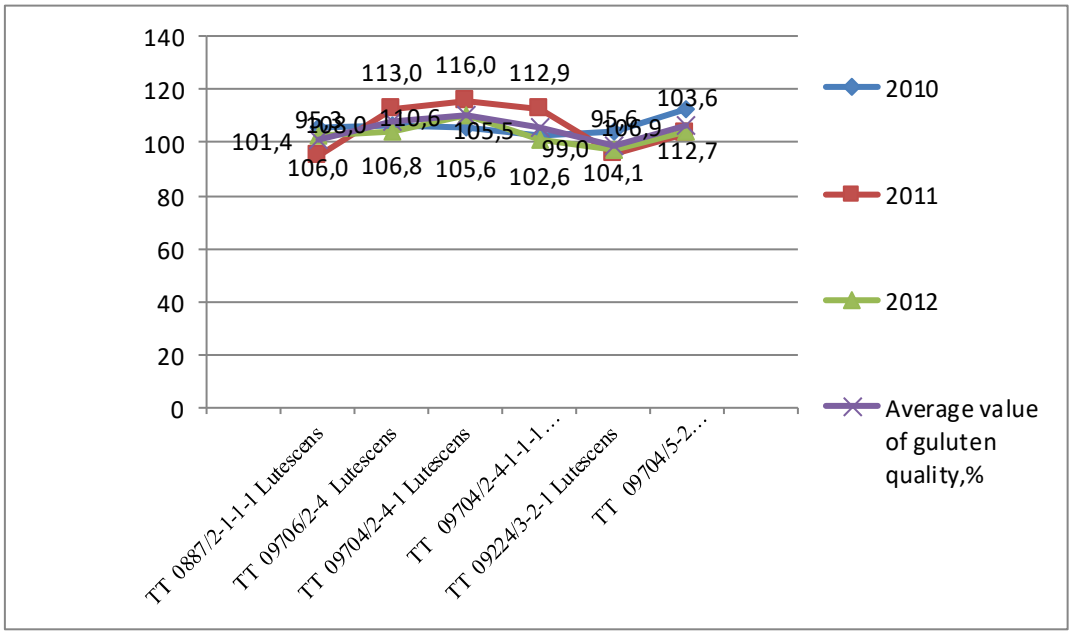

Fig. 4. Indicators of GQI of adaptive varieties

As can be seen in Fig. 5, the improvement in growing conditions over the years has positively affected the sedimentation indices of only one line, TT 0887/2-1-1-1 lutescens. So, in 2010, sedimentation rates for this line was 22.5 ml., and, in 2011-2012 this indicator increased to 34.5 ml. The exception was also the line TT 09706/2-4 lutescens, when the value of the sedimentation (35.2 ml.) only in 2012 was very different from the previous ones (22.5 ml).

Thus, the study of both groups (adaptive and non-adaptive) varieties and lines did not reveal a significant difference in the years between groups in terms of the quality of grain, as can be seen in the gluten content (Fig.6). The only significant deviation in the gluten content by year that was observed in 2012 at the TT 09214/3-1-1 albidum line seems to be due to either a successful combination of the passage of the development stage during the growing season with the weather condition prevailing in the given year or own responsiveness of the genotype to change the weather conditions, or is the result of an accidental technical error that requires additional verification.

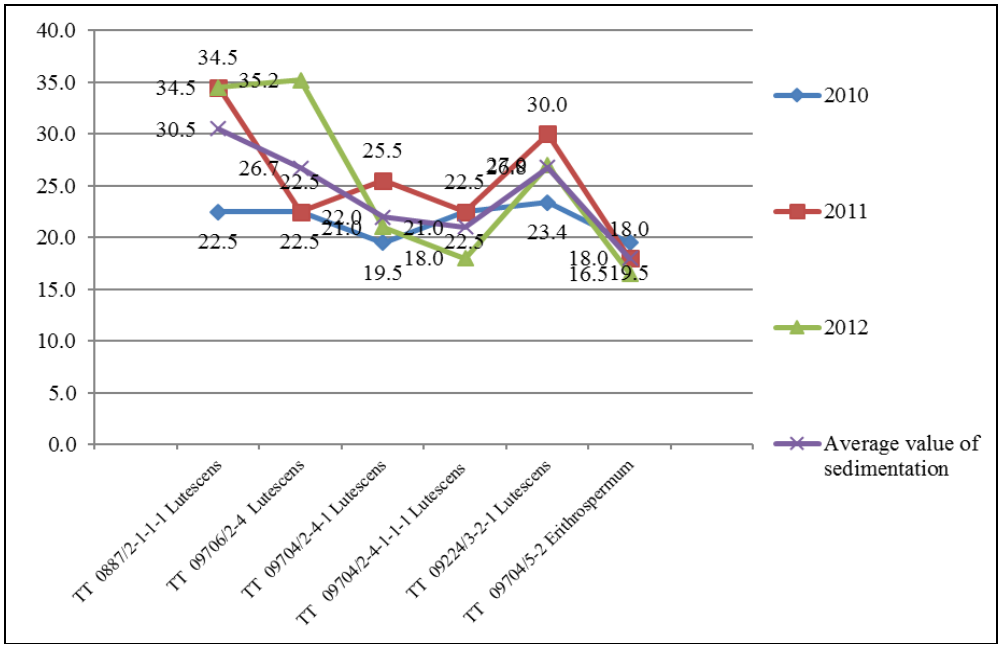

Fig. 5. Sedimentation indicators of adaptive varieties, ml.

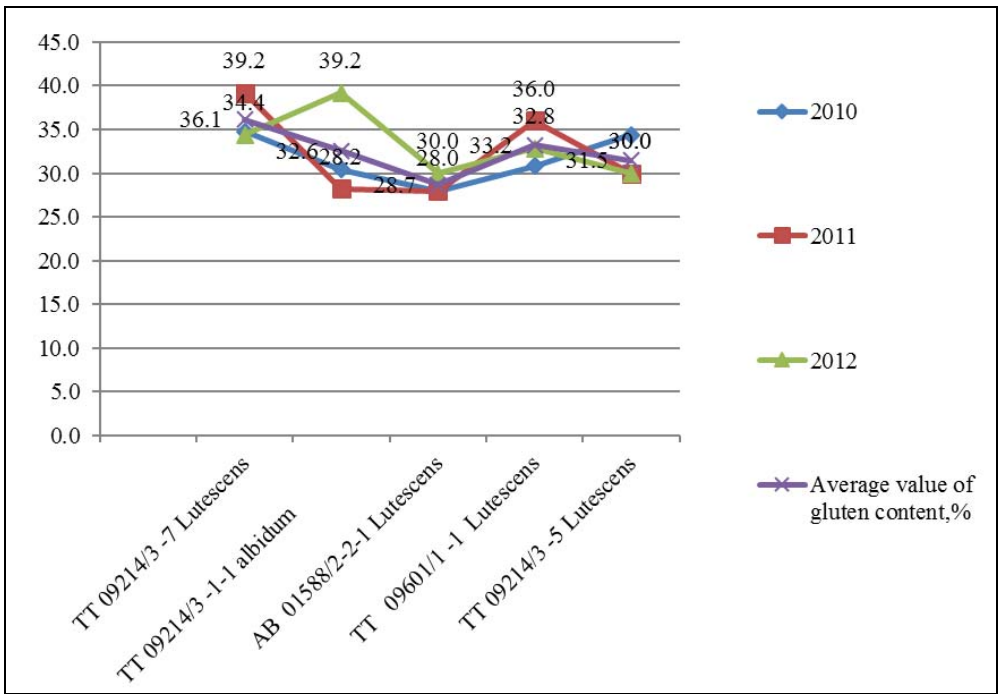

Fig. 6. Gluten content of non-adaptive varieties, %.

The study revealed that a differentiated approach to the selection of varieties for cultivation, especially in regions with an unstable climate over the years is very important. This approach helps eliminate the risk associated with the negative impact of weather conditions.

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Payızlıq Yumşaq Buğdanın Perspektiv Xətlərinin Adaptivliyinin Düzən Qarabağ Şəraitində Qiymətləndirilməsi

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Tədqiqat payızlıq buğdanın adaptiv perspektiv xətlərinin müəyyənləşdirilməsi məqsədi ilə aparılmışdır. 21 valideyn forması arasında növdaxili çarpazlaşdırılmadan əldə edilən yumşaq buğda sortnümunələrində adaptivlik tədqiq edilmişdir. Düzən Qarabağ şəraitində yumşaq buğdanın müxtəlif persüpektiv sortnümunələrində çoxillik müşahidələr göstərir ki, heç də sortnümunələrin hamısı becərildiyi eyni şəraitdə eyni dərəcədə özlərinin məhsuldarlıq potensialını realizə edə bilmir. Bununla belə, illər üzrə az sayda sortnümunələrözünün məhsuldarlığını qoruyub saxlaya bilmişlər.İllər üzrə məhsuldarlığın qeyri-sabitliyi tədqiq edilmiş sortların əksəriyyətinin kifayət qədər yüksək adaptivliyinin olmadığını göstərir. Bəzi perspektivsortnümunələr müxtəlif illərin becərmə şəraitinə neytral reaksiyaları ilə fərqlənmişlər.

Açar sözlər: *Növdaxili çarpazlaşdırılma, adaptasiya, perspektiv xəttlər, sortnümunələr, yumşaq buğda, məhsuldarlıq, seçmə*

Оценка Адаптивности Перспективных Линий Озимой Мягкой Пшеницы в Условиях Равнинного Карабаха

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Работа проведена с целью выявления степени адаптивности перспективных линий озимой пшеницы. Изучена адаптивность сортообразцов мягкой пшеницы, полученных от внутривидового скрещивания родительский форм. Многолетние наблюдения показали, что не все перспективные сортообразцы мягкой пшеницы могут в равной степени реализовать свою потенциальную урожайность в условиях равнинного Карабаха. Вместе с тем, небольшое число сортообразцов сохранило свою урожайность. Исследования по выявлению причин нестабильности урожая в зависимости от года выращивания указывают на отсутствие достаточной адаптивности у большинства сортов. Некоторые перспективные сортообразцы отличались нейтральной реакцией на изменяющиеся по годам условия культивирования.

***Ключевые слова**: Внутривидовое скрещивание, адаптация, перспективные линии, сортообразцы, мягкая пшеница, урожайность, отбор*

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Assessment of Biodıversıty and Ecosystem Services in Azerbaijan: Challenges and Experıences

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The aim of this paper is to establish a brief overview on the status of biodiversity and ecosystem services in Azerbaijan in the context of international science-policy interface within modern global concepts. These issues are discussed using the results of the analysis of state or public reports and scientific literature on biodiversity in Azerbaijan, as well as the outcomes of own studies the drivers of change and trends in biodiversity within the framework of the strategy and policy for biodiversity conservation. The existing knowledge gaps and needs for filling them have been also identified.

Keywords: *Biodiversity, ecosystem services, science-policy interface, conservation strategy, Azerbaijan*

The conservation and sustainable use of natural resources is one of the sources for social, political, and economic challenges of the 21st century. In the context of the rapid degradation of nature, it is still important to assess drivers, impacts and responses to biodiversity that is considered as an integral part of natural resources. In order to a clear understanding of these issues in Azerbaijan we here propose a brief insight into biodiversity in the context of ecosystem services, and the main assessments on biodiversity.

Biodiversity is the living fabric of our planet that includes variability within species, between species (genetic diversity) and of ecosystems. It may underpins some essential services to humanity, from material goods (for example, food, timber, medicines, and fiber) to underpinning functions (flood control, climate regulation, and nutrient cycling), and nonmaterial benefits such as recreation (MA, 2005). Biodiversity can also contribute to agriculture through pollination and pest control (Hooper et al., 2005), provide carbon storage and sequestration (MA, 2005), and positively affect human physical and mental health (Barton and Pretty 2010). The economic value of benefits from biodiverse natural ecosystems may be 10 to 100 times the cost of maintaining them (TEEB, 2009).

Threats to biodiversity. However, many of these benefits, known as "ecosystem services," are in serious threat because of the increased effect of biotic factors, anthropogenic pressure and natural disasters. Over-exploitation of natural resources, expanding land-use change invasive alien species, pollution, climate change, especially degradation, and fragmentation of habitats are all key pressures affecting biodiversity loss around the world (MA 2005; Butchart et al., 2010; IPBES 2018; Ali-zade and Salimov, 2015).

These impacts shows a continued, dramatic overall decline richness in global and local biodiversity, resulting in unprecedented losses in biodiversity at all levels, from genes and species to entire ecosystems with including ecosystem processes and functions. Due to the most recent assessments of some taxonomic groups, 11% of the world's of legumes are threatened with extinction (Brummitt et al., 2015), as are 41% of amphibians (IUCN), 15% of reptiles (Böhm et al., 2013), 14% of birds (IUCN), 25% of mammals (IUCN), 16% of pteridophytes (Brummitt et al., 2015), 18% of monocots (Brummitt et al., 2015), 40% of gymnosperms (Brummitt et al., 2015), and 1616 63% of cycads. If trends will continue at such a rate, the worldwide and local loss of biodiversity and degradation of ecosystems will cause a mass extinction event, and also reducing the services they can provide (Fig. 1).

Biodiversity and ecosystem services decline not only an irreversible loss of the Mother Earth, but also flag other important issues, such as the threat to future food security and good quality of life that nature provides represent everything from the food we eat to the air we breath (Diaz et al., 2015; Cardinale et al., 2012; Hooper et al., 2012).

Therefore decision makers need to be sufficiently and clearly informed on how biodiversity underpins these services, the demand for them, the capacity of ecosystems to provide them and the pressures reducing that capacity. This implies the existence of a scientifically based programs assessing the biodiversity and ecosystem services considering them as a part of an interconnected system.

International Science-Policy interface for conservation of biodiversity and ecosystem services. As a result of wide political recognition regarding the imperative to reduce anthropogenic

environmental impacts, and other drivers influence on biodiversity, the United Nations Convention on Biological Diversity (CBD) were adopted in 1992.

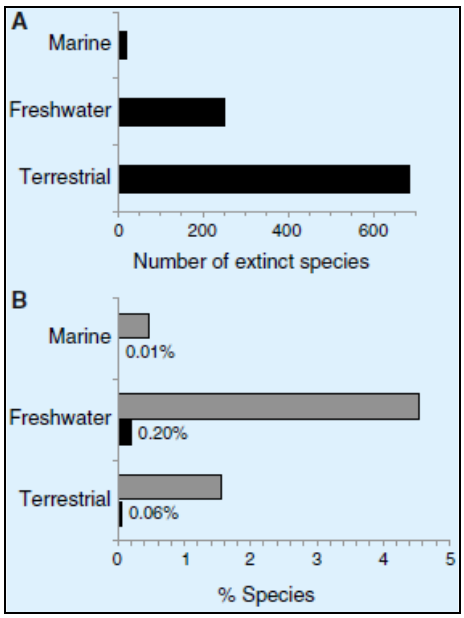

Fig. 1. Extinctions. (A) The number of extinct species amongst the 226,000 marine, 126,000 freshwater and 1,150,000 terrestrial named species. (B) The percentage of species in these environments that are extinct (black bars) and threatened (hollow bars). Source: Costello (2015).

There is an increasing array of national, regional, and international policy mechanisms aimed at biodiversity conservation; for example, 87% of the signatories to the CBD have now developed National Biodiversity Strategies and Action Plans (NBSAPs). Thus, NBSAPs are the principal instruments for implementing the Convention at the national level (Article 6) and have frameworks for tackling biodiversity loss at local scales (CBD, 1995; Butchart et al., 2010).

The first comprehensive large-scale international biodiversity assessment was the Global Biodiversity Assessment (GBA 1995) which for the first time, mobilize teams of experts involving some 300 authors from over 50 countries, and covering many different disciplines in the biological, economic and social sciences to evaluate the global status of biodiversity. GBA had almost no impact on policy formulation, because it did not have a mechanism for involving many (multiple) stakeholders, including decision-makers and was not an intergovernmental process (Watson and Gitay, 2007).

Millennium Ecosystem Assessment (MA, 2005) was another major, one-time global biodiversity assessment designed to respond to the scientific needs of the biodiversity-related conventions. It was carried during 2001-2005 and assesses the status and trends in biodiversity, ecosystems and their services, possible future scenarios, options for action. The MA has had little impact on policy formulation and decision-making because their findings were formally approved by their Board and not Governments.

The most comprehensive assessment on biodiversity and ecosystem services to date is the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) that was launched in 2012. The IPBES is an independent intergovernmental body which serve as an assessment mechanism by synthesizing global, sub-regional and regional assessments, as well as assessments on specific thematic and methodological issues such as pollination, scenarios and models, land degradation and restoration, values, invasive alien species, and sustainable use of wild species. The IPBES provides policymakers with objective scientific knowledge foundations for better policy through science for the conservation and sustainable use of the planet's biodiversity, ecosystems and their services, long-term human well-being and sustainable development. To guide the assessment process IPBES has developed and applied a Conceptual Framework (CF) that is a highly simplified model of the complex interactions between the nature and human societies (Díaz et al., 2015; Fig. 2). The IPBES CF for biodiversity and ecosystems services provide an integrated view of the biodiversity knowledge–policy interface with the consideration of diverse disciplines in the sciences and humanities (natural, social, engineering, health sciences, history), as well as broad range of stakeholders (the scientific community, governments, international organizations, indigenous and local communities), and their different knowledge systems (sciences and humanities, indigenous, local and practitioners' knowledge). The IPBES CF provides structure and comparability to the assessments at different spatial scales, on different themes, and in different regions.

The IPBES scientifically credible and policy-relevant assessments do not generate new data, but seek to create new understandings of the causes of the loss of nature and nature's contributions to people through synthesis and sorting of academic literature, as well as insights from indigenous and local knowledge using different methods. The broad range of stakeholders – contributors (scientists, research and educational institutions, indigenous, local and practitioners' knowledge holders), and end users (governments, multilateral environmental agreements, UN agencies, inter-governmental organizations, non-governmental organizations (NGOs), other practitioners within the private sector and the public) were involved in these assessments.

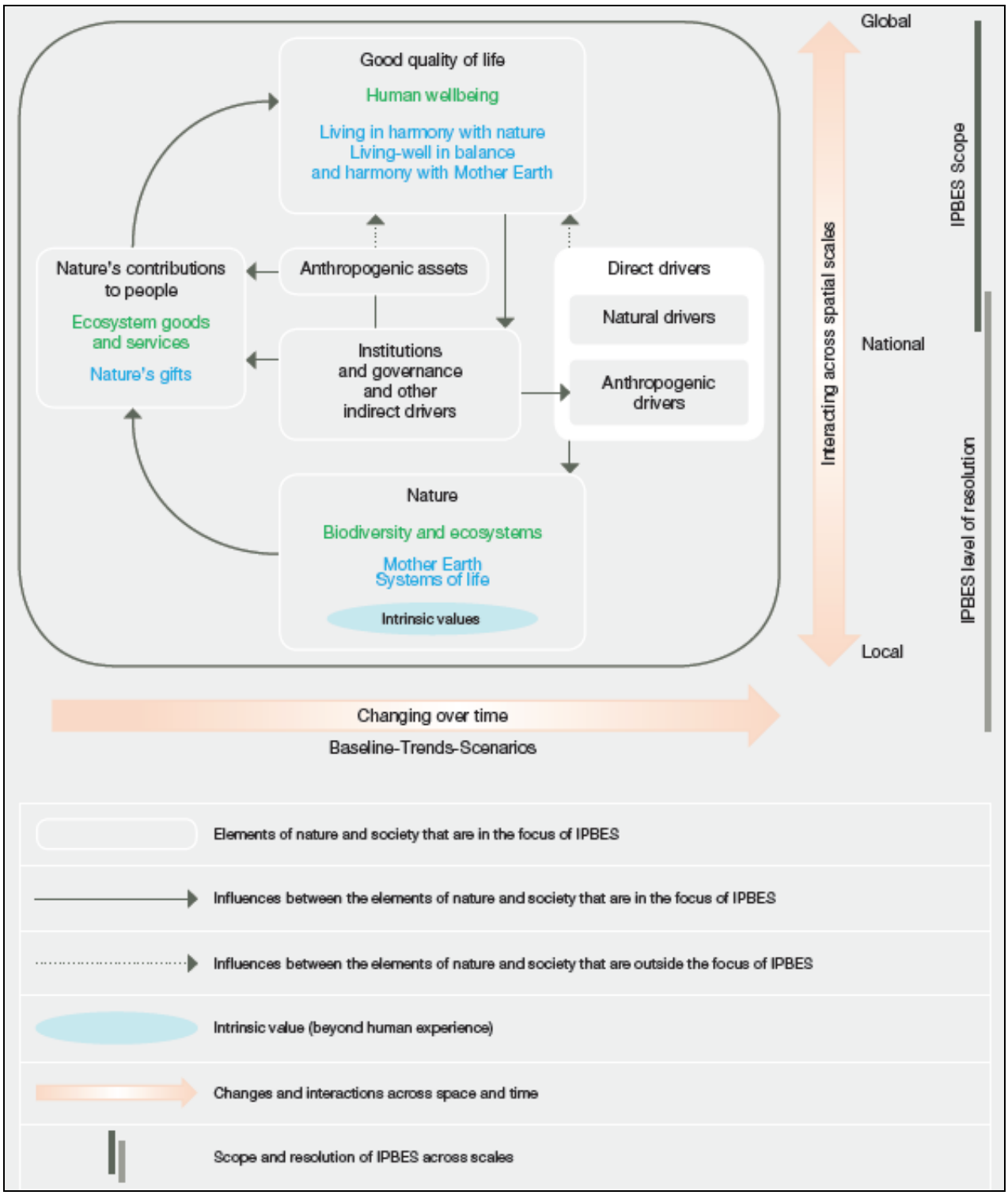

Fig. 2. The IPBES Conceptual Framework (CF) (Texts: in **black** – inclusive category labels intelligible for all stakeholders; in **green** – category labels in western science; in **blue** – category labels of other knowledge systems). Source: Díaz *et al*. (2015).

For example, the IPBES Regional Assessment for Europe and Central Asia (IPBES 2018) is based on a request from Governments, multilateral environmental agreements and other stakeholders to investigate the following key policy questions:

– How do biodiversity and ecosystem functions and services contribute to the economy, livelihoods, food security, and good quality of life in the regions, and what are the interdependences among them?

– What are the status, trends and potential future dynamics of biodiversity, ecosystem functions and ecosystem services?

– What are the pressures driving the change in the

status and trends of biodiversity, ecosystem functions, ecosystem services and good quality of life?
- What are the actual and potential impacts of various policies and interventions on the contribution of biodiversity, ecosystem functions and ecosystem services to the sustainability of the economy, livelihoods, food security and good quality of life?
- What gaps in knowledge need to be addressed in order to better understand and assess drivers, impacts and responses of biodiversity, ecosystem functions and services?
- How can ecosystems those underpinning ecosystem-based adaptation to climate change and nature-based solutions to sustainable development, be protected through investments, regulations and management regimes for terrestrial, freshwater, coastal and marine systems?

Answering the specific key questions offers important knowledge concerning progress toward the Aichi Biodiversity Targets, the Sustainable Development Goals, and national policies (IPBES, 2018).

The two flagship global reports - Global Biodiversity Outlook (GBO) series and the Global Environment Outlook (GEO) series are the periodic publications of the Convention on Biological Diversity and UN Environment Programme (Urho 2009), respectively. GBO is a periodic report that summarizes the latest data on the status and trends of key drivers for biodiversity loss, and effectiveness of implementation measures being taken by the global community for conservation and sustainable use of biodiversity, and also draws conclusions relevant to the further implementation of the Convention. The first report was published in 2001. The upcoming fifth edition, GBO-5, will be launched in 2020. It is important for the post-2020 global biodiversity framework and reporting on a target-by-target analysis of progress towards the achievement of the Strategic Plan for Biodiversity 2011-2020 and its Aichi Biodiversity Targets, and their contribution to the Sustainable Development Goals; provide data on the thematic, regional and global assessments of the IPBES and any relevant scenario analysis and modelling of biodiversity and ecosystem services undertaken as part of these assessments.

GEO was initiated at the request of the UNEP Governing Council in 1995 to create scientific information on the state of the world's environment and aims to facilitate the interaction between science and policy via informing not only governments but also various stakeholders such as the youth, businesses and etc. It provides an independent integrated assessment of the social, economic and environmental trends, the effectiveness of the policy response to address these environmental challenges and the possible pathways to be achieve various internationally agreed environmental goals over the past two decades. The first publication was in 1997.

In this article we discussed the scope and achievements on assessment of biodiversity in the context of ecosystem services, using the result of the analysis of state or public reports and scientific literature on biodiversity in Azerbaijan, as well as the outcomes of own studies. We draw on a broad range of these efforts through outline the key challenges, experiences and perspectives in Azerbaijan.

STATUS AND TRENDS OF BIODIVERSITY CONSERVATION IN AZERBAIJAN

Along with the diverse geographic, biological and climatic conditions in Azerbaijan has provided the higher levels of biological diversity in its flora and fauna over time. Biodiversity of Azerbaijan with the rich natural resources is the source of our present and our future, and provides valuable material (e.g. food, medicinal resources), regulating (e.g. pollination, air and freshwater quality regulation) and non-material contributions to people (e.g. learning and inspiration). It supports sustainable development which is essential for the quality of life as they have economic, social and cultural values (IPBES, 2018).

The issues on conservation and sustainable use of biodiversity are reflected in the government's main strategic documents. As a parties to the United Nations Convention on Biological Diversity (CBD, 1992), Azerbaijan ratified the Convention in 2000, and formally recognised the provisions and principles of the Convention and therefore its national legal frameworks comply with the requirements of the convention.

Strategy and policy framework. Since gaining independence, the Nationwide Leader Heydar Aliyev developed. The principles of the Azerbaijan's national policy in the field of environmental protection and ecology. On the initiative and under leadership of the academician Jalal Aliyev developed the National Program on genetic resources of biodiversity, and to fulfill commitments during the research on biodiversity and conservation, which was the most important requirement of time. At present, Mr. President Ilham Aliyev attaches great importance to the protection and rational use of biodiversity in our country (Ali-zade and Salimov, 2015).

Environmental protection and use of natural resources in the country are based upon the principles declared in the Constitution of the Azerbaijan Republic (1995).

Issues related to environment policy for protection and sustainable use of natural resources in Azerbaijan has been also reflected in other legislative documents (Government of Azerbaijan 2014) such as:

- *NATIONAL STRATEGY of the Republic of Azerbaijan on Conservation and Sustainable Use of Biodiversity for 2017-2020 (2016);*
- *The State Programme for Poverty Reduction and Sustainable Development in the Azerbaijan Republic* (SPPRSD, 2008-2015);
- *The State Programme for the Socio-economic Development of the Regions of the Azerbaijan Republic* (2009-2013);
- *The State Strategy on Use of Alternative and Renewable Energy Sources* (2012-2020);
- *The State Program on the reliable food supply of population in the Azerbaijan Republic* (2008-2015);
- *The National Program on forest restoration and expansion*;
- *National Program "On Environmentally sustainable social and economic development (2003);*
- *State Program on Efficient Use of Summer Winter Pastures, Hayfields and Prevention of Desertification in the Republic of Azerbaijan (2004);*
- *Comprehensive action plan on improvement of the environmental situation for 2006-2010 in the Republic of Azerbaijan*

On October 3, 2016, the President of the Republic of Azerbaijan endorsed an updated National Biodiversity Strategy and Action Plan (NBSAP) for the 2017-2020, including national biodiversity targets, with the global framework being taken into account. The document formulates a comprehensive policy and defines national priorities in order to "*sustainable use of genetic resources; conservation of biodiversity and transfer to future generation; improving biodiversity monitoring systems; promotion of environmental education including biological diversity and ecosystem services; ensuring transition to a "green economy"; reducing the negative impacts and threats to biodiversity, and strengthening institutional capacities in the planning, management and use of biodiversity*".

Beside the abovementioned documents, it is necessary to add that the National Caspian Action Plan (2002) and the Framework Convention for the Protection of the Marine Environment of the Caspian Sea (2006) are key instructions and policies to maintain legislative environment focused on conservation actions in Azerbaijan's territorial waters of the Caspian Sea. The recently adopted the Convention on the legal status of the Caspian Sea (Aktau, 2018) aims strengthening governmental capacities on rational management of Caspian Sea resources, as well as exploration, protection and conservation of its environment.

Drivers of change in biodiversity. Globally across all regions, as well as in Azerbaijan, biodiversity loss and natural habitat decline directly negatively impacts on the nature's contributions to people and good quality of life, respectively (Government of Azerbaijan, 2014). The main drivers and pressures on ongoing biodiversity loss and ecosystem decline in Azerbaijan are:

- land degradation;
- habitat fragmentation;
- unsustainable levels of natural resource use;
- pollution;
- invasive alien species;
- climate change.

Trends in biodiversity. The Greater and Lesser Caucasus regions and Talysh Mountains are distinguished with the highest level of the flora and fauna diversity. In Azerbaijan, nearly 5,000 plant species occur, representing around 65 % of the Caucasus' plant diversity. Of these approximately 200 species are national endemics and 950 species Caucasian endemics (Solomon et al., 2013). There are 107 mammals, over 394 of birds, 154 reptiles, around 10 of amphibians and 102 species of fishes, and approximately 25,000 species of invertebrates in Azerbaijan.

At present, because of the lack of regular monitoring and limited data, there is scarce information available on dynamics of populations of species listed in the Red Book of Azerbaijan (RDB 2013). Currently 213 (80%) plant species are evaluated as threatened (25 critically endangered, 53 endangered and 135 vulnerable). Another 52 plant species are listed as near threatened About 26 species of the evaluated fungi and lichens (96%) are threatened (Fig. 3).

36 vertebrate (25%) and 15 invertebrate (60%) species are listed as threatened, respectively. There is a remarkably high percentage of insect species with unknown population trend, and considered as not evaluated (about 67% of invertebrates) (Fig. 3).

Based on the surveys conducted, these high percentage indicate a significant increase in the number of threatened species which were listed in the first edition of Red Book of Azerbaijan (1989), and require under various projects to develop a regular and extensive research and monitoring capacity of the various institutions of the Azerbaijan National Academy of Sciences.

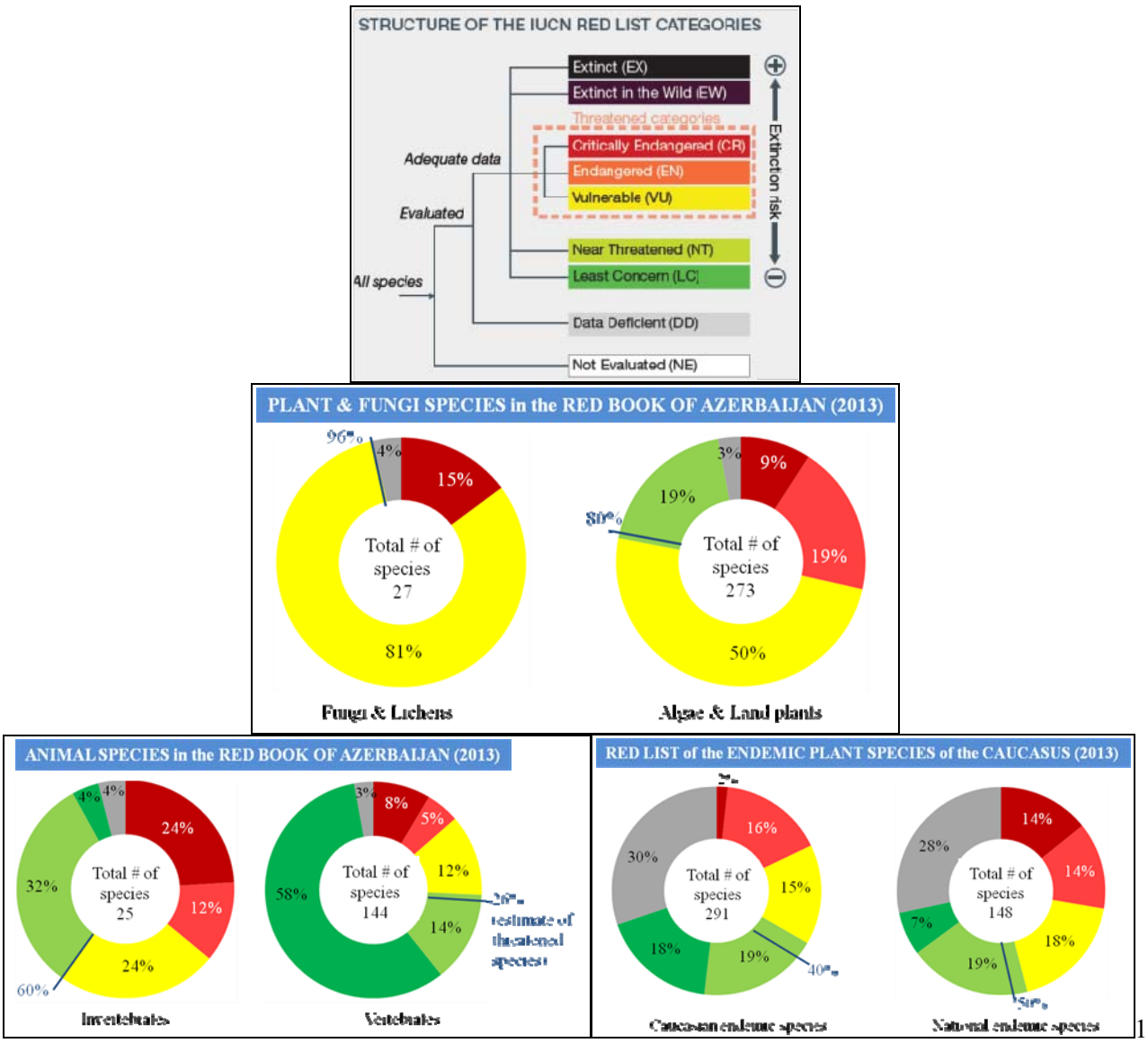

Fig. 3. Extinction risk of species in Azerbaijan according to IUCN Red List Categories.
The blue bar is the best estimate of the proportion of threatened and extinct species, assuming that the same proportion of DD species is threatened or extinct as of species with sufficient data (i.e., EX, CR, EN, VU, NT, LC). Only species in comprehensively assessed taxonomic groups are considered. Source: Red Book of Azerbaijan (RDB 2013) and Red List of the Endemic Plants of the Caucasus (Eds. Solomon et al. 2013).

The plants and animals of the various ecosystems provide people with many resources necessary for their daily lives and with economic values as well. Products of a number of wild and cultivated plants are widely used as a food source such as wild fruits, nuts, berries, mushrooms, and edible greens, tubers, and other plant products that are seen in markets. Some of them used as timber materials, medicinal plants and etc. A 1547 medicinal plant species which belonging to 740 genera and 78 families, were reported as diuretic (444 species), antibacterial (362), anti-inflammatory (249) and having other healing effects.

The natural pastures of Azerbaijan also provide fodder for domestic sheep, goats and cattle. Due to for their caviar, sturgeon fishes have a high economic value in Azerbaijan. Therefore overfishing is still the main threat to Caspian fishes. Besides overfishing of freshwater fish species, a main threat for them is the destruction or modification of their habitat, including a change in the river continuum with the construction of dams and weirs that fragment populations.

Migratory and resident birds, particularly waterfowl, are widely and illegally hunted throughout the country, providing food for villagers and also seen for sale along roads through some districts. Forest animals like wild boar, Caucasian tur and smaller game are also hunted for meat by locals (Foster-Turley and Sultanov, 2010).

Land-use change, including agricultural intensification and urbanization has a multi-scale impact on biodiversity and ecosystem services in both directions. In despite of there are examples of

sustainable agriculture policies and practices (e.g. organic farming) in recent years, land conversion and land-use change due to industrial, infrastructural and other forms of economic development poses a particular threat to loss of (semi-) natural habitats, including valued wildlife species of flora and fauna (IPBES, 2018).

Land area in Azerbaijan was reported at 82663 km^2 (95.45 % of total area) in 2016, according to the World Bank collection of development indicators, compiled from officially recognized sources (Fig. 4).

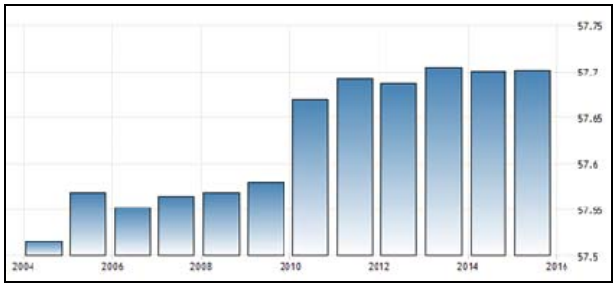

Fig. 4. Land area in Azerbaijan (% of land area). Source: World Development Indicators database.

47698 km^2 or 57.7% of land area is classified as agricultural land which is divided into arable land, permanent crops land, and permanent pastures and meadows. According to estimates, agricultural land use has expanded between 2004 and 2016. These trends are consistent across the agricultural land types (Table 1).

Table 1. Trends in agricultural land use types in Azerbaijan (2004-2016).

Agricultural land type	Profile	2004-2016
Arable land	*land cultivated for crops like wheat, maize, and rice that are replanted after each harvest*	↗
Permanent cropland	*land cultivated for crops like citrus, that are not replanted after each harvest, and includes land under flowering shrubs, fruit trees, nut trees, and vines*	↗
Permanent pastures and meadows	*land used for at least five years or more for forage, including natural and cultivated crops*	↗

Source: World Development Indicators database. ↗ indicate moderate and consistent increase in indicator.

According to the World Bank collection of development indicators, since the 1990s, the forest area in Azerbaijan are expanded up to 11394 km^2, which means it comprises 13.78% of land area of country (Fig. 5).

Intensive plantation forestry, including planting of greenery urban areas using introduced exotic plant species can be give rise to undesirable, and hazardous effects to loss of habitat and associated species turnover.

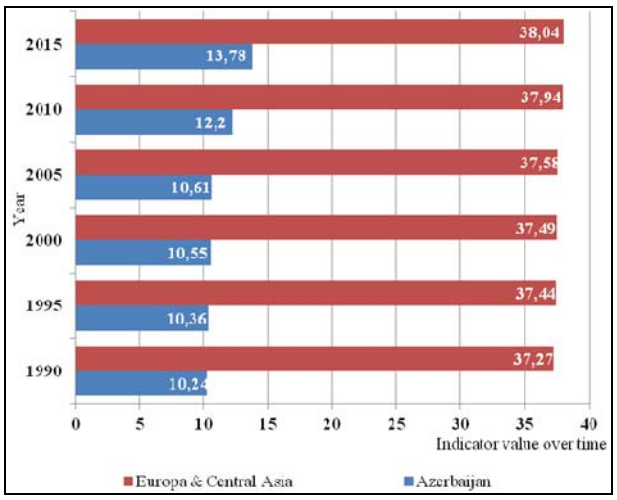

Fig. 5. Forest area in Azerbaijan (% of land area). Source: World Development Indicators database.

One key indicator for the land use change is the habitat fragmentation and land degradation, including extensive salinization, widespread soil erosions, large-scale use of fertilizers and pesticides, as well as socio-economic drivers including the increasing demand for living space per person, and increased mobility and growth of transport infrastructure. Hence, increasing intensity of salinization and erosion of soils tend to be result decline of the productive soil layer with its water regime that lead to desertification and deforestation.

In Azerbaijan ~42.5 % (3.6 million ha) of total areas are eroded, and ~7% (0.3 million ha) of total areas are salinized, as a result of which the widespread degradation of agricultural land is expected (Government of Azerbaijan 2014).

In recent decades there is a trend to expand the protected areas in conservation of nature, including biodiversity and nature's contribution to people. Protected nature areas for the end of 2017 cover 10,3 % of the country territory, where there are 11 state natural reserves, 9 national parks and 24 state natural sanctuaries (Fig. 6).

But it is significant fact that protected areas alone cannot prevent biodiversity loss, particularly for migratory species or habitats or species particularly sensitive to environmental change (Mora & Sale, 2011; Strayer & Dudgeon, 2010). A global systematic review shows that individual protected areas were effective at protecting habitats, particularly forests, but less effective at conserving populations of species (Geldmann et al., 2013).

Agriculture is a highly climate sensitive sector, and therefore, Azerbaijan's rural population and their livelihoods are vulnerable to climate change. The impacts of climate change may have severe consequences for the nature, including biodiversity and nature's contribution to people. The vulnerabilities (high risk of natural disasters, severity of impacts from anthropogenic activities)

and the effects of the changing climate functionally affecting ecosystems and eventually causing decline in biodiversity and ecosystem services.

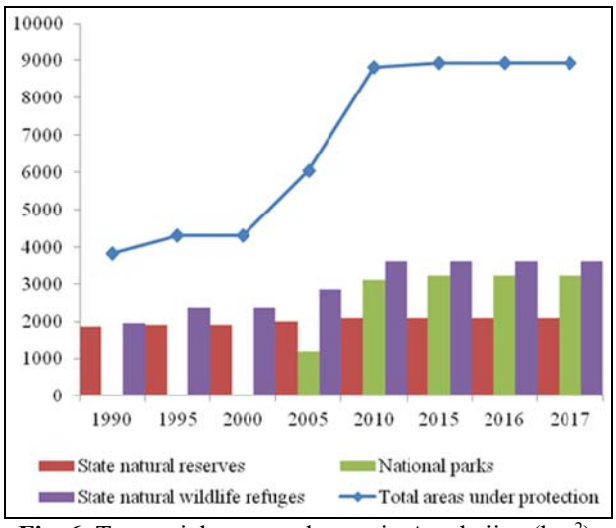

Fig. 6. Terrestrial protected areas in Azerbaijan (km^2). Source: Database of the State Statistical Committee of the Azerbaijan Republic.

It is important to evaluate how climate has varied and changed in the past. The monthly mean historical rainfall and temperature data can be mapped to show the baseline climate and seasonality by month, for specific years, and for rainfall and temperature. As a result of the analysis of possible climate changes in Azerbaijan the temperatures are increasing (Fig. 7). The chart below shows mean historical monthly temperature and rainfall for Azerbaijan during the time period 1961-1990 and 1991-2015.

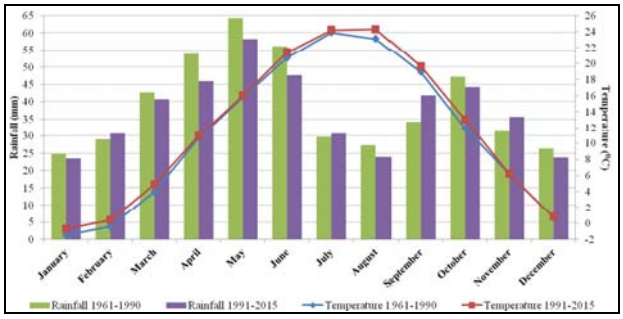

Fig. 7. Average Monthly Temperature and Rainfall for Azerbaijan from 1961-1990 and 1991-2015. Source: World Development Indicators database.

Studies suggested that the temperatures are expected to continue increasing, and while the trends and scenarios for average precipitation are more varied, they are tending to decrease (Table 2). Climate models indicate an average annual increase of 1.5-1.6°C by 2021-2050 and 3-6°C by 2070–2100 across the entire country. Maximum temperatures are also predicted to increase and may reach 47-53°C. There is less certainty about precipitation trends. (Shatberashvili et al., 2015). Extreme weather events in the future may lead to directly impact the overall water balance, and increasing the hazards, and the various risks related to the economy, including the agriculture, human health and safety.

Climate change leads to more extreme and less predictable weather events (heat waves, droughts, floods, heavy precipitation, and wind storms) that impact biodiversity across ecosystems. These trends will cause to shift seasonal timing, growth and productivity, species ranges and habitat location, which affect biodiversity, agriculture, forestry and fisheries. Many species with limited capability to migrate or adapt fast enough for corresponding to projected climate change.

Table 2. Observed climate change and scenarios for Azerbaijan.

	Observed	Scenarios
Temperature	↑	↑
Extreme temperature (+)	↑	↑
Precipitation	↓	↑↓

Source: Second National Communication of Azerbaijan, 2010. (↑- indicates increasing trend, ↓- decreasing trend, ↑↓- mixed trend).

Invasive alien species are among the important direct drivers of loss of biodiversity and nature's contributions to people across Europe and Central Asia as well as in Azerbaijan, especially in combination with other direct drivers (IPBES, 2018).

Invasive alien species generally tend to have negative effects on biodiversity, including displacement and extinction of native species, gene pollution, homogenization of communities, modification of biological interactions, communities, habitats and ecosystem functions, with consequences for human health; and agricultural and economic production (Katsanevakis et al., 2014; Vilà et al., 2010). Some alien species, and even some invasive alien species, have positive impacts, which include provision of the habitat; increasing local species richness and associated ecosystem services, with subsequent economic gains; ecosystem engineering; and aesthetic and cultural value (Schlaepfer et al., 2011). Data limitations across Azerbaijan impede assessment of trends associated with invasive alien species. Priority should be given to improving the evidence-base for impacts of invasive alien species.

Progress towards the 2011-2020 Aichi Biodiversity Targets. The Strategic Plan for Biodiversity 2011-2020, including its 20 Aichi Biodiversity Targets under five Strategic Goals provides a framework for the management and policy development on biodiversity within a framework of United Nation Systems.

There is a lack of well established both

quantitative indicators and qualitative information, as well as absence of a consolidated biodiversity monitoring system suitable for statistical extrapolation to judge progress towards the Strategic Plan for Biodiversity 2011-2020 and its Aichi Biodiversity Targets (Government of Azerbaijan, 2014). Therefore assessment of country report on progress in the implementation of the Aichi Targets relies on more qualitative systematic review of the literature (Table 3).

Table 3. Summary of Azerbaijan's current state of progress towards the Aichi Targets.

Strategic goal A: address the underlying causes of biodiversity loss by mainstreaming biodiversity across government and society	1		↔		
	2		↔		
	3	↕			
	4		↔		
Strategic goal B: reduce the direct pressures on biodiversity and promote sustainable use	5		↔		
	6		↔		
	7		↔		
	8		↔		
	9		↔		
	10			√	
Strategic goal C: to improve the status of biodiversity by safeguarding ecosystems, species and genetic diversity	11		↔		
	12			√	
	13			√	
Strategic goal D: Enhance benefits to all from biodiversity and ecosystem services	14		↔		
	15			√	
	16				
Strategic goal E: Enhance implementation through participatory planing, knowledge management and capacity-building	17			√	
	18		↔		
	19		↔		
	20		↔		

Notes: √ symbols indicate successes and positive trends (~51-75% good progress) in the achievements of targets; ↔ shows mixed and variable (~26-50% fair or reasonable progress) in the achievements of targets; and ↕ indicate trend little or failure (~0-25% limited progress) in the achievements of targets. Grey colors without any symbol indicate lack of evidence (insufficient information to score progress.

Filling knowledge and capacity gaps. The lack of availability of comprehensive and up-to-date biodiversity data, information and knowledge remains a challenge. Therefore, there is need for better understanding, quantification and integrating monitoring system of biodiversity in the context of ecosystem services. A few scientific researches have been conducted on biodiversity focused on how direct and indirect drivers impact, status and trends, and a plausible scenarios. Moreover, systematic and integrated investigations, as well as biodiversity monitoring of fungi, plants, animals, species of Caspian Sea and inland freshwaters, and soil organisms are required to better assess this issue for the whole Azerbaijan. There is also necessary to integration of indigenous and local knowledge systems and scientific knowledge to better understand the diverse values of nature and nature's contributions to people. There is also a significant gap in the institutional cooperation between Ministry of Ecology and Natural Resources and various scientific institutions of ANAS in terms of scientific credible assessment projects on biodiversity and ecosystem services.

During the last decades, the scientific-research works and cooperations carried out at the Institute of Botany within the framework of memorandums, contracts and agreements signed with various international scientific organizations have a significant role for the scientific bias of the conservation, sustainable use and development of plant diversity in Azerbaijan as well as implementation of the relevant tasks set out in various State Programs and Action Plans (Ali-zade and Salimov 2015; 2016).

The Institute of Botany has been actively participated in the work programs of regional assessment of biodiversity and ecosystem services for Europe and Central Asia (2015-2017) and Global Assessment (2016-2018).

Acknowledgment. The authors are thankful to the IPBES Secretariat, for the opportunities to participate in the expert evaluation and the final document of the platform that scientifically credible up-to-date assessment of available knowledge regarding biodiversity and ecosystem services. And also are grateful to the Presidium of ANAS for supporting researches in this direction.

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Azərbaycanda Biomüxtəliflik və Ekosistem Xidmətlərinin Qiymətləndirilməsi: Çağrışlar və Təcrübə

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Məqalədə müasir qlobal konsepsiyalar çərçivəsində beynəlxalq elm-siyasət interfeysi kontekstində Azərbaycanda biomüxtəliflik və ekosistem xidmətlərinin vəziyyəti haqqında qısa məlumat verilmişdir. Hökumət və ya ictimai hesabatların, elmi ədəbiyyatın təhlili nəticəsində, eləcə də müəlliflərin öz tədqiqatlarının nəticələrinə görə, bioloji müxtəlifliyin mühafizəsi strategiyasının və siyasətinin həyata keçirilməsi çərçivəsində biomüxtəlifliyin dəyişməsinə təsid edən hərəkətverici qüvvələr və tendensiyalar müəyyən edilmişdir. Bu sahədə mövcud bilik çatışmazlığı və onların həlli yolları aşkarlanmışdır.

***Açar sözlər**: Biomüxtəliflik, ekosistem xidmətləri, elm-siyasət interfeysi, mühafizə strategiyası, Azərbaycan*

Оценка Биоразнообразия И Экосистемных Услуг в Азербайджане: Вызовы и Опыт

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В статье рассматривается состояние биоразнообразия и экосистемных услуг в Азербайджане в контексте современных глобальных представлений и международного интерфейса науки и политики. На основе анализа государственных документов и научной литературы, а также результатов собственных исследований, определены движущие силы и направления изменения биоразнообразия в рамках реализации стратегии и политики по сохранению биоразнообразия, выявлены возможные пробелы и пути пополнения знаний.

***Ключевые слова**: Биоразнообразие, экосистемные услуги, наука, политика, движущие силы, направления, стратегия сохранения, Азербайджан*

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Biological and Phytosenological Features of the *Orchidaceae* Juss. Family Species New for the Nakhchivan Autonomous Republic Flora

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This article presents systematic, biological and phytosenological characteristics of species *Neotia ovata* (L.) Bluff & Fingerh of the *Neottia* Guett genus, *Orchis simia* Lam. of the *Orchis* L. genus and species *Anacamptis pyramidalis* (L.) L.C.Rich of the *Anacamptis* genus.

Keywords: *Orchidaceae, Orchid, biological features, phytocenosis, oval secret flower, Listera, Neottia, ovata, Orchis Simia Lam, Orchis simia, Anacamptis pyramidalis, pyramidal orchid*

INTRODUCTION

The diversity of plant species in the Nakhchivan Autonomous Republic is primarily related to the diversity of eco-geographical conditions, the historical development of the kaynazoy Pleistocene eras, especially the development history of the biological components of landscape, as well as to its location on the borders of three botanical regions, and having numerous altitudes zones. In a very short distance, it has different natural-geological features, ranging from Arazboyu plains to the Gapijik peak, where the desert, semi-desert, mountain desert, xerophyte bush, subalpine forest, alpine, rocky and glacier landscapes are located (Babayev, 1999).

The research object is located on the west slope of the Zangezur mountains, in the upper and lower parts of the Duylunchay basin. The main part of the area bordered by the peaks and mountains such as Tokhumgedik (3106 m), Khoshkeshin (2081 m), Yokhush (2364 m), Pazbashi (2613 m), Gendagh (1780 m) etc., is located in the cold climate zone, with characteristic dry summer. Optimal radiation, temperature, and humidification here, make favorable vegetation condition for *Orchidaceae* species. Compared to other places of Azerbaijan, the radiation amount of 145 kcal / cm^2 is relatively more than at other relevant altitudes. The absolute maximum temperature is +320°C, the minimum temperature is -190°C and the average annual temperature is +110 C. The average thickness of the snow cover varies from 19 to 24 cm depending on the altitude change. Snow cover prevents the root system of perennial plants and provides enough humidity in the soil. On the active layer, the humidity in the brown soils reaching to 30-35mm ensures normal growth of the plants (Mirzəyev, 1972).

One of the most important plant species in the Nakhchivan Autonomous Republic is *Orchidaceae* Juss. One of the species included into the *Orchidaceae* family is *Neotia* Guett. Representatives of this species have their own unique beauty and spreading areal.

MATERIALS AND METHODS

The article is based on the researches carried out in the Nakhchivan Autonomous Republic. The *Neotia ovata* (L.) Bluff & Fingerh species of the *Neottia* Guett. genus, *Orchis simia* Lam. species of the *Orchis* L. genus that grow on the western slope of the Zengezur mountains, on the upper part of the Duylunchay basin, in the midland and highland and the Mezre village and the *Anacamptis pyramidalis* (L.) L.C. Rich species of the *Anacamptis* genus from the Batabat massif have been taken as the research objects.

The data of previous researches collected in "The Azerbaijan Flora" (Флора Азербайджана, 1952), "The USSR Flora" (Флора СССР, 1935), materials of Euro+Med Plantbase - the information resource for Euro-Mediterranean plant diversity and the information base of the APG IV system were used in the current research.

In addition, there have been studied materials collected by researchers working in the field of biology in the autonomous republic in different years.

DISCUSSION AND CONCLUSIONS

Most of the *Orchidaceae* species are of decorative importance, and all of them deserved to be protected.

Genus: *Neottia* Guett. - Neottia. The height is 20-40 cm. Rooty stem is horizontal and intense and covered with thick root cover. The plant is yellowish-brown. The leaves are scabbard and

round. The flower group is thick (the bottom is empty), and it is15-20 cm long. The perianth are dark-light brown; the leaves are 4-5 mm in length and they are oval. The labium is 10 mm in length, and darker than others. Spread in the mountain forests.

The *Neottia ovata* (L.) Bluff & Fingerh species including into the *Neottia Guett* genus are perennial, mesophytes that grow in the upper part of the Duylunchay basin, in the midland and highland, around the Mezre village of the Ordubad region. They are observed in the lowland, in the mixed forests that are favorable for this area. They occur in some areas (2-3 pieces) or in small groups in the walnut and quince gardens that are newly planted in meadows. In the lower and middle mountain ranges, mountain slopes they are observed in the mixed forests, shrubberies, meadows, shady places, in small groups (2-3 pieces). The period of flowering and fruiting is May and June (Figure 1).

Fig. 1. *Neottia ovate.*

Neottia ovata (L.) Bluff & Fingerh. - Oval bird nest orchid is a perennial herb. The root is short and thick (Fig. 1). The leaves are situated by 2 opposite each other, wide-oval-ellipse, width up to 6 cm and thick. There are 1-3 round leaves in the upper part of stem. The flower group is long and empty, the bract is oval. Flower stem is short and periant leaves are 3-4mm in length and they are green. Two outer leaves are strait, oval and blunt, but two inner leaves are lanceolate shape.

Inflorescence is a brush shape with a lot of flowers. The flowers are long enough, small, curved, round, sometimes greenish, yellowish-green or dark violet. The torus is approximately the same in length, oval, elliptic or scarecrow, free. The labium is 10 mm in length, yellowish, two blunt lobed. Labium is actually 2or 3 times longer than outer part of the torus, flat, without spur, lingulate or wedgeshaped, with two wings. The stalk is strait and short. The beak is geared. The pollen bowl is on the top. The height is 20-40 cm. It spreads in the humid forests and meadows. It is observed in the upper part of the Duylunchay basin of the Ordubad region, on the banks of the Mezre river, in the lowlands and midlands, mountain slopes, in the mixed forests, shruberries, meadows, in the shady places in smal groups (2-3 pieces).

Oval bird nest orchid is a short rhizome perennial herb, hemicryptophyte, 20-30 cm, flowering period is May. It is observed in the middle mountainous ranges, in the woods, in humid places, in shady forests, in the shrubberies. It is pluregional. This species has been described in Europe. It has spread in the west, north, the center of the lesser Caucasus, and in Lenkoran in Azerbaijan. It is found in the Caucasus in Dagestan, East, West Transcaucasia and inTalish region. Common spreading: Europe, North, South West (Turkey), East Asia, North America.

It should be noted that according to molecular-philogenic studies the *Listera* genus can be included into the genus *Neottia* Guett. (Chase et al., 2003). Such kind of combining was also proposed on the basis of the analysis of morphology of flowers (Szlachetko, 1995) (Определитель сосудистых растений Тамбовской области, 2010).

In some sources, *Listera ovata* (L.) R.Br. that was called *Neottia ovata* (L.) Bluff & Fingerh species was included into the *Listera* R.Br. the genus. (Флора СССР, 1935; Флора Азербайджана, 1952; Миняев и Конечная, 1976; Саксонов и Конева, 2006; Перебора, 2007).

Euro + Med PlantBase - The information resource of the Euro-Mediterranean plant diversity estimates the latest information from all regional and national flora. According to that source and the APG IV system, the genus *Listera* R.Br. including into the genus *Neottia Guett*. Hist. Acad. Roy. Sci. Mém. Math. Phys. (Paris, 4to) 1750: 374 was included into 1754. Species *Listera ovata* (L.) R.Br. adopted as a species *Neottia ovata* (L.) Bluff & Fingerh. Comp. Fl. German., Ed. 2, 2: 435 was adopted as a synonym for the species 1838 (http://ww2.bgbm.org/EuroPlusMed/PTaxonDetail.asp?NameId=90573&PTRefFk=8000000) (Fig. 1).

Genus: *Orchis* L. - All flower perianth leaves, or only three upper leaves have been folded as helmets. The two inner floral lines of perianth are linear-lengthened and form a helmet. The labium is three or four sliced, tourned downwod, upper part is bare or covered with small suckings, flat and folded, cylindrical, cone-shaped or curved; the capsule stalk is short.

The pollen grows with an abutment and usually it is ellipsoid with parallel slots. There is a cranberry outcrop locating between its slots; pollinators provided with two gloves, which are located in a double-glove pocket, are pin shaped. The ovary is

folded. The pistil opening is covered. The root is round, oval or ellipsoid. The plant has underground roots.

Orchis simia Lam, a new species for the Nakhchivan flora, discovered by us - mainly grows in shrubberies, forest foliage and mountain meadows. In the low and middle mountain ranges, mountain slopes, in mixed forests, shrubs, meadows, shady areas were observed in smaller groups (2-3 individuals). Flowering and fruiting season is April-May (Figure 2).

O. simia Lam., Fl. France, III, 507 (1778). - O. tephrosanthos. ViII, M. B., 364; Ledeb., IV, 62. is a perennial plant. Roots are oval or elliptic. The stem is 25-50cm in height. The leaves are 4-5 lancet shaped, with 5.5-15 cm length and 2-3.5 cm width. The leaves are blunt or blunty-sharpened. There is 1-2 vaginas covered with leaves above them, on the stem.

Fig. 2. *Orchis simian.*

Inflorescence is a thick, multiflowered. It is 3-7 cm in height and 3-4 cm in width in the sprouting period. The bract is 1.25-3 (4) cm and it is oval or oval-lancet, sharpened, whitish. Outer leaves of the periant are pale-purple-pinky or pale-purple-violet, basically adjacent, oval-lancet, strongly sharpened, with three vessels, 1-1.4 cm in length. The periants are not equal. The inner two leaves of the periants are sharpened, with one vessel, white and a bit shorter than the outer leaves. The labium is pale-pinky or pale-rosy. Its central lobe is a bit pale-violet till the last slices. It is 7.5 mm in length with 2 narrow-wound folded slots, and with a longitudinal-linear middle end, with two long-linear folded slices 8-11 mm long and between them there are small teeth 2-3 mm in length; the length of the labium is 1.4-1.5 cm; The spur is 4-5 mm in length and 1.5- 1.75 mm in thickness, twice shorter than the ovary, down and slightly twisted (Флора СССР, 1935).

It spreads throughout the Caucasus, Lankaran. Common Spreading zones: Atlantic and Central Europe, the Mediterranean Basin, Balkans, Less Asia - South Europe.

There has been provided information on *Orchis* L. genus belonging to the Nakhchivan AR flora in the previous sources (Talıbov and IIIbrahimov, 2008).

For the first time, as a result of the research conducted in the Batabat massif, the *Anacamptis pyramidalis* (L.) L.C. Rich species of the Anacamptis. – Anacamptis genus was found to spread in the Nakhchivan flora.

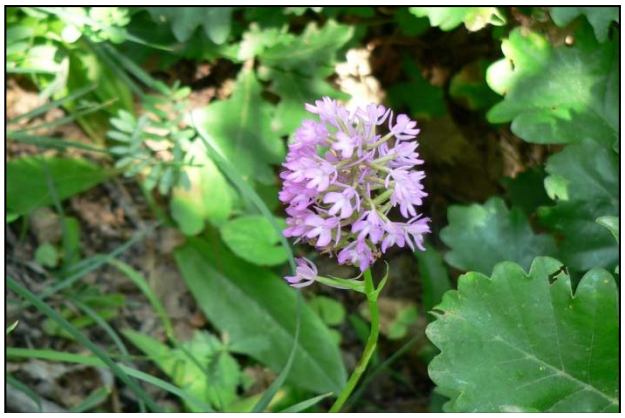

Fig. 3. *Anacamptis pyramidalis.*

Genus: *Anacamptis* Rich. - Anacamptis. The flower is red, sometimes pink or white, with a length of 5-6 mm. The parianth leaves are in the shape of oval-triangular. The labium is deep in a three-sided area, its slots are tight and long, and the labium end is covered with comb shaped protuberances. The spur is thin. The flower group is in the form of a dense, oval- pyramidal 4-5 cm length, and 3 cm width. The leaves are narrow-lancet shaped, 30-60 cm in height. Spread in shrubberies and medows in the middle mountain ranges.

Anacamptis pyramidalis (L.) L.C. Rich. – *Pyramidal orchid* is a perennial plant. The rubes are whole, round or sometimes elliptical. The stem is 25-65 cm in height. The leaf line is in the shape of a wedge covering the body, slightly narrower or slightly sharpened, and the top is the vagina shaped. Flower group is thick, multicolored, oval-pyramidal. The bract is long-sharpened and shorter than the flowers. Flowers are purple-red, sometimes red and even white. The outer leaves (perianth) are 4-6 mm in length, medium-sized, the sides are often two, the entrances are single-core, nearly equal to those in the overs. The labium is triangular with long slices, rounded or round cut. The spur is 13-14 mm in length.

The species have spread in the Samur-Shabran Plain - The Greater Caucasus (GC) (Guba Mountain Massif) - GC, East - GC, West - Kura - Plateau - Lesser Caucasus (LC), North - Central Asia -

Lankaran Mountains - Lankaran Plains - Low Mountain Range up to the middle mountain ranges - on forest edges, shrubberies, orchards and grassy rocks. Common spreading zones: Atlantic and Central Europe, Western and Eastern Mediterranean, Balkan Peninsula - Lesser Asia, Iran. It has been described in Switzerland (Picture 3).

Based on literary sources and researches in the Nakhchivan Autonomous Republic, the genus and species included into the *Orchidaceae* Juss family are classified as follows:

Super Ordo*: Lilianae*
Ordo: *Asparagales*
Familia: *Orchidaceae* Juss.
Subfam .: *Neottioideae* Lindl.
1. Genus: *Epipactis* Zinn
1 (1) *Epipactis microphylla* (Ehrh.) Sw.
2 (2) *E. palustris* (L.) Grantz
3 (3) *E. veratrifolia* Boiss. et Hohen.
2. Genus: *Neottia* Guett.
4(1) *Neottia ovata* (L.) Bluff & Fingerh.
Subfam .: *Orchidoideae*
3. Genus: *Planthera* Rich.
5 (1) *Planthera chlorantha* (Custer) Reichenb.
4. Genus: *Dactylorhiza* Neck. ex Nevski
6 (1) *Dactylorhiza euxina* (Nevski) Czer.
7 (2) *D. salina* (Turcr. ex Lindl.) Soo
8 (3) *D. iberica* (Bieb., Willd.) Soo
9 (4) *D. romana* (Sebast.) Soo [D. flavescens (C. Koch) Holub]
10 (5) *D. umbrosa* (Prof. & Kir.) Nevski (1937)
11 (6) *D. urvilleana* (Stand) H. Baumann et Künkele
12 (7) *D. osmanica* (Kinge) P.F. Hunt & Summerh.
5. Genus: *Orchis* L.
13 (1) *Orchis mascula* L.
14 (2) *O. punctulata* Stev. ex Lindl.
15 (3) *O. simia* Lam.
6. Genus: *Anacamptis* (L.) Rich.
16 (1) *Anacamptis coriophora* (L.) R. M. Bateman, Pridgeon & M.W.Chase
17 (2) *A. laxiflora* (Lam.) R.M. Bateman, Pridgeon & M.W.Chase
18 (3) *A. palustris* (Jacq.) R.M. Bateman, Pridgeon & M.W.Chase, 1997
19 (4) *A. pyramidalis* (L.) Rich.
7. Genus: *Ophrys* L.
20 (1) *Oprhys apifera* Huds.
8. Genus: Gymnadenia R.Br.
21 (1) *Gymnadenia conopsea* (L.) R.Br.

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Naxçıvan Mutar Respublikasının Florası Üçün Yeni Olan Səhləbkimiıər - *Orchidaceae* Juss. Fəsiləsi Növlərinin Bioloji və Fitosenoloji Xüsusiyyətləri

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Məqalədə Səhləbkimilər (*Orchidaceae* Juss.) fəsiləsinin *Neottia* Guett cinsinə aid *Neotia ovata* (L.) Bluff & Fingerh, *Orchis* L. cinsinin *Orchis simia* Lam. və *Anacamptis* Rich. cinsinin *Anacamptis pyramidalis* (L.) L.C.Rich növlərinin sistemayik, bioloji və firosenoloji xüsusiyyətləri haqqında məlumatlar təqdim olunmuşdur.

***Açar sözlər**: Orchidaceae, səhləb, bioloji xüsusiyyətlər, fitosenoz, ovalvarı gizli çiçək, Listera, Neottia ovata, Orchis simia* Lam*., Anacamptis pyramidalis, piramidal səhləb*

Биологическая и Фитоценологическая Характеристики Новых для Флоры Нахчыванской Автономной Республики Видов Семейства Орхидные - *Orchidaceae* Juss

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В статье представлены данные о систематических, биологических и фитоценологических особенностях представителей семейства Орхидные (*Orchidaceae* Juss.): вида *Neotia ovata* (L.) Bluff & Fingerh, относящегося к роду *Neottia* Guett, *Orchis simia* Lam. – род *Orchis* L. и *Anacamptis pyramidalis* (L.) L.C.Rich –род *Anacamptis* Rich.

***Ключевые слова:** Orchidaceae, орхидея, биологические особенности, фитоценоз, скрытый цветок овальной формы, Listera, Neottia ovata, Orchis simia* Lam*., Anacamptis pyramidalis, пирамидальная орхидея*

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Creation and Development of the Information System of the Plant Genepool of Azerbaijan

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The Information System that provides optimal monitoring of the plant genetic resources and enables us utilize the research findings regarding the spread, preservation and morpho-agronomic, biogeographic, technological, biochemical and molecular traits, genetic diversity and modifications of crops and crop wild relatives in Azerbaijan in the process of collection, storage, analysis, breeding and in the other scientific works in a centralized space and standardized form, as well as a number of databases covering various traits of domestic plant gene pools, and the bases of characterization and evaluation of 13 species of grain cereals and leguminuous crops conserved in the National GeneBank were created, developed and integrated into the international systems.

Keywords: *Information system, database, genetic resources, genepool, characterization, evaluation, plant*

INTRODUCTION

In the 80-90s of the last century, the notable Azerbaijani scientist of biology and agricultural sciences, academician Jalal Aliyev called attention to the necessity of the information systems for reliable conservation and sustainable use of biological diversity, raised concerns over creation of the information bases that cover passport and comprehensive research data of the plant genepool samplings and put forward his perspicacious recommendations. He stressed the need for creation of the information systems aimed at on time revealing of the overwhelming risks related to PGR and their root causes, simulating the expected development of resources, suggesting recommendations on the basis of the predictions, gathering information in a centralized and standardized manner for information provision of decision-making mechanisms. Later the thesis emphasizes that the information system creates opportunities for studying and efficient management of the bioresources were put forth number of times (Thysen, 2000; Guarino et al., 2002; Əliyev və b., 2008; Descriptors – BI). The related priorities specified by J.Aliyev were systemized afterwards into an appropriate action plan of which key priorities were reflected in the national strategies and state programs (Əliyev, Əkpərov, 2002).

In the study of genetic resources that are the carriers of informative aspects of animate nature, are applied the methods typical for the field, as well as informational-analytical software programs and databases based on standardization of quality and quantity indicators (Наумов, Вендров, 1991; Thysen, 2000; Winfried, 2006).

Special descriptors (Germier, Frese, 2001; Descriptors – BI, 1982-2018; FAO/BI Multi-Crop Passport Descriptors list, 2015) developed by FAO and Global Biodiversity are applied to study genetic diversity and modification and morpho-agronomic, biogeographical and so forth traits (Guarino et al., 2002; Akparov et al., 2013), whereas phenotypical, metabolic, biochemical and molecular characterizations are performed via various markers (RFLP, protein fractions, microsatellites, RAPD, SSR, EST, SNP). It is tremendously necessary to organize such researches as a database so that they can be digitalized and collected in a centralized space and standard forms, stored, manipulated and more efficiently used in the other researches (Thysen, 2000; Germier, Frese, 2001; Guarino et al., 2002; Winfried, 2006; Əliyev və b., 2008). Such databases of the germplasms enable us to automate analyses and selections, eliminate the need for multiple researches, and save funds and time.

Characterization of plant genetic resources is the major description of germplasm for breeding. Their morpho-agronomic, biochemistry and molecular evaluation also plays an important role for creation new valuable varieties. With the support of information technologies, it can be more effective use of characterization and evaluation data.

The researches we have conducted for long years have basically aimed at development of the bases of an information system that posseses all the foregoing traits, and creation of passport, taxonomic and ecological databases, as well as agronomic, characterization, evaluation, genomics ones on the basis of an enhanced software.

MATERIALS AND METHODS

The research materials of the national information system on PGR include passport, ecological, climatic, geological and geobotanical, areological and taxonomic storage, recovery, exchange, introduction and reintroduction, characterization and evaluation data standardized by relevant technologies with regard to the collections of the national genepool, scientific and national breeding varieties, wild crop relatives, herbarium funds and plant samplings. The objects of the system are the expeditions, reports and description lists on study of genepool materials, electronic maps, historical and archeological materials, initial registration and quarantine protocols, collectors, farmers, experts and their PGR-related knowledge, organizations, projects, programs, publications, catalogues, other references and materials (Акпаров, Мамедов и др., 2007; Akparov et al., 2008).

In the creation of the multi-table and multi-level databases were used the Database Management Systems and other program packages (Visual FoxPro, dBase, MS Access, MySQL, SQL Server, Apachi, Oracle, MS Excel və s.), other open-source software and database servers and internet resources, and SQL language (Structured Query Language) applied in built-in software writing (Наумов, Вендров, 1991; Сосински, 1997; Каратыгин и др., 1999; Дейт, 2005; Кузнецов, 2007; Germier, Frese, 2001; GENESYS; ECPGR Germplasm Databases).

The gathering, determination and analysis of the taxonomic data included into the system were put into practice through online version of the Index Kewensis system, GRIN-Taxonomy site, Vascular Plant Families and Genera, Mansfeld's World Database, other relevant internet resources and related databases (The Index Kewensis; GRİN Taxonomy; Mansfeld's World Database, 2012; Vascular Plant Families and Genera).

International passport and characterization descriptors (as well as genetic marker) were used for standardization, digitalization, collection and processing of research results for studying of plants (Descriptors – BI, 1982-2018; FAO/BI Multi-Crop Passport Descriptors list, 2015; GENESYS; ECPGR Germplasm Databases). In the analysis of the informational analysis of the national genepool were applied the quality factors and indexes on evaluation of genetic diversity of collections and ecosystems (Əliyev və b., 2008), whereas GIS software tools (Guarino et al., 2002; Winfried, 2006; Madurika, Hemakumara, 2017) and statistic processing packages of data were used in drawing up electronic areal maps of plant diversity, transferring and analysis of the ecogeographic, geobotanical and agronomic data and creation of relevant databases. The World Information and Early Warning System (WIEWS) of FAO, European Internet Search Catalogue – EURISCO on PGR and European Cooperative Program for Plant Genetic Resources (ECPGR Germplasm Databases), World Information Center for PGR (GENESYS) were used for integration into the international systems of the information system and work on the online mode.

Because the National Information Sharing Mechanism has the Web-based program, it needs a Web browser. High versions of Explorer or Mozilla Firefox etc. can be used. The software was created by using non-licensed, open source software (for example, Java Servlet and Java Server Pages), Web-server - Tomcat 4.1.24 and database server McKoi v.94.

RESULTS AND DISCUSSION

Functional blocks of the information system and creation of its fundamental structure. The biodiversity can be much better conserved by organization of electronic information resources on the biological systems in a different way and by securing information flow, in consideration of which were specified the key principles of the information flows that provide an efficient and comfortable access to resources, freedom of action in the rich information space for users, operativeness in information search and selection and inquiry, settlement of maintenance of actual condition of all information resources and provision of uninterrupted work of the documentation and information flow of genepools, and organizational structure and software were created. Such structure enables us to gather and analyze complex passport, morphological, botanical and ecnological data, including but not limited to characterization and evaluation and genomix data. The key principles of user-system and system-user interactice model were developed within the information system on PGR.

The Information System for PGR of Azerbaijan consists of the National Information Sharing Mechanism on PGRFA and its internet-based databases and other multiple file groups, multi-table and multifunctional Central Database of the national genepools, characterization and evaluation databases integrated into its structure and other software tools and relevant interfaces.

The Information System can be divided into three main functional blocks: 1) Inventarization (information gathering) block; 2) Analytical block; 3) Organizational-management block. Applying information technologies, all blocks have been closely associated with the software tools.

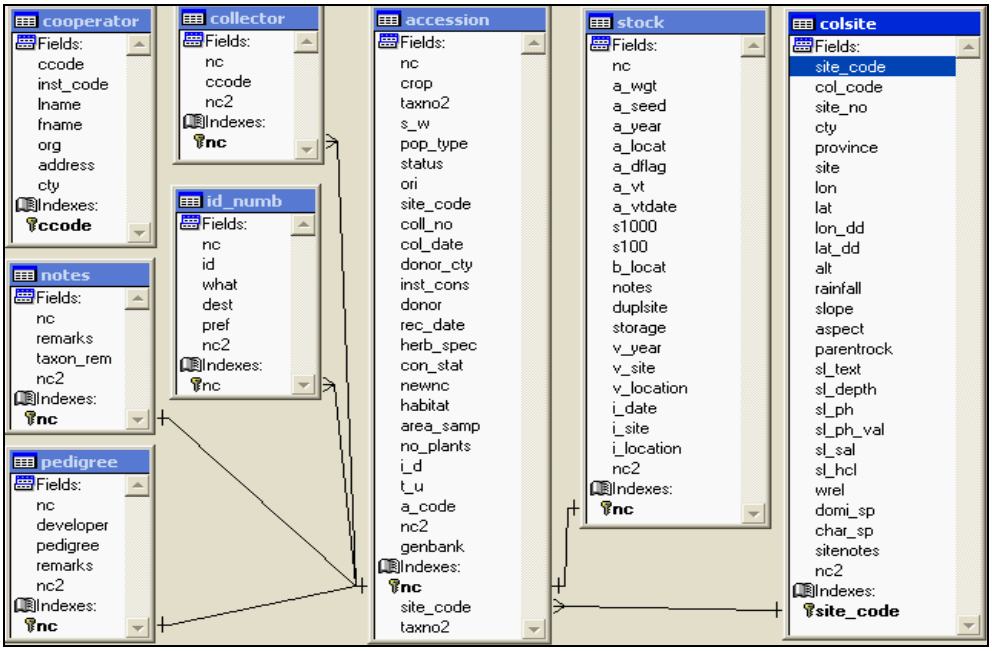

Fig. 1. A view of the internal structure of the database.

The Central Information Base mainly comprises of software made by modern technologies, and tables coordinated by special key fields and cover the most various issues of the PGR. The data on passport, ecological, botanical details and storage of the plant samplings have been systemized separately. Besides, the tables were projected and coordinated by coding and indexes to store additional information on subjects, taxon names and countries that collect and conserve plant samplings (Figure 1).

The Central Database can also be used for statistic analysis. The selections and surveys once made are saved and thus, no need arises to make such surveys once again.

Database of the National Genebank was established within the structure of the Central Database. The structure of the database that contains 14 tables, explanations and additional blocks, search and report forms adapted to the nature of the GeneBank activities has an efficient structure and interface to reach data consecutively and in a quick way, and provides better management of data describing the germplasm.

The internet-based and multi-table National Information Sharing Mechanism that secures high quality management of PGR research activities cover the data on *in situ* and *ex situ* conservation of PGR, their study, breeding and seed-growing. Through their research, regular strategic analyses are conducted on the condition of the PGR in Azerbaijan. Using keys in an online mode, it is possible to make searches and selections for all databases or their separate parts on the search system page.

The intellectual solutions blocks of the system we have created enable us to achieve a development plan of the condition on the basis of integral evaluation and expert system (knowledge database) of the prediction characteristics. The creation of the knowledge base built in accordance with the principles that enable us to protect and efficiently use the PGR create suitable condition for revealing, display and solution of the most serious problems of protection of plant biodiversity, and provide sustainable development of the genetic resources. The base acts on the basis of decision-making computerization, at the time of which creation there were taken into account expert answers to the questions on PGR in various forms.

Ecogeographical analyses within the information system. The organization of the data in geographic systems was believed to be necessary in studying of the space-time aspects of the development of plant diversity. Having applied the Central Database tools, the places where national genepool samplings are found, passport, characterization and evaluation data have been included into the distribution maps of the species. There were analyzed the possibility on availability of the materials collected upon addition of the climatic characteristics of the territory or the variation of the quality indicators in respect of the height above sea level. The genetic erosion of the country was evaluated on the basis of various data gathered to provide information support of the strategic planning and by specifying precisely nature and range of problems and the root of dangers for biodiversity.

In the 10 regions of the republic, a big amount of data that cover more than 200 farms and 678 points on local, traditional and national selection of

pomegranate, as a sample, were gathered and analyzed. It turned out to be possible to specify the diversity of national selection varieties, modification dynamics of diversity, condition of on-farm protection on lands and the distribution properties of the varieties. Besides, there were analyzed the relations among species diversity, absolute height, disease catching, local bioclimatic indicators and such other elements. The richness indexes of the Margalef (10,28), Shannon-Viner (2,972) and Simpson (0,901) proved the availability of variable diversity in different regions (Figure 2.).

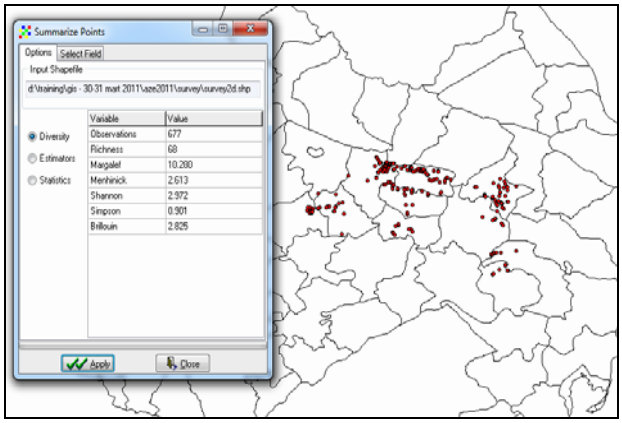

Fig. 2. Evaluation of the variable diversity in the regions of study.

Climatic data and other indicators prove that, the domestic pomegranate varieties have reached a respective adaptivity as a result of utilization of wild crops and multi-century breeding. According to the researches, in the territories where the amount of annual rainfall is higher, the rate of disease catching is high too.

The data received from the researches have been included into the relevant database together with the other agronomic data.

Content (accession, taxonomical) of the Central DB. Original crops, CWR, traditional and modern varieties are widely presented in the Central Database of which principal part constitute the data on cereals, leguminous and technical plants, fruits and berries of high priority.

There are more than 13500 plant accessions included in the database and conserved in the main *ex situ* collections of Azerbaijan until the present day. These accessions refer to 113 families, 443 genera, 871 species, and 304 variations. Of these, 5135 accessions are cereals, 1513 legumes, 751 fodders (forages), 873 vegetable-melons, 1586 technical plants, 2414 fruits, 786 medicines and etc. More than 7900 seed samples are in the medium-term storage cell.

According to the biological status of the registered samples from the CDB, 4861 were classified as modern scientific varieties, 2365 of the folk selection, 3652 constant research materials, 2205 CWR and etc.

Families Poaceae, Malvaceae, Fabaceae, Rosaceae, Vitaceae, Solanaceae, Lythraceae, Apiaceae, Cucurbitaceae, Betulaceae, Moraceae have been represented with more accessions in the central database of the *ex situ* collections of the Republic. The species *Gossypium hirsutum, Triticum aestivum, Zea mays, Vitis vinifera, Pyrus communis, Triticum status, Malus domestica, Hordeum vulgare, Phaseolus vulgaris, Punica granatum, Corylus avellana, Gossypium barbadense, Medicago sativa, Ficus carica,* Cicer *arietinum* differ with more accessions.

Characterization – evaluation databases. The characterization bases of the wheat, barley, triticale, rye, bean, cowpea, vetch, pea, lentil, broad bean and grass pea (13 species, 2186 accessions) were created in the structure of the Central Database via FoxPro program (Table 1).

A fragment of the small programs used in creation of such databases is given below:

Table. Species and accession content of characterization and evaluation databases

N	Botanical name of the species	Common name of the plant	Number of evaluated accessions	Number of traits for evaluation
1.	*Lathyrus sativus* L.	Grass pea	67	25
2.	*Phaseolus vulgaris* L.	Green bean	93	18
3.	*Lens culinaris* Medic.	Lentil	85	34
4.	*Cicer arietinum* L.	Chickpea	209	34
5.	*Zea mays* L.	Maize	177	10
6.	*Vicia faba* L.	Broad bean	89	24
7.	*Vigna unungiucullata* (L.)Walp.	Cowpea	25	18
8. 9.	*Triticum aestivum* L. *Triticum durum* Desf.	Common and durum wheat	1033	10
10.	*Hordeum vulgare* L.	Barley	194	22
11.	*Triticale*	Triticale	19	12
12.	*Secale*	Rye	135	12
13.	*Vicia*	Vetch	60	24
		Total	**2186**	

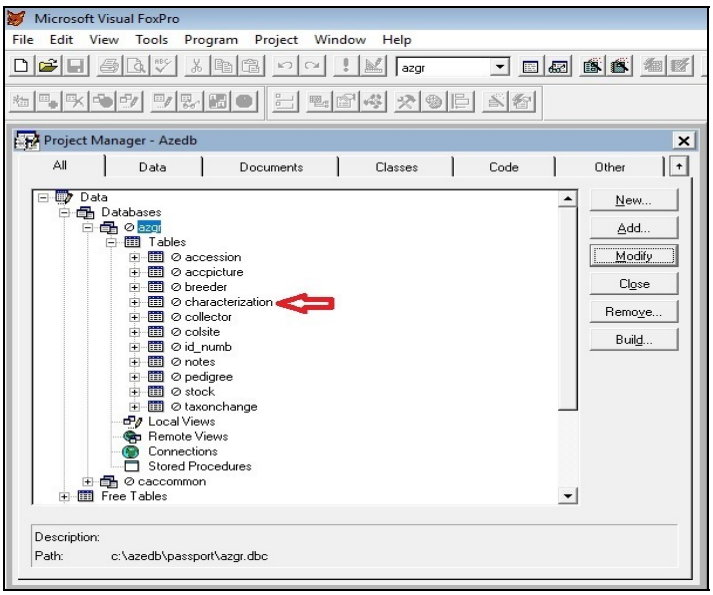

Fig. 3. Description of the characterization table in the given database.

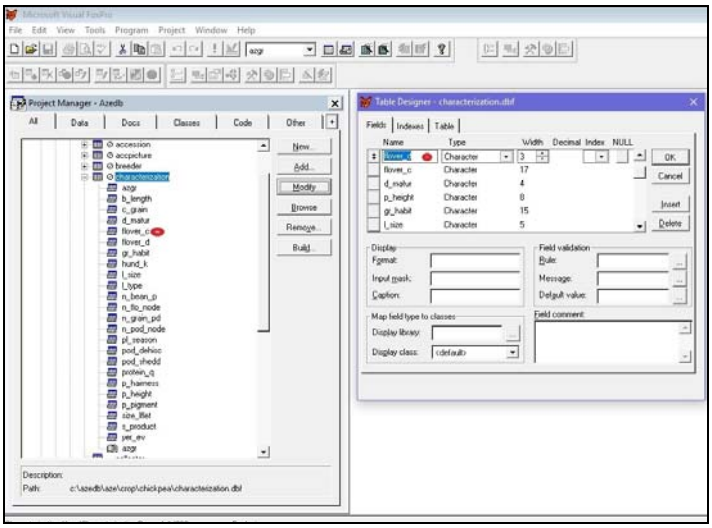

Fig. 4. Fields of table that makes the characterization database.

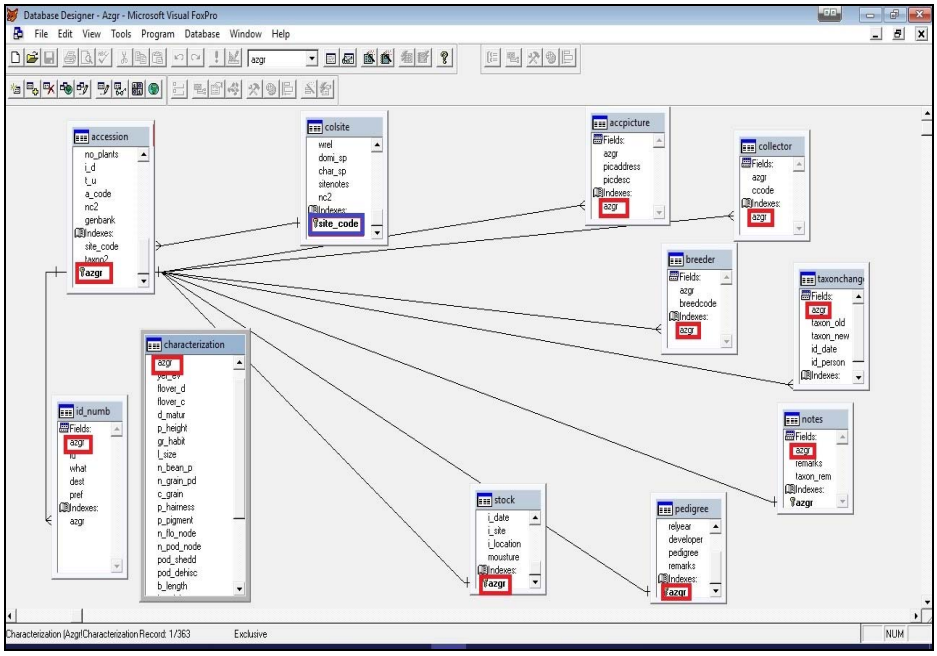

Fig. 5. Coordination of the other tables per AzGR attributive, including characterization table of the National Database.

AZE: wheat, wheat accession in Azerbaijan collection, C:\cac\AZE\cropsel\wheat\SELECTIONS; C:\cac\AZE\crop\wheat\DB_CROP.dbf C:\cac\AZE\cacsel\db_user.dbf; SELECT nc FROM accession WHERE taxno2 IN (SELECT taxno2 FROM taxon2 WHERE atc ('wheat',tax_name) > 0.

"CHARACTERIZATION" tables of different structure per plants were created in every database to store characterization and evaluation data (Fig. 3, 4). The relations between all tables that make up the base have been built by national AzGR indicator (Fig. 5).

Under guidance of the breeders and experts, the appropriate characterization and evaluation databases of separate plants and plant groups take a defined shape and are developed on the basis of morphological, physiological, technological, biochemical immunological and genetic study of cereals and leguminous plant collections of the National GeneBank.

CONCLUSIONS

There were created, for the first time, the Information System on PGR of Azerbaijan that provides the gathering, systemization, storage, spread, exchange and optimal management of the data on plant genetic resources, the Central Database that is an integral part of the Information System and the other databases that cover the data space on such resources in different directions. There were established the National Information Exchange Mechanism that provides coordination of movement dynamics and management of PGR, and the database that helps decision-making mechanisms on regular monitoring of the condition of genepool and its sustainable use.

In Azerbaijan, the relations among the spread and agronomic indicators of the national breeding varieties, adaptability of varieties and forms and agroclimatic gradient were studied for the first time in the background of the bioclimatic data of the regions where pomegranate, as a sample, is grown, and the diversity of which conservation is the highest priority was ascertained.

There were created characterization and evaluation databases of wheat, barley, maize, triticale, rye, cowpea, chickpea, lentil, broad bean and grass pea conserved in the National GeneBank.

Created database system is an open access for country and Institutional users (breeders, scientists, PhD students, specialists), and in near future will be online like a web-based Information system for all users in the world.

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Azərbaycanın Bitki Genefondu İnformasiya Sisteminin Yaradılması Və İnkişafı

Z.İ. Əkpərov, İ.A. Mirzəliyeva, A.T. Məmmədov

AMEA Genetik Ehtiyatlar İnstitutu

Azərbaycanın mədəni bitkilərinin və onların yabanı əcdadlarının yayılması, mühafizəsi, morfo-aqronomik, biocoğrafi, texnoloji, biokimyəvi və molekulyar xüsusiyyətləri, genetik müxtəlifliyi və dəyişkənliyi üzrə tədqiqat nəticələrinin vahid məkanda və standart formalarda toplanması, saxlanması, təhlili, seleksiya işlərində və digər elmi işlərdə daha səmərəli istifadəsinə imkan verən, bitki genetik ehtiyatlarının optimal idarə olunmasını təmin edən İnformasiya Sistemi və onun strukturunda milli bitki genefondunun müxtəlif cəhətlərini əhatə edən çoxsaylı məlumat bazaları (o cümlədən, Milli Genbankda mühafizə edilən 13 dənli taxıl və paxlalı bitki növü üzrə səciyyələndirmə və qiymətləndirmə bazaları) yaradılmış və beynəlxalq sistemlərə inteqrasiya edilmişdir.

Açar sözlər: *İnformasiya sistemi, məlumat bazası, genetik ehtiyatlar, genefond, səciyyələndirmə, qiymətləndirmə, bitki*

Создание И Развитие Информационной Системы Генофонда Растений Азербайджана

З.И. Акпаров, И.А. Мирзалиева, А.Т. Мамедов

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Создана и интегрирована в международные системы Информационная Система и входящие в её структуру многочисленные базы данных, охватывающие различные аспекты национального генофонда (в том числе, характеристические и оценочные базы 13 видов зерновых и зернобобовых растений, сохраняемых в Национальном Генбанке), обеспечивающие оптимальное управление генетическими ресурсами растений, позволяющие в едином информационном пространстве и в стандартных формах сбор, хранение, анализ, рациональное использование в селекционных работах и в других научных работах данных по результатам исследований по распространению, защите, морфоагрономическим, биогеографическим, технологическим, биохимическим и молекулярным характеристикам, по генетическому разнообразию и изменчивости культурных растений Азербайджана и их диких сородичей

Ключевые слова: *Информационная система, база данных, генетические ресурсы, генофонд, характеристика, оценка, растение*

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Determınatıon of Qualıty Indıcators of Basıc Feed Crops Belongıng to A Varıety of Herbs ın Summer Pastures

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The article provides information on the nutritional value of various herbs, collected from the north-eastern regions of Lesser Caucasus Azerbaijan and summer pastures of Talish region (Masally, Lankaran and Yardimli). Studies have shown that, vitamin "C" is mainly observed in representatives of Rosaceae (6-44%). It is relatively less in the representatives of other families (2-9%). The chemical composition of the fodder herbs belonging to various herb groups is calculated based on the absolute dry mass. In the flowering phase of plants, the amount of ash protein, proteins, lubricants and insecticides in various organs was studied and their nutritional value was determined on that basis.

Keywords: *Summer pastures, fodder crops, chemical composition*

INTRODUCTION

In connection with the development of civilization ecological condition changes gradually and various negative circumstances arise everywhere in the world. In this case actual problems emerge, such as the rise of the photosynthetic coefficient, the high productivity of soil for the full provision of the population with valuable food products caused by drought, energy, efficacious effect of active irradiation (Batisse, 2001). Taking into account that the development of livestock is not possible without balanced feed and the main part of human nutrition is animalistic nutrition, then it would be challenging to estimate the importance of plants. One of the actual issues is protein problem (Eryashev, 2003). Plants are considered to be the source of protein up to now. Traditional high-rise cultivated plants are mainly representatives of the legume family and great work is being done through the increase of their productivity (Lavrenko, 1995). But this cannot fully satisfy the protein as well. Therefore, we have reviewed several representatives of the families in order to search for new sources.

The main purpose of this research is to define chemical composition and economic indices of the valuable species of summer pastures in a complex way, and at the same time to identify more promising ones, defining types of high nutritional value on their basis.

MATERIALS AND METHODS

The research was carried out in 2017-2018. The plants were collected mainly from the Shahbuz region of the Autonomous Republic of Nakhchivan, the northern regions of the Lesser Caucasus and the Talish summer pastures (Masalli, Lankaran and Yardimli). The nutritional value and chemical composition of the fodder plants that are important in feeding animals and grown in summer pastures were investigated (Lidzhiyeva et. al, 2005). Feeding quality of the raw materials of the plants collected from different regions of Azerbaijan was tested. Assessment of feeding quality of plants, fractional structure of phytocturity, chemical composition of nitrogen, ash element, surface phytocturity and other analyses were carried out according to commonly accepted methods (under publication, 1982). The essential oil of fragrant plants has been studied by the Qinzberg method (Goryayev et. al., 1962). Greasy acids and vitamin C have been obtained from some of the major types of feeding according to the methods (Yermakov et al., 1972). For the determination of vitamin C leaf, stem and seed samples were cut in the porcelain dish or glass plate using stainless or chrome plated knife. Taking into account that iron traces, especially copper, accelerate the destruction of vitamin C, the process should be carried out very fast. As the average sample (if the plant is large) is 10-15 copies, a certain part should be taken so that all the tissue parts of the given object are included in this section. For example, one from the main part to the top (end) part, and the other one perpendicular to the first cut. The obtained tissues should be quickly cut in the porcelain dish and mixed. The whole or half of the average-size leaf sample was crushed into pieces and weighed on a techno-chemical scale to determine ascorbic acid.

RESULTS AND DISCUSSION

First of all, the amount of ascorbic acid found in general strengthening vitamins has been studied for feeding and health of animals. The determination method is based on reductive properties of ascorbic acid. Blue 2,6-dichlorphenolindophenol (indicator) is transformed into colorless compounds thanks to plant extracts (Tilmans reaction). Two types of the 2,6-dichlorophenolindophenol reactions exist: as a result of Type 1 reaction, the pH of the environment changes (as in ordinary asymmetric indicators), in which the blue color of the alkaline environment is intensely transmitted to light red of the acidic environment. The color change occurs between pH 4 and pH 5, and the indicator is purple. In the 2nd reaction dark blue color of the indicator becomes colorless. The last reaction is used for the determination of ascorbic acid. The acid extracts are titrated with the indicator until the pink color appears.

Vitamin "C" was found to be 890 mg% in leaves of *Potentilla reptans* L. belonging to the Rosaceae Juss. Family collecting in June, 291 mg% in leaves of *Filipendula vulgaris* Moench., up to 117 mg% in leaves of *Geum urbanum* L., 30 mg% in *Arctium transcaucasicum* DC. leaves belonging to the Asteraceae Dumort. family, 65.8 mg% in leaves of *Pyrethrum leptophyllum* Stev. ex. Bieb., it reaches 50-70% in *Taraxacum officinale* Wigg. leaves, 87.5 mg% in *Telekia spesiosa* Baumg. leaves, 40 mg% in green mass of the *Hieracium umbellatum* L. plant in flowering phase, 180 mg% in leaves of *Rumex confertus* Willd. belonging to the Polygonaceae Juss. family, 50 mg% in *Nepeta grandiflora* Bieb. belonging to the Lamiaceae Lindl. family, in large amount in green leaves of *Bunias orientalis* L. belonging to the Brassicaceae Burneett family. It was defined that there are 173 mg% vitamin "C" in the early flowering phase, in *Galium verum* L. belonging to the Rubiaceae Juss family and 33.5 mg% in the late flowering phase, 108 mg% in leaves of *Campanula rapinculoides* L. belonging to the Campanulaceae Juss. family, 144.5 to 400 mg% in leaves of *Campanula latifolia*.

As you can see, there are enough vitamin C in food ingredients for the feeding of the animals in the summer pastures, it is 6-90% in the Rosaceae family members, but it varies between 2-9% in the other species (Fig. 1).

In the flowering phase of plants, the amount of ash protein, proteins, lubricants and insecticides in various organs was studied and their nutritional value was determined on that basis (Table 1).

As seen in the table, the summer feed, rich in vitamin C, has a high nutritional value of the main feed crops. In addition, the sources of literature (Biologically active., 2001) and the results of our long-term research have been focused on, and full information on the main feed crops of summer pastures has been obtained. Presence of protein and vitamins in the surface part of the *Potentilla reptans*type indicates the quality of the excretion. It is a growing and herbaceous plant. It is eatable as its root splinters contain starch. It was defined that, there is 35% to 45% vaccine preparation in the roots of the genus *Geum rivale* L. species and there is 45% to 48% vaccine preparation in the roots and leaves of the genus *Rumex confertus* Willd.It was defined that there is 5.03% essential oil in *Salvia verticillata* L. flowering phase. V.Khalilov indicates that there is 15.1% vitamin "E" in the plant. (Khalilov, 2012).There is 0.6% essential oil in *Clinopodium vulgare* L. species. Essential oil is obtained from the plant in the flowering and seeding phases. *Nepeta pannonica* L. And *Nepeta grandiflora* Bieb. are plants with essential oil.

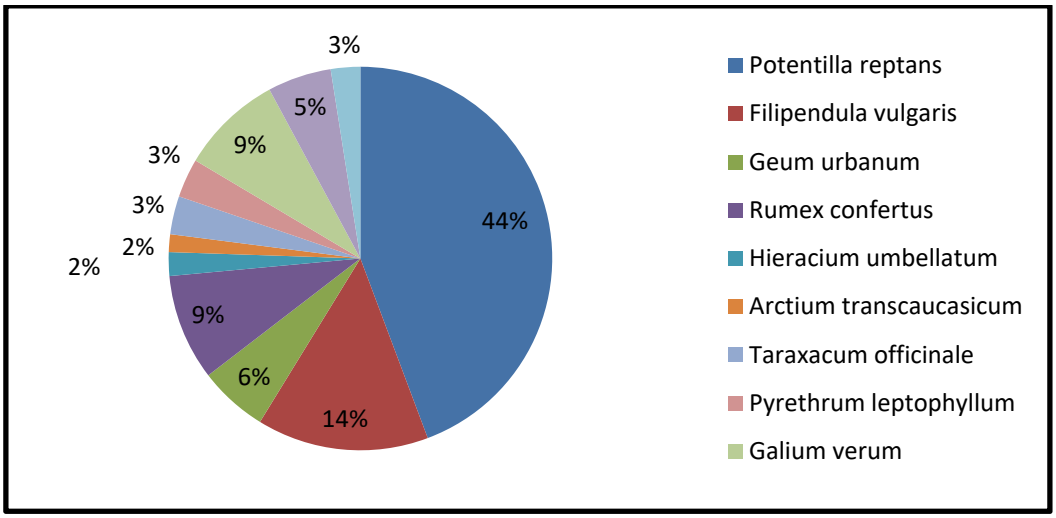

Fig. 1. Determination of Vitamin C in various plants of summer pastures.

Table 1. Chemical composition of feed crops belonging to various grass groups of summer pastures per an absolute dry mass, %.

Herbs	Protein	Nitrogen-free extractivessubstances	Oils	Cellulose	Ash	Flowering phase
Veronica anagillis – aguatica	16.4-17	50	6.6	18.9	6.3	surface part
Hypericum perforatum	12-12.5	57	5	22	4	surface part
Symphytum caucasicum	-	-	3.4-3.5	-	-	surface part
Hesperis matronalis	14-15	43	3-3.12	-	10	surface part
Anthriscus nemorosa	10	45	6-6.21	28	-	surface part
Salvia verticillata	-	-	5.03-5.05	-	-	leaf
Leontodon hispidus	18,6	44.1	3.8	18.8	14.7	surface part
Potentilla reptans	11	54.5	4-4.1	20.45	-	surface part
Galium ruthenicum	10.9	55.2	4	22.7	7.2	leaf
Galium verum	13.8	42	3.5	30.3	10.4	leaf
Galium tenuissimum	13.4-16.7	38.7	4.5	28.1	12	leaf
Arctium transcaucasicum	11.5	4.3	4.1	-	14.4	leaf
Arctium lappa	18.4	43.2	1.5	22.3	14.6	leaf
Achillea millefolium	19-55	41.05	3.90	22.45	13.5	surface part
Taraxacum stevenii	18.7-25.0	-	-	14.8-26.5		leaf
Pimpinella rhodantha	8-9	51	4	29		surface part
Chamaesciadium acaule	14.1	40.8	3.5	28.8	12.8	surface part
Centaurea fischeri	12.3	49.3	3.2	27. 6	8.5	leaf
Campanula tridentata	13.5	51	4.9	20.6	10	surface part
Campanula rapinculoides	11.3	51	-	2.9	9.9	surface part
Heracleum sosnowskyi	16	58	2.6	1.1	11.5	leaf

In summer pastures, animals are fed with all three of these species. It was defined that there is 4.7% oil and 8.7% vitamin "E" in *Heracleum trachyloma* Fish et Mey. species flowering phase. There are large quantities of carbohydrates, proteins, fats, ash compounds (microelements such as copper, magnesium, cobalt, iodine, molybdenum, iron and selenium), vitamins A, C, E in the composition of *Heracleum sosnowskyi* Manden. The calcium content fully meets the zootechnical requirements of feeding. *Arctium transcaucasicum* DC. slightly differs from *Arctium lappa*species for its chemical composition. Acylloid is found in leaves and flowers. There are 75% of inulin, essential oil, gum and spicy substances in the root of the plant. There are alkaloids and essential oil in composition of *Arctium lappa* L. which has a fodder value in its green mass. *Galium ruthenicum* Willd., *Galium tenuissimum* Bieb. and *Galium verum*has have average fodder quality due to 9.8% protein in their composition. *Campanula aucheri* is considered as one of the best fodder plants because of its chemical composition.

In the chemical composition of *Bunias orientalis* L. in flowering phase (absolute dry substance in %): protein 2.5, and it contains fatty oil in seeda. There are 4.2% oil and 16.5% vitamin "E" in the composition of *Alchemilla retinervis* Bus. in flowering phase. There are up to 17% fatty oil or 4-6% essential oils in the seed composition of *Carum carvi* L.. Essential oils consist of ketone-carbon and terpene-limonene. Protein amount is high, especially in leaf composition of *Rumex confertus* Willd., especially it is rich in proteins (91%). *Achillea millefolium* L. collected from a height of 2000 m is an import medical herb, rich in the chemical composition and essential oils in the bubble stage. It must be added to the animal feed ration. *Taraxacum officinale* Wigg. species composition collected in different phases show that, this plant is rich in proteins and oil substances, however the amount of cellulose is low. These figures once again prove the high fodder importance of themedicinal herb. There is spiciness in its leaves, but there is no alcoholoid in its leaves and root. According to some authors, there is alcholoid called teraxine in its root. The amount of protein in *Taraxacum stevenii* DC. composition is 18.7-25.0; and cellulose is less- 14.8-26.5%. *Leontodon hispidus* L. grows very fast after feeding, the chemical analyses show that, this is an important fodder plant in the vegetation phase. It is mentioned that, there is a few alcoholoid in the plant. *Doronicum oblongifolium* DC. chemical composition of leaves and body show that, there are 2.2 times more ash, 2 times more protein, 2.5 times more cellulose in leaves in comparision with body. The amount of oils are the same at both organs of plant (leaf and stem). There are 5.7-5.8% oils in stem and leaves of *Doronicum macrophyllum* Fisch. ex Hornem. as in long doronicum. The analyses of chemical composition of *Centaurea fischeri* Schlecht. collected from subalpine grassy of Dashkasan district show that its composition is fodder important (for absolute dry substance). There are 0.7-0.9% essential oils in baskets of *Pyrethrum leptophyllum* Stev. ex. Bieb. in the flowering phase

and this is used in production of camphor. According to the literature, it was defined that, there is alcholoid in its composition. *Campanula tridentata* and *Campanula rapinculoides* L. are important in term of food. It was noted that, alcholoid is found in leaves and flower groups of *Telekia spesiosa* Baumg. The amount of protein is much in young plants of *Cirsium arvense* (L.) Scop., but the amount of cellulose is less. There are 8.1% protein, 28.4% cellulose, 10% ash, 6.1% oils, 47.4% non-nitrogen extractive substances in the composition of the rough plant. There are vitamins C and A in the composition of *Cirsium arvense* species. There are reports on alcholoid called sirzin in the composition of desert gingival. There is 27.2% fatty oil in seeds of the plant. There are 21.55% protein, 15.95% ash in the leaves and 6.1% protein, 7.6 % ash in the stem of *Cirsium vulgare* (Savi) Ten during the flowering phase.

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Yay Otlaqlarının Müxtəlifot Qrupuna Aid Əsas Yem Bıtkılərının Keyfiyyət Göstəricilərinin Təyini

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Məqalədə Azərbaycanın Kiçik Qafqaz şimalı-şərq rayonlarında və Talış ərazisinin (Masallı, Lənkəran və Yardımlı) yay otlaqlarından toplanılan müxtəlifot yem qrupu bitkilərinin qidalılıq dəyəri barədə məlumat verilmişdir. Tədqiqatlar göstərmişdir ki, «C» vitamini əsasən Rosaceae fəsiləsi nümayəndələrində (6-44%) daha çox müşahidə edilir. Digər fəsilələrin nümayəndələrində (2-9 %) nisbətən azdır. Yay otlaqlarının müxtəlifot qrupuna aid yem bitkilərinin kimyəvi tərkibi mütləq quru kütləyə görə hesablanmışdır. Bitkilərin çiçəkləmə fazasında müxtəlif orqanlarında külün, proteinin, zülalın, yağların və azotsuz ekstraktiv maddələrin miqdarı öyrənilmiş və bu əsasda onların qidalılıq dəyəri təyin edilmişdir.

Açar sözlər: *Yay otlaqları, yem qrupu, kimyəvi tərkib*

Определение Показателей Качества Основных Кормовых Растений, Относящихся к Группе Разнотравья Летних Пастбищ

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В статье представлены данные о пищевой ценности растений кормовой группы разнотравья, собранных в Азербайджане на летних пастбищах северо-восточных районов Малого Кавказа и Талыша (Масаллы, Ленкорань и Ярдымлы). Исследования показали, что витамин «С» чаще встречается у представителей семейства *Rosaceae* (6-44%). У представителей других семейств (2-9%) он выявлен в меньшей степени. Химический состав кормовых культур летних пастбищ, относящихся к разнотравью, рассчитывался на основе абсолютной сухой массы. Количество золы, белков, жиров и безазотистых экстрактивных веществ определяли в фазе цветения в различных органах растений и на основании полученных результатов определялась их пищевая ценность.

Ключевые слова: *Летнее пастбище, кормовая группа, химические свойства*

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Significance of the Initial Material in Developing New Short Wheat Varieties

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Heights of 464 local and foreign, durum and bread wheat varieties of various geographical origin were determined in 2016-2017 vegetation years. From 259 bread wheat varieties 12.3% (32) appeared to be semi-dwarf (51-80 cm), 87.7% (227) of the genotypes were middle-height (81-110 cm). From 205 durum wheat genotypes 14.6% (30%) was semi-dwarf (51-80 cm), 81.0% (166) middle-height (81-110 cm) and 4.4% (9) was tall (111-140). Semi-dwarf bread and durum wheat genotypes were chosen as a genetic source and used in hybridization for breeding short varieties.

Keywords: *Breeding, bread wheat, durum wheat, plant height*

INTRODUCTION

Cereal plants and their products are known to be indispensable in the world agricultural system, including the Azerbaijan economy. As wheat is a main food plant in our country, increasing its production is an urgent issue and the development of this area is one of the priority directions. Due to the diversity of soil and climatic conditions in Azerbaijan, one of the main tasks facing the selectionists is developing 70-100 cm bread and durum wheat varieties, for irrigated and wetland areas, which are adaptable to high agraphone and resistant to lodging. Whereas, for dryland and rainfed zones medium height varieties, resistant to frost, drought and diseases, with high grain quality and productivity have to be developed.

Since the beginning of the last century researchers have been paying attention to short wheat genotypes. Prominent scientists, such as N.I.Vavilov and J.A.Aliyev noted the importance of short varieties in developing high productive wheat genotypes. In short varieties the ratio of grain product to straw is approximately 1:1 and in tall varieties more photosynthetic products are expended to straw than to grain (Aliyev, 1983; Recomendations for ...., 1984; Vavilov, 1985). According to D.J.Miralles et al. the study of wheat varieties having various morphophysiological properties showed that plant height profoundly affected productivity. According to the results of the experiments performed for the last 20 years, for the potential productivity, the optimal plant height should be 70-100 cm. When plants are shorter, in spite of the increasing agricultural index, a decrease in biomass per unit area is observed (Miralles and Slafer, 1995; Villegas et al., 2001).

MATERIALS AND METHODS

The investigations were conducted at the experimental station of the Research Institute of Crop Husbandry under irrigated conditions in 2016-2017. Seeds of 464 local and foreign, durum and bread wheat varieties of various geographical origin were sowed in the first decade of November, in 2016, using leguminous plants as predecessors. Sowing norm for bread and durum wheat genotypes was accepted as 400 grains, having germination ability, per $1m^2$ area. Multiple seedlings were observed in the second decade of November.

Samples were taken from bread wheat varieties Murov 2, Fatima and durum wheat varieties Barakatli-95 and Garabagh. A complex fertilizer (nitrophoska) -100 kg per a hectare was applied before sowing. In the early spring during the tillering phase 90 kg ammonium nitrate fertilizer (NH_4NO_3) was applied per hectare-determined as kilograms of active substance per hectare. During the vegetation period samples were watered at the leaf tube formation, earing and grain filling stages and respective agrotechnical care was provided in the experimental field. Using the available method heights of the studied plants were determined (Musayev et al., 2008).

RESULTS AND DISCUSSION

Heights of the studied wheat varieties were 93.6 cm and 90.8 cm in Murov 2 and Fatima, respectively. Whereas, heights of other varieties were in the range 67-100 cm. From 259 bread wheat genotypes 12.3% (32) was found to be semi-dwarf (51-80 cm), and 87.7% (227) medium-height

plants (81-110 cm) (Fig. 1).

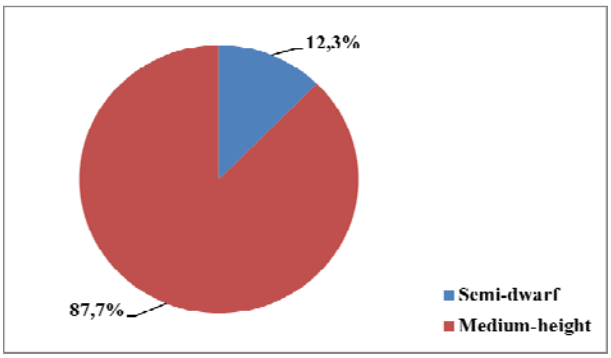

Fig. 1. Heights of bread wheat varieties.

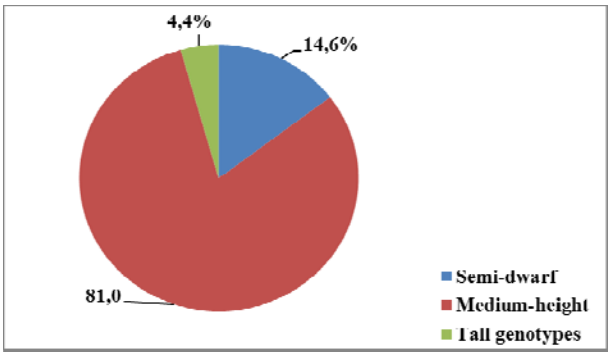

Fig. 2. Heights of durum wheat variety samples

P-7, N12 (SIMMIT)- 69 cm; P-3, N25 (SIMMIT)-70 cm; Spichka (Russian)-74 cm; Grom (Russia)-76 cm; Shafag 79.3 cm; Nurlu 99 (Azerbaijan)-80 cm etc. were found to be semi-dwarf (69-80cm) varieties. Murov- 81.5 cm; Markhal-82.1; Zirva 85 (Azerbaijan)-92.3 cm; Vassa (Russia)-90.2 cm; Guneshli (Azerbaijan)-95.4 cm; Saratovskaya-29 (Russia)- 110 cm etc.- medium height varieties (81-110 cm).

From the studied 205 durum wheat varieties, 14.6% (30) was semi-dwarf (51-80 cm), 81.0% (166) medium height (81-110 cm) and 4.4% (9) tall (111-140 cm) (Fig. 2).

Heights of durum wheat genotypes Barakatli 95 and Garabagh were 91.8 cm and 93.8 cm, respectively. Whereas, heights of the other varieties changed in the range 70-136 cm. P-11, N10 (SIMMIT)-70 cm; P-10, N78 (SIMMIT)-73 cm; 16 W. Durum Entri 88-78 cm; 16 W. Durum Entri 89-78 cm appeared to be semi dwarf (51-80 cm). Garagylchyg 2 - 95 cm; Tartar 2 (Azerbaijan)-89 cm, Zatino (France)- 92 cm etc.- medium height varieties. v.coemlesens (Azerbaijan)-132 cm, *v.apulicum* (Azerbaijan)-136 cm etc.-tall varieties.

As a result of the research semi-dwarf bread and durum wheat genotypes were chosen as a genetic source and used in hybridization for breeding short varieties.

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Alçaqboylu Yeni Buğda Sortlarının Yaradılmasında Başlanğıc Materıalın Əhəmıyyətı

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Azərbaycan Respublikası KTN Əkinçilik Elmi Tədqiqat İnstitutunun Bitki fiziologiyası və biotexnologiya şöbəsi

Məqalədə 2016-2017-ci vegetasiya ilində bərk və yumşaq buğdanın 464 yerli və müxtəlif coğrafi mənşəli xarici sortnümunələrinin boylarının tədqiqinin nəticələri öz əksini tapmışdır. Tədqiq edilən 259 yumşaq buğda genotiplərinin 12,3%-i (32-si) yarımkarlik (51-80 sm), 87,7%-i (227-si) ortaboylu (81-110 sm), 205 bərk buğda genotiplərinin 14,6%-i (30-u) yarımkarlik (51-80 sm), 81,0%-i (166-sı) ortaboylu (81-110 sm) və 4,4%-i (9-u) isə hündürboylu (111-140 sm) olmuşdur. Tədqiqatlar nəticəsində yarımkarlik yumşaq və bərk

buğda genotipləri genetik mənbə kimi seçilərək alçaqboylu sortların yaradılması məqsədi ilə hibridləşmədə istifadə edilmişdir.

Açar sözlər: *Seleksiya, yumşaq buğda, bərk buğda, bitkinin boyu*

Значение Исходного Материала При Выведении Новых Низкорослых Сортов Пшеницы

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Рост 464 местных и зарубежных сортов твердой и мягкой пшеницы различного географического происхождения определяли в 2016-2017 вегатационные годы. Из 259 сортов мягкой пшеницы 12,3% (32) оказались полукарликовыми (51-80 см), 87,7% (227) - среднерослыми (81-110 см), а из 205 генотипов твердой пшеницы 14,6% (30%) оказались полукарликовыми (41-80 см), 81,0% (166)-среднерослыми (81-110 см) и 4,4% (9) высокорослыми (111-140). В качестве генетического источника были выбраны полукарликовые генотипы мягкой и твердой пшеницы, которые использовались в гибридизации для выведения низкорослых сортов.

Ключевые слова: *Селекция, мягкая пшеница, твердая пшеница, рост растения*

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Significance of Polyene Antibiotics in Increasing Membrane Permeability and in the Treatment of Animal and Plant Infections

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Using polyene antibiotics (PAs) in combination with dimethyl sulfoxide (DMSO) was shown to increase ion permeability of membranes and biological activity of antibiotics sharply. The comparative physical and chemical characteristics of dimethyl sulfoxide and PAs were determined. The effects of a complex relation between PAs and the bilayer lipid membranes (BLM) were studied. The parameters of biological activity of PAs and BLM were determined. It was shown that among all the studied PAs, amphotericin B and levorin were the most effective. Ion permeability of BLM was shown to depend on the concentrations of amphotericin B, levorin and cholesterol. On the basis of PAs, biological active preparations were developed against viral, bacterial and fungal diseases.

Keywords: *Polyene antibiotics, dimethyl sulfoxide, amphotericin B, levorin, lipid membranes, membrane permeability, animal and plant infections*

INTRODUCTION

Polyene antibiotics (PAs) are one of the most effective compounds in the fight against fungal infections (Borowski, 2000). The main representatives of PAs are amphotericin B, nystatin, mycoheptin and levorin. PA molecules contain a lactone ring, a conjugated system of double bonds and a hydrophilic chain consisting of hydroxyl and carbonyl groups. The biological activity of PAs depends on the presence of a certain structure in the membranes of sterol cells (Récamier et al., 2010). Polyenes are more sensitive to membranes containing ergosterol (Ciesielski et al., 2016). Due to this distinctive feature, polyenes are successfully used in medicine for therapeutic purposes. Currently, amphotericin B and nystatin are mainly used in the treatment of systemic fungal infections. A comparative analysis of the biological activity of amphotericin B and nystatin shows that amphotericin B is about 6 times more effective against most fungi than nystatin (Aszalos et al., 1985). The BLM method showed that the conductivity of the amphotericin channel is about 10 times higher than that of the nystatin channel (Gasymov, 2009). Amphotericin B and nystatin are very close to each other in their chemical structures, but membranes with cholesterol are more sensitive to amphotericin B than to nystatin. According to the studies, the presence of a certain number of double bonds in the chromophores of PAs is an important factor determining their biological activity (Samedova et al., 2018). Amphotericin B and nystatin differ in the number of double bonds in the chromophore structures of polyene molecules. Nystatin has less double bonds than amphotericin B, which is the reason of the markedly less biological activity of nystatin. PAs were not chosen randomly as objects of the study. The peculiarity of PAs is that they constitute the only class of compounds in nature that forms molecular-sized channels in the cell and lipid membranes selectively permeable to ions and organic compounds (Ibrahimova et al., 2006; Gasymov, 2009; Récamier et al., 2010; Cohen, 2010). The studies of the molecular mechanism of the interaction of PAs with membranes showed that polyenes in combination with sterols create channels with certain structures in the membranes (Gasymov, 2009). However, in spite of the presence of a large amount of PAs and their derivatives, none of them can prevail the effectiveness of the action of amphotericin B and levorin in the treatment of systemic fungal infections. In recent years, scientists aimed at obtaining new medicinal forms of PAs and the development of new ways of their delivery to the affected organs and tissues. Interest in antifungal preparations has increased due to the widespread HIV infection, which appeared to be sensitive to the presence of fungal infections in the organism (De Marie et al., 1994). About 90% of HIV-infected patients were found to be infected with a fungal infection, because of the weakening immune system of the organism (Mamidi et al., 2002). In addition, patients are administered immunosuppressive preparations in the transplantation of various organs and bone marrow. However, they create conditions

for the appearance of HIV and fungal infections in patients (Sepkowitz, 2002). Currently, researches focus on a more comprehensive study of the action mechanisms of PAs at the molecular level. Growing interest of scientists is largely due to the decoding chemical structures of PAs and the development of ways for the modification of the polyene molecule (Zotchev, 2008; Baginski, Czub, 2009). The use of antibiotics with a known molecular structure allows conducting researches at the molecular level. The main goal of the research was to determine the intensification degree of the biological activity of PAs by studying the physicochemical properties of PAs in combination with DMSO.

MATERIALS AND METHODS

PAs dissolve well in DMSO. Polyenes have amphoteric properties and when ionized, they form a cation in an acidic medium, and an anion in an alkaline medium (Yu, Quinn, 1994). In combination with DMSO, polyenes are a liquid with a dark yellow color, bitter taste and a specific smell.

When preparing active forms of PAs, it is necessary first to convert the antibiotic from the powdery form (crystalline) to the molecular form. Wherein the conversion of the antibiotic into the most effective form occurs. After thoroughly mixing PA with DMSO, the composition is kept for 24 hours at room temperature. Then the liquid is filtered and stored in a dark, cool place. The obtained stock solution is ready for using. The use of PA in this combination of components is highly effective. The biological activity of PAs is determined by the method of bilayer lipid membranes (BLM) (Ibrahimova et al., 2006). BLM was obtained from total phospholipids isolated from cells by applying a drop of phospholipids to a hole in the teflon cell. Total phospholipids were purified from cholesterol and other neutral lipids by acetone washing and stored at 0°C at a concentration of 20 mg/ml in a chloroform-methanol solution in a volume ratio of 2:1. The integrated conductivity of the membranes was studied depending on the concentration of the antibiotic in the mode of fixation of the potential. At a certain concentration of the antibiotic, maximum membrane conductivity is achieved, which is considered as the active component of the PAs. The main information about the mechanism of PAs functioning in membranes was obtained using the BLM method. The method is based on the ability of PAs to dramatically increase the permeability of lipid membranes for the corresponding ions by registering changes in the electrical conductivity of the membranes. Studies of the integrated conductivity and measurement of the membrane potential were carried out in the mode of fixation of the potential and current using an electrometric DC amplifier Keithley-301 (USA).

RESULTS AND DISCUSSION

Dimethyl sulfoxide $(CH_3)_2SO$ is obtained by oxidizing dimethyl sulfide $(CH_3)_2S$ with nitric acid (Vaisman, Berkowitz, 1992). At present, for this purpose hydrogen peroxide H_2O_2 is used as an oxidizing agent. Dimethyl sulfoxide is the first member of the homologous series of sulfoxides R_2SO. As a result of their further oxidation, R_2SO_2 sulfones are formed. The chemical structure of DMSO contains two methyl groups, sulfur and oxygen (Fig. 3). DMSO is a clear, colorless, slightly bitter-tasting liquid, with a specific smell and it is highly water soluble (Yu, Quinn, 1994). Organic sulfoxides have a pyramidal structure with a sulfur atom at the top. In sulfoxides (RR′SO), the radicals R and R ′ differ from each other and exist in two optically active forms. The DMSO molecule is amphiphilic and highly polar. The negative pole of the dipole is on the oxygen atom. DMSO has an ordered structure, because of the temperature dependence of the refractive index, density, viscosity and other characteristics. DMSO is a protophilic solvent, and therefore, its associates are easily destroyed by the addition of substances that are proton donors. DMSO absorption spectrum in the wavelength range of 350 nm - 220 nm appeared to be optically transparent. A high degree of solubility of a number of organic compounds in DMSO is usually used to study their physicochemical characteristics and molecular structure (Yu, Quinn, 1994). Table 1 shows some physical characteristics of DMSO and water. The relatively high boiling point and large latent heat of vaporization (53 J/M at 25 ° C) indicate that DMSO molecules are strongly associated (Vaisman, Berkowitz, 1992). DMSO has properties such as amphiphilicity, polarity, high resorption.

Table 1. Physical properties of DMSO and water

Physical properties	DMSO	Water
Molecular weight	78.13	18.02
Density at 20◦C	1.1014	18.02
Melting point 20◦C	18.4	0.00
Boiling point	189.0	100.00
Surface tension at 20◦C (10^{-3}x Pa x c)	46.2	72.75
Viscosity at 20◦C(10^{-3}x Pa x c)	2.20	1.002
Dielectric constant at 20◦C	48.9	80.20

Studies of the biological activity of PAs showed that these compounds specifically interact with sterols of antibiotic-sensitive organisms, such as fungi and protozoa (Grayetal., 2012). Studies of the molecular mechanism of interaction of PAs with

membranes showed that polyenes create channels in the membranes through which ions and intracellular components can diffuse from the cells into the external medium, leading to cell lysis (Cohen, 2010).The biological activity of PAs is assumed to depend on the nature of the intermolecular interactions between the charged groups of antibiotic molecules and phospholipids. Probably, the incorporation of antibiotics into the membrane occurs as a result of the formation of a hydrogen bond between PAs and the phosphate groups of phospholipid molecules. A comparative analysis of the biological activity of amphotericin B and nystatin shows that amphotericin B is more effective against most fungi than nystatin (Aszalosetal., 1985). It was found that the polyene chains in nystatin A1 and amphotericin A are the same and antifungal activities of these two antibiotics are identical (Aszalosetal, 1985). According to these data, the presence of a certain number of double bonds in the chromophores of PAs is an important factor determining their sensitivity to membranes. There is a direct relationship between the number of double bonds in the chromophore and the biological activity of antibiotics – high biological activity corresponds to the high number of double bonds in the chromophores of PAs (Gasymov, 2009). Levorin has a higher selectivity of action on the membrane and differs from other PAs by its high solubility in water. The structure of the lipid bilayer, as well as the structure of the penetrating molecules, is an important factor determining the permeability of water-soluble compounds. A high degree of resorption of DMSO molecules is attributed to the fact that the value of the dielectric constant of DMSO is between that of water and fats (Table 1). This indicates that DMSO enhances the permeability of a large number of medicinal compounds through biological membranes, and also contributes to their quite deep penetration into the cell (Ibrahimova et. al. 2002).

For the first time, the physicochemical properties of amphotericin B and levorin in combination with DMSO and their mixed solutions in various ratios were studied. The dependence of the conductivity of bimolecular membranes on the concentration of amphotericin B and levorin was studied. Amphotericin B dramatically increases membrane permeability to ions, water, non-electrolytes and organic compounds. The dependence of the membrane conductivity on the concentration of amphotericin B increases in proportion to the 8-10th degree and this degree depends on the structure of the PA molecules. The sharp dependence of the conductivity of the membranes on the concentration of amphotericin B allows us to suggest that ion permeability is associated with the formation of oligomeric structure in the membranes of the polyene channels. Probably, the system responsible for the selective permeability of membranes is localized in the hydrophilic chain of the amphotericin B molecule. With the increasing concentration of DMSO in an aqueous solution, the efficiency of assembly of polyene channels and the stability of the channels in the conducting state increase. At a concentration of $1 \cdot 10^{-6}$ mamphotericin B reduces (10^5-10^6 times) the initial specific resistance of membranes ($1\text{-}5 \cdot 10^{-8} Om \cdot cm^2$), prepared from common phospholipids. The dependence of the conductivity of bimolecular membranes on the concentration of amphotericin B was studied at various concentrations of cholesterol in the membranes. The addition of cholesterol to phospholipids increases the effectiveness of the antibiotic. Membranes in the presence of amphotericin B are selectively permeable to monovalent anions. However, in the study of aromatic antibiotics, it was found that, unlike amphotericin B, levorin causes selective permeability not for anions, but for alkali metal cations. This antibiotic differs from nystatin, amphotericin B and mycoheptinum by the presence of an additional aromatic group- ρ-aminoacetophenone, which contains positively charged nitrogen. In the presence of levorin, with increasing cholesterol concentration, membrane conductivity increases. Studies of the dependence of the conductivity of membranes on the concentration of levorin led to the assumption that there are molecular-sized channels in the membranes that induce ion permeability. Selective permeability for cations is thought to be associated with the formation of negatively charged pores in the membranes. Probably, the transfer of cations through membranes occurs in the hydrophilic parts of the channel. Essential information on the mechanism of membrane permeability in the presence of aromatic antibiotics can be obtained from data on the transfer of small ions through the membrane, such as guanidine and hydrazine. In the presence of levorin, these ions penetrate the membrane much better than K^+ and Na^+ ions. The presence of the same number of double bonds in the chromophores of amphotericin B and levorin is an important factor determining their high sensitivity to membranes. The most effective of the studied PAs appeared to be amphotericin B and levorin. Dimethyl sulfoxide (DMSO) plays a special role in the formation of the amphotericin and levorin channels inside the membrane. DMSO has the ability to dramatically enhance the biological activity of PAs and induce selective permeability for ions and organic compounds in membranes.

The results of the experiments suggest that the

mechanism of selective action is based on the specific interaction of antibiotic molecules with membranes. The chromophores of PA molecules, interacting with phospholipids, form a channel in a 1:1 stoichiometric ratio. The stoichiometric coefficient of assembly of single channels for various PAs can be different and range from 3 to 17 (Ibrahimova et al., 2006). It should be noted that the molecular structure of the hydrophilic part of the channel has not yet been established due to the lack of appropriate methods for determining the exact localization of the molecular groups in the internal cavity of the channel. According to the research, the internal diameter of the channel is 7-10 Å (Ibrahimova et al., 2006). A computer analysis showed that during the formation of the ion channel in the presence of an amide derivative of amphotericin B, the ionizing groups of molecules can be turned both inward and outward, i.e. polar groups can be in two conformational forms, due to the rotation of mycosamine around the glycosidic bond. There is an assumption that the biological activity of PAs may depend on the nature of the intermolecular interactions between the charged groups of antibiotics and phospholipids. The incorporation of antibiotics into the membrane occurs as a result of the formation of a hydrogen bond between PAs and the phosphate groups of phospholipid molecules.

THE PRACTICAL SIGNIFICANCE OF THE RESEARCH

Antibiotics in animal and crop husbandry became widespread after the adverse effects of using some preparations had been established. Because, suppressing phytopathogenic microflora, they poison useful species of birds and animals that feed on pollinated plants. Compared with other substances, antibiotics have a number of valuable advantages in the fight against phytopathogenic microorganisms. Antibiotics act selectively and, suppressing the development of phytopathogenic bacteria and fungi, they are practically harmless to plants and animals (Lewis, Papavizas, 1987; Ibrahimova et al., 2014). The absence of toxicity is required when choosing antibiotics. For example, PAs used in low concentrations (10^{-6}-10^{-4}M), are non-toxic for plants and animals. Most antibiotics penetrate into the tissues of animals and plants and are well absorbed. The concentration of antibiotics necessary to suppress pathogenic microflora in animal and plant tissues depends on the properties of the antibiotic and external conditions. PAs were used as the basis for the development of effective antiviral, antibacterial and antifungal preparations. Based on the data obtained, the minimum concentration of the antibiotic corresponding to its maximum biological activity was calculated. New active compounds, which have the ability to effectively and selectively suppress pathogenic infections, were revealed in the group of PAs. The preparations were found to suppress viral and fungal infections of plants. Spraying a solution of the preparation on plants affected by a viral and fungal infections leads to the effective destruction of plant infections. Laboratory analysis of a soil sample on which vegetables were grown showed that soil contains a small amount of nitrogen, a large amount of phosphorus and a small amount of potassium, and pH of the soil sample was slightly alkaline. Table 2 shows mineral elements in the soil composition, based on soil gradation. Despite the missing mineral elements in the soil, where vegetables were grown, studies have shown the high efficiency of the preparations against pathogenic microorganisms of vegetable cultures. It should be noted that the preparations have the ability to completely inhibit the growth of the tobacco mosaic virus (*Tobacco mosaic virus*) (Ibrahimova et. al., 2014). Infected plants after the treatment are not only cured, regeneration of plants faded from infection also occurs. The antiviral and antifungal effects of infanvir are attributed to the binding of the antibiotic with the membranes with the subsequent formation of a complex in them. This complex is a molecular-sized channel formation, which is reflected in the inhibitory effect of the preparation on the reproduction of viruses and fungal cells. The proposed preparation is non-toxic and harmless, which contributes to its rational use in agriculture for growing vegetable and fruit crops.

Table 2. Mineral composition of soil based on soil gradation.

Sample name	pH	The degree to which soil is provided with mineral elements on the basis of soil gradation			Conductivity on particle size distribution of the soil (mS)	NaCl (mg/kg) Standart 150-300	KCl (mg/kg) Standart 350-700
		nitrogen, 40-120 mg/kg	phosphorous, 15-60 mg/kg	potassium, 300-600 mg/kg			
		Index of mineral sample provision					
		nitrogen, N/NH_3 mg/kg	phosphorous, P_2O_5 mg/kg	potassium, K_2O mg/kg			
Soil	7.55	7.76	133.32	212.08	1.18	520	516

For the first time the effect of preparations against viral, staphylococcal and fungal infections has been studied. The effect of the preparation formed by Streptomyces microorganisms on a number of pathogens - Staphylococcus, Escherichiosis, Candida, opportunistic bacteria and Coxsacke A, ECHO virus and type I and II herpes simplex virus has been investigated. Antimicrobial activity of preparations has been studied in various test systems. It was found that in low doses (10^{-7}-10^{-6} M) the preparations had antibacterial and antifungal effects on the cultures *Salmonella typhimuium, Pseudomonas aeruginosa*, *Proteus vulgaris, Escherichia coli,* gram-positive cultures *Staphylococcus aureus* and cells of the fungus *Candida albicans.* They also have an antiviral effect on Coxsacke A 20, ECHO9 virus and type I and II herpes simplex virus. Eurasian patents were obtained for both preparations.

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Membran Keçiriciliyinin Artırılmasında və Heyvan və Bitki İnfeksiyaların Müalicəsində Polien Antibiotiklərin Rolu

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Göstərilmişdir ki, polien antibiotiklərin (PA) dimetilsulfoksidlə (DMSO) kompleks istifadəsi nəticəsində membranların ion keçiriciliyini və antibiotiklərin bioloji aktivliyini kəskin sürətdə artırır. PA və DMSO- in müqayisəli fiziki-kimyəvi xüsusiyyətləri göstərilmişdir. DMSO və PA–nın bimolekulyar lipid membranları BLM-lə kompleks əlaqəsinin təsir effekti tədqiq edilmişdir. BLM-lə PA-nın bioloji aktivliyinin parametrləri təyin olunmuşdur. Göstərilmişdir ki, bütün öyrənilmiş PA-dən ən effektivi amfoterisin B və levorindir. BLM-nın ion keçiriciliyinin amfoterisinin B və levorinin membranlarda mövcud olan xolesterinin konsentrasiyasından asılılığının nəticələri verilmişdir. PA-nın əsasında heyvan və bitkilərdə olan virus, bakteriya, göbələk xəstəliklərinə qarşı bioloji aktiv preparatlar yaradılmışdır.

Açar sözlər: *Polien antibiotiklər, dimetilsulfoksid, amfoterisin B, levorin, lipid membranları, membran keçiriciliyi, heyvan və bitki infeksiyaları*

Значение Полиеновых Антибиотиков в Увеличении Мембранной Проводимости и при Лечении Животных и Растительных Инфекций

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Показано, что при комплексном использовании полиеновых антибиотиков (ПА) с диметилсульфоксидом (ДМСО) резко усиливается ионная проницаемость мембран и биологическая активность антибиотиков. Представлены сравнительные физико-химические характеристики ДМСО и ПА. Рассмотрены эффекты комплексного взаимодействия ДМСО и ПА с бислойными липидными мембранами (БЛМ). Методом БЛМ определены параметры биологической активности ПА. Показано, что из всех изученных ПА самыми эффективными оказались амфотерицин В и леворин. Изложены результаты зависимости ионной проводимости БЛМ от концентрации амфотерицина В и леворина и от концентрации холестерина в мембранах. На основе ПА разработаны эффективные мембраноактивные препараты против вирусных, бактериальных и грибковых заболеваний животных и растений.

Ключевые слова: *Полиеновые антибиотики, диметилсульфоксид, амфотерицин В, леворин, липидные мембраны, проводимость мембран, животные и растительные инфекции.*