European Cereals Genetics Co-operative Newsletter 2019

Proceedings of the 17th International EWAC Conference

3 – 8 June 2018

Bucharest, Romania

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Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany

and

National Agricultural Research and Development Institute, Fundulea, Romania

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Edited by

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and
National Agricultural Research and Development Institute, Fundulea, Romania


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INSTITUTUL NATIONAL DE CERCETARE DEZVOLTARE AGRICOLA
FUNDULEA

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Preface

A. Börner

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The 17th EWAC International Conference was organised by Matilda Ciucă and her colleagues in Bucharest, Romania from June 3 – 8, 2018. The organizing bodies were the National Agriculture Research and Development Institute, Fundulea and Ministry of Agriculture and Rural Development by the ADER116 project. It was the first EWAC conference in Romania since it was founded in 1967 (Table 1).

Table 1: Years and venues of EWAC Conferences.

1967	Cambridge	UK
1970	Weihenstephan	Germany
1974	Novi Sad	Yugoslavia
1979	Cambridge	UK
1981	Wageningen	The Netherlands
1984	Versailles	France
1987	Martonvasar	Hungary
1991	Cordoba	Spain
1994	Gatersleben	Germany
1997	Viterbo	Italy
2000	Novosibirsk	Russia
2002	Norwich	UK
2005	Prague	Czech Republic
2007	Istanbul	Turkey
2011	Novi Sad	Serbia
2015	Lublin	Poland
2018	Bucharest	Romania

Since 2016 EWAC is a working group 'Cereals Genetic Stocks' of the Cereals Section of the 'European Association for Research on Plant Breeding' (EUCARPIA). Fifty-five participants from 12 countries did attend the conference comprising 22 lectures and 15 poster presentations.

Under the general Motto 'Cereals for Tomorrow' two main subjects were discussed:

- Genetic gains through novel diversity and tools
- New approaches for cereals improvement and the future contribution of genetic stocks

The scientific programme but also the local organisation of the conference were excellent. Many thanks to Matilda Ciucă and her team for preparing and running this successful conference in a very kind and friendly atmosphere. We did enjoy the days in Bucharest and Fundulea very much.

Just before the conference Elena Khlestkina offered to organise the next EWAC Conference at the Vavilov Research Institute of Plant Genetic Resources in St. Petersburg in 2021.

We are looking forward to the 18th EWAC Conference.

Examples of successful collaboration (2015 - 2018)

A. Börner[1], U. Lohwasser[1], E. K. Khlestkina[2], T. A. Pshenichnikova[2], S. V. Osipova[3], S. Misheva (Landjeva)[4], M. R. Simon[5]

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In this review we will list examples of fruitful co-operation between 2015 (last EWAC conference in Lublin) and 2018. We will focus on joint work done on resistance to biotic (leaf rust, *Septoria tritici blotch, Pyrenophora tritici-repentis*) and abiotic (drought, salt, cadmium) stresses, but also on grain quality, flowering time and leaf pubescence. Loci responsible for the traits mentioned were identified performing bi-parental QTL mapping, genome wide association mapping but also by exploiting genetic stocks (isogenic lines, introgression lines, single chromosome recombinant lines, single chromosome recombinant DH lines). It is clearly demonstrated that cereals genetic stocks are an important resource for present and future genetic and molecular studies and that collaborative projects within EWAC are very successful, even more than 50 years after the foundation of the European Co-operative.

2015

Simón M R, Ayala F M, Moreno M V, Lohwasser U, Börner A: Mapping QTL for resistance against *Pyrenophora tritici-repentis* in wheat. Cer. Res. Commun. 43 (2015) 649-660.

Tan spot, caused by the fungus *Pyrenophora tritici-repentis* (Died.) Drechs is an important foliar disease of wheat (*Triticum aestivum* L.). From a set of phenotypically and molecularly characterized set of Argentinean isolates, two isolates H0019 and H0120 which do not correspond to known races of the pathogen were selected. Segregation for resistance among a set of recombinant inbred lines bred from the cross 'W7984' × cv. 'Opata 85' was used to identify the basis for resistance at the seedling stage, against those fungal isolates (H0019 and H0120), across three independent environments. On the basis of the mean performance across all three environments, a QTL against chlorosis located on the 6AS and linked to the RFLP locus *Xksuh4c* was significant for both isolates (with a LOD of 3.76 for isolate H0019 and 5.87 for H0120).

Pshenichnikova T A, Khlestkina E K, Landjeva S, Doroshkov A V, Kartseva T, Börner A, Simonov A V, Shchukina L V, Morozova E V: Genetic dissection of earliness by analysis of a recombinant chromosome substitution double haploid mapping population of bread wheat (*Triticum aestivum* L.) in different geographic regions. Euphytica 206 (2015) 191-202.

The synchronization of flowering with the time of most favorable temperatures and light has substantial effects on grain yield and quality. In bread wheat, the major developmental genes determining vernalization requirement (*Vrn-1*) and photoperiod response are used in breeding cultivars adapted to different latitudes and climates. Fine regulation of flowering date is

provided by minor genes whose allelic variation is not well studied. Although spring cultivars Saratovskaya 29 (S29) and Yanetzkis Probat (YP) carry the same two dominant *Vrn-1* alleles, YP is several days later in flowering compared to S29. The aim of the current study was to establish the chromosomal and map locations of loci determining this difference. Inter-cultivar single chromosome substitution lines S29(YP) and a set of recombinant chromosome substitution double haploid lines grown at three contrasting climatic and geographic locations in Western Siberia and Europe. The substitution line S29(YP 4D*7A) carrying the entire donor chromosome 4D and an additional fragment of chromosome 7A showed the largest delay in flowering at all sites. A quantitative trait locus (QTL) between microsatellite markers *Xgwm0089* and *Xgwm4736* on chromosome 4D was detected only in Europe following substantially earlier sowing. It was manifested under increasing day length, and, therefore, was regarded as a photoperiod response locus. Another QTL associated with $(TG)_{8 \text{ or } 9}$-$(CG)_3$ polymorphism of the *TaFTA* gene on chromosome 7A was effective under both long and short days thus representing an intrinsic earliness per se gene. This knowledge could aid the fine regulation of flowering in cultivars tailored for growing in specific agro-climatic conditions.

Shoeva O Y, Kukoeva T V, Börner A, Khlestkina E K: Barley *Ant1* is a homolog of maize *C1* and its product is part of the regulatory machinery governing anthocyanin synthesis in the leaf sheath. Plant Breed. 134 (2015) 400–405.

Anthocyanins contribute to plants' defence against a number of abiotic and biotic stress agents. The anthocyanin pigmentation of the barley leaf sheath is genetically determined by *Ant1,* a gene which maps to a region of chromosome 7HS delimited by the microsatellite loci *Xgbms0226* and *Xgbms0240*. The sequence of the maize gene *C1* (encoding an R2R3 MYB factor regulating anthocyanin synthesis) was used for the PCR-based cloning of *Ant1*. In *ant1* genotypes, no transcript is generated in the leaf sheath, whereas the gene is active in the presence of the dominant allele. A comparison of the coding and promoter sequences of *Ant1* (which induces purple pigmentation in the leaf sheath) and *ant1* (which does not) showed that the key polymorphisms lay in the promoter sequence. The transcription of four anthocyanin synthesis structural genes (*Chi, F3h, Dfr, Ans*) was dependent on the allelic status of *Ant1*.

2016

Shoeva O Y, Mock H-P, Kukoeva T V, Börner A, Khlestkina E K: Regulation of the flavonoid biosynthesis pathway genes in purple and black grains of *Hordeum vulgare*. PLoS One 11 (2016) e0163782.

Barley grain at maturity can have yellow, purple, blue, and black pigmentations which are suggested to play a protective role under stress conditions. The first three types of the colors are caused by phenolic compounds flavonoids; the last one is caused by phytomelanins, oxidized and polymerized phenolic compounds. Although the genetic basis of the flavonoid biosynthesis pathway in barley has been thoroughly studied, there is no data yet on its regulation in purple and black barley grains. In the current study, genetic model of *Hordeum vulgare* 'Bowman' near-isogenic lines (NILs) was used to investigate the regulation of the flavonoid biosynthesis in white, purple, and black barley grains. Microsatellite genotyping revealed donor segments in the purple- and black-grained lines on chromosomes 2H (in region of the *Ant2* gene determining purple color of grains) and 1H (in region of the *Blp* gene determining black lemma and pericarp), respectively. The isolated dominant *Ant2* allele of the purple-grained line has high level of sequence similarity with the recessive Bowman's *ant2* in coding region, whereas

an insertion of 179 bp was detected in promoter region of *ant2*. This structural divergence between *Ant2* and *ant2* alleles may underlie their different expression in grain pericarp: Bowman's *Ant2* is not transcribed, whereas it was up-regulated in the purple-grained line with coordinately co-expressed flavonoid biosynthesis structural genes (*Chs*, *Chi*, *F3h*, *F3'h*, *Dfr*, *Ans*). This led to total anthocyain content increase in purple-grained line identified by ultra-performance liquid chromatography (HPLC). Collectively, these results proved the regulatory function of the *Ant2* gene in anthocyanin biosynthesis in barley grain pericarp. In the black-grained line, the specific transcriptional regulation of the flavonoid biosynthesis pathway genes was not detected, suggesting that flavonoid pigments are not involved in development of black lemma and pericarp trait.

Osipova S, Permyakov A, Permyakova M, Pshenichnikova T, Verkhoturov V, Rudikovsky A, Rudikovskaya E, Shishparenok A, Doroshkov A, Börner A: Regions of the bread wheat D genome associated with variation in key photosynthesis traits and shoot biomass under both well watered and water deficient conditions. J. Appl. Genet. 57 (2016) 151-163.

A quantitative trait locus (QTL) approach was taken to reveal the genetic basis in wheat of traits associated with photosynthesis during a period of exposure to water deficit stress. The performance, with respect to shoot biomass, gas exchange and chlorophyll fluorescence, leaf pigment content and the activity of various ascorbate-glutathione cycle enzymes and catalase, of a set of 80 wheat lines, each containing a single chromosomal segment introgressed from the bread wheat D genome progenitor *Aegilops tauschii*, was monitored in plants exposed to various water regimes. Four of the seven D genome chromosomes (1D, 2D, 5D, and 7D) carried clusters of both major (LOD >3.0) and minor (LOD between 2.0 and 3.0) QTL. A major QTL underlying the activity of glutathione reductase was located on chromosome 2D, and another, controlling the activity of ascorbate peroxidase, on chromosome 7D. A region of chromosome 2D defined by the microsatellite locus *Xgwm539* and a second on chromosome 7D flanked by the marker loci *Xgwm1242* and *Xgwm44* harbored a number of QTL associated with the water deficit stress response.

2017

Gerard G S, Börner A, Lohwasser U, Simón M R: Genome-wide association mapping of genetic factors controlling Septoria tritici blotch resistance and their associations with plant height and heading date in wheat. Euphytica 213 (2017) 27.

Septoria tritici blotch (STB), caused by the ascomycete fungus *Zymoseptoria tritici* (also known as *Mycosphaerella graminicola*), is one of the most devastating foliar wheat diseases worldwide. Host resistance is the most effective strategy for management of the disease. A factor that complicates the determination of resistance is its reported interaction with heading date (Hd) and plant height (Ph). In this study, we report findings from a genome-wide association study of resistance to STB in a world-wide collection of 96 wheat accessions. The collection was evaluated under conditions of artificial infection for seedling and adult plant STB resistance, Hd and Ph in field trials. Marker-trait associations (MTAs) were detected using a mixed linear model. STB disease severities showed significant phenotypic variation. In total, 73 MTAs involving STB resistance were detected. The chromosomal locations of some of them were similar to known *Stb* genes or quantitative trait loci; whereas others were detected in new genomic regions. The field experiment showed evidence of genetic association between STB resistance and Hd, but only for a few genotypes. This was corroborated at the molecular level,

where a total of eight genomic regions associated with STB resistance were located in similar positions to MTAs for Hd. New genomic regions associated with STB resistance found here could be useful in wheat breeding aimed at controlling STB after validation in relevant genetic backgrounds.

Dobrikova A G, Yotsova E K, Börner A, Landjeva S P, Apostolova E L: The wheat mutant DELLA-encoding gene (*Rht-B1c*) affects plant photosynthetic responses to cadmium stress. Plant Physiol. Biochem. 114 (2017) 10-18.

The sensitivity to cadmium (Cd) stress of two near-isogenic wheat lines with differences at the *Rht-B1* locus, *Rht-B1a* (tall wild type, encoding DELLA proteins) and *Rht-B1c* (dwarf mutant, encoding modified DELLA proteins), was investigated. The effects of 100 μM $CdCl_2$ on plant growth, pigment content and functional activity of the photosynthetic apparatus of wheat seedlings grown on a nutrient solution were evaluated through a combination of PAM chlorophyll fluorescence, oxygen evolution, oxidation-reduction kinetics of P700 and 77 K fluorescence. The results showed that the wheat mutant (*Rht-B1c*) was more tolerant to Cd stress compared to the wild type (*Rht-B1a*), as evidenced by the lower reductions in plant growth and pigment content, lower inhibition of photosystem I (PSI) and photosystem II (PSII) photochemistry and of the oxygen evolution measured with Clark-type and Joliot-type electrodes. Furthermore, the enhanced Cd tolerance was accompanied by increased Cd accumulation within mutant plant tissues.

The molecular mechanisms through which the *Rht-B1c* mutation improves plant tolerance to Cd stress involve structural alterations in the mutant photosynthetic membranes leading to better protection of the Mn cluster of oxygen-evolving complex and increased capacity for PSI cyclic electron transport, protecting photochemical activity of the photosynthetic apparatus under stress. This study suggests a role for the *Rht-B1c*-encoded DELLA proteins in protective mechanisms and tolerance of the photosynthetic apparatus in wheat plants exposed to heavy metals stress.

Shchukina L V, Pshenichnikova T A, Chistyakova A K, Khlestkina E K, Börner A: Properties of grain, flour and dough in bread wheat lines with *Aegilops markgrafii* introgressions. Cereal Res. Commun. 45 (2017) 296-306.

Various milling parameters, wet gluten content and key dough properties were analyzed for two sister lines of bread wheat with *Ae. markgrafii* introgressions in genetic background of cultivar Alcedo carrying a set of sub-chromosomal alien segments on chromosomes 2AS, 2BS, 3BL, 4AL and 6DL. The lines revealed higher grain vitreousness, larger particle size of flour, and higher wet gluten content in grain compared to cv. Alcedo. The flour from these lines also showed excellent water absorption and developed more resilient dough. The introgressions in the Alcedo genome caused no reduction in 1,000-grain weight. General improvement of the grain technological properties appears to be the result of introgressions into 2AS, 2BS and 3BL chromosomes. Coincidence of locations of *Ae. markgrafii* introgressions in chromosome with the QTLs positions for technological traits, revealed in bread wheat mapping populations, is discussed.

Glagoleva A, Shmakov N, Shoeva O, Vasiliev G, Shatskaya N, Börner A, Afonnikov D, Khlestkina E K: Metabolic pathways and genes identified by RNA-seq analysis of barley near-isogenic lines differing by allelic state of the *Black lemna and pericarp* (*Blp*) gene. BMC Plant Biol. 17 (2017) 182.

Some plant species have 'melanin-like' black seed pigmentation. However, the chemical and genetic nature of this 'melanin-like' black pigment have not yet been fully explored due to its complex structure and ability to withstand almost all solvents. Nevertheless, identification of genetic networks participating in trait formation is key to understanding metabolic processes involved in the expression of 'melanin-like' black seed pigmentation. The aim of the current study was to identify differentially expressed genes (DEGs) in barley near-isogenic lines (NILs) differing by allelic state of the *Blp (black lemma and pericarp*) locus.

RNA-seq analysis of six libraries (three replicates for each line) was performed. A total of 957 genome fragments had statistically significant changes in expression levels between lines BLP and BW, with 632 fragments having increased expression levels in line BLP and 325 genome fragments having decreased expression. Among identified DEGs, 191 genes were recognized as participating in known pathways. Among these were metabolic pathways including 'suberin monomer biosynthesis', 'diterpene phytoalexins precursors biosynthesis', 'cutin biosynthesis', 'cuticular wax biosynthesis', and 'phenylpropanoid biosynthesis, initial reactions'. Differential expression was confirmed by real-time PCR analysis of selected genes.

Metabolic pathways and genes presumably associated with black lemma and pericarp colour as well as *Blp*-associated resistance to oxidative stress and pathogens, were revealed. We suggest that the black pigmentation of lemmas and pericarps is related to increased level of phenolic compounds and their oxidation. The effect of functional *Blp* on the synthesis of ferulic acid and other phenolic compounds can explain the increased antioxidant capacity and biotic and abiotic stress tolerance of black-grained cereals. Their drought tolerance and resistance to diseases affecting the spike may also be related to cuticular wax biosynthesis. In addition, upregulated synthesis of phytoalexins, suberin and universal stress protein (USP) in lemmas and pericarps of the *Blp* carriers may contribute to their increased disease resistance. Further description of the DEGs haplotypes and study of their association with physiological characteristics may be useful for future application in barley pre-breeding.

Permyakova M D, Permyakov A V, Osipova S V, Pshenichnikova T A, Shishparenok A A, Rudikovskaya E G, Rudikovsky A V, Verkhoturov V V, Börner A: Chromosome regions associated with the activity of lipoxygenase in the genome D of *Triticum aestivum* L. under water deficit. Russ. J. Plant Physiol. 64 (2017) 28-40.

Quantitative trait loci (QTLs) associated with the phenotypic expression of the activity of different forms of lipoxygenase (LOX) under water deficit were detected in the chromosomes of the D-genome using intogression lines of common wheat *Triticum aestivum* L. Chinese Spring (Synthetic 6x). QTL associated with the activity of seed soluble LOX was identified on the short arm of chromosome 4D. The activity of membranebound form of enzyme in the seedlings was mapped to the short arm, while that of a soluble form was on the long arm of chromosome 5D. Two regions responsible for the activity of soluble LOX in the leaves were found on the short arm of chromosome 2D. Three QTLs associated with the activities of chloroplast LOXs were found on the same chromosome: the activity of the soluble form was linked to *Xgwm261* and *Xgwm539* markers, and the membrane form to *Xgdm93* marker. QTLs for the activities of both soluble and membrane-bound LOX in the leaves were identified in the

centromeric region of chromosome 7D. The activities of two membrane enzymes in the leaves were linked to *Xgdm130* marker on the short arm of this chromosome. Loci associated with the activity of different LOX forms colocalized with QTLs for the shoot mass, gas exchange parameters, chlorophyll fluorescence, content of photosynthetic pigments, and grain productivity of wheat. A correlation between these parameters and the LOX activity was detected and it was shown that various forms of the enzyme were differentially involved in the adaptation of wheat plants to water deficit. The current paper discusses their presumed physiological role.

Pshenichnikova T A, Doroshkov A V, Simonov A V, Afonnikov D A, Börner A: Diversity of leaf pubescence in bread wheat and relative species. Genet. Resour. Crop Evol. 64 (2017) 1761–1773.

Interspecific hybridization and polyploidization are the characteristic processes of evolution in the world of plants. The allopolyploid genomes undergo numerous structural rearrangements associated with the adaptation of separate genomes to each other. An important issue is to establish which part of the total diversity of genes characteristic of the ancestral forms have been preserved and manifested in the complex genomes. The aim of this work was to compare the diversity of the adaptive morphological trait-leaf pubescence among the relatives and the ancestors of hexaploid wheats to establish the variability of its phenotypic manifestation as a result of evolution and domestication. This was achieved through the study of quantitative characteristics of leaf pubescence among 47 representatives of di-, tetra- and hexaploid species of genera *Triticum* and *Aegilops*, the donors of the individual genomes of the allopolyploid *Triticum* species. Quantification of leaf pubescence was based on automated counting of the trichome number (*N*) and trichome length (*L*) estimation on a leaf fold and calculation of the pubescence index *H* (*L*/*N*). The species with different sets of elementary genomes differed for the type of pubescence. The ploidy level affected only the trichome length and the index of pubescence $H_{L/N}$. The density of the hairs was affected by the individual genomes A and B, whereas genome D significantly influenced all of the studied pubescence parameters. The diploid species showed the largest variability while the cultivated durum wheats lacked pubescence. Bread wheat demonstrated pubescence characterized by a close correlation between *N* and *L*.

Simonov A V, Chistyakova A K, Morozova E V, Shchukina L V, Börner A, Pshenichnikova T A: The development of a new bread wheat genotype carrying two loci for endosperm softness. Vavilov J. Genet. Breed. 21 (2017) 341-346.

The technological purpose of grain and flour wheat is largely determined by the grains endosperm structure. Its variability among wheat varieties depends mainly on the multiple allelism for a single *Ha* locus on chromosome 5D leading to a continuous variation of the trait. The grain endosperm can vary from hard and vitreous suitable for yeast baking to soft and floury favorable for confectionery and technical purposes. Furthermore, these traits, especially vitreousness, are strongly influenced by the growth conditions. Earlier, the *Ha-Sp* locus was introgessed into chromosome 5A of the bread wheat line 84/98w from Aegilops speltoides Tausch., which reduces endosperm hardness and vitreousness, like the dominant allele of the Ha locus. This paper is the first to describe the obtaining and testing of the supersoft lines combining in their genotype the homoeoallelic loci *Ha-Sp* of the line 84/98w and Ha of the soft grain cultivar Chinese Spring. The lines were isolated from 6–8 generations of self-pollinated F2 hybrids. They consistently exhibit a greater grain softness than the parental forms under both greenhouse and field conditions. These lines can be used in the breeding of wheat cultivars, the

flour of which will not require chemical baking powder in the confectionery industry. It is also possible to use them for technical purposes for the production of bioethanol. In addition, these lines may serve as a genetic model for the study of the functional activity of homoeoallelic genes in the complex polyploid genomes of plants.

Strygina K V, Börner A, Khlestkina E K: Identification and characterization of regulatory network components for anthocyanin synthesis in barley aleurone. BMC Plant Biol. 17 (2017) 184.

Among natural populations, there are different colours of barley (*Hordeum vulgare* L.). The colour of barley grains is directly related to the accumulation of different pigments in the aleurone layer, pericarp and lemma. Blue grain colour is due to the accumulation of anthocyanins in the aleurone layer, which is dependent on the presence of five *Blx* genes that are not sequenced yet (*Blx1*, *Blx3* and *Blx4* genes clustering on chromosome 4HL and *Blx2* and *Blx5* on 7HL). Due to the health benefits of anthocyanins, blue-grained barley can be considered as a source of dietary food. The goal of the current study was to identify and characterize components of the anthocyanin synthesis regulatory network for the aleurone layer in barley.

The candidate genes for components of the regulatory complex MBW (consisting of transcription factors **M**YB, **b**HLH/MYC and **W**D40) for anthocyanin synthesis in barley aleurone were identified. These genes were designated *HvMyc2* (4HL), *HvMpc2* (4HL), and *HvWD40* (6HL). *HvMyc2* was expressed in aleurone cells only. A loss-of-function (frame shift) mutation in *HvMyc2* of non-coloured compared to blue-grained barley was revealed. Unlike aleurone-specific *HvMyc2*, the *HvMpc2* gene was expressed in different tissues; however, its activity was not detected in non-coloured aleurone in contrast to a coloured aleurone, and allele-specific mutations in its promoter region were found. The single-copy gene *HvWD40*, which encodes the required component of the regulatory MBW complex, was expressed constantly in coloured and non-coloured tissues and had no allelic differences. *HvMyc2* and *HvMpc2* were genetically mapped using allele-specific developed CAPS markers developed. *HvMyc2* was mapped in position between SSR loci *XGBS0875-4H* (3.4 cM distal) and *XGBM1048-4H* (3.4 cM proximal) matching the region chromosome 4HL where the *Blx*-cluster was found. In this position, one of the anthocyanin biosynthesis structural genes (*HvF3'5'H*) was also mapped using an allele-specific CAPS-marker developed in the current study.

The genes involved in anthocyanin synthesis in the barley aleurone layer were identified and characterized, including components of the regulatory complex MBW, from which the MYC-encoding gene (*HvMyc2*) appeared to be the main factor underlying variation of barley by aleurone colour.

2018

Gerard G S, Kobiljski B, Lohwasser U, Börner A, Simón M R: Genetic architecture of adult plant resistance to leaf rust in wheat association mapping panel. Plant Pathol. 67 (2018) 584-594.

Leaf rust caused by *Puccina triticina* is one of the most destructive fungal diseases of wheat (*Triticum aestivum*). Adult plant resistance (APR) is an effective strategy to achieve long-term protection from the disease. In this study, findings are reported from a genome-wide association study (GWAS) using a panel of 96 wheat cultivars genotyped with 874 Diversity Arrays

Technology (DArT) markers and tested for adult leaf rust response in six field trials. A total of 13 quantitative trait loci (QTL) conferring APR to leaf rust were identified on chromosome arms 1BL, 1DS, 2AS, 2BL, 2DS, 3BS, 3BL, 4AL, 6BS (two), 7DS, 5BL/7BS and 6AL/6BS. Of these, seven QTLs mapped close to known resistance genes and QTLs, while the remaining six are novel and can be used as additional sources of resistance. Accessions with a greater number of combined QTLs for APR showed lower levels of disease severity, demonstrating additive and significant pyramiding effects. All QTLs had stable main effects and they did not exhibit a significant interaction with the experiments. These findings could help to achieve adequate levels of durable resistance through marker-assisted selection and pyramiding resistance QTLs in local germplasm.

Jusovic M, Velitchkova M Y, Misheva S P, Börner A, Apostolova E L, Dobrikova A G: Photosynthetic responses of a wheat mutant (*Rht-B1c*) with altered DELLA proteins to salt stress. J. Plant Growth Reg. 37 (2018) 645-656.

Salinity increases in the world's land area and significantly affects the rate of photosynthesis and corresponding plant growth. In this study, the impact of salt stress (200 mM NaCl equivalent to an electrical conductivity of 18.6 mS cm^{-1}) on the photosynthetic apparatus and some growth parameters were investigated in wheat DELLA mutant (*Rht-B1c*) and wild-type (*Rht-B1a*) seedlings grown on a half-strength Hoagland solution. Results revealed that salt toxicity was alleviated in the *Rht-B1c* mutant compared to the *Rht-B1a* wild type, as manifested by less-reduced leaf pigment content, relative water content, and photochemical activity of photosystem II (PSII) and photosystem I (PSI) after a 9-day salt exposure of plants. Compared to the wild-type wheat, a higher capacity for PSI-dependent cyclic electron flow, preventing the photosynthetic apparatus from oxidative damage, was observed in the mutant plants before and after salt treatment. In addition, an increase of PsaB proteins was detected in the mutant plants after long-term salt stress unlike the wild type. The observed higher oxidation level of P700 ($P700^+$) in the mutant was consistent with higher abundance of PSI-related protein complexes. The data demonstrated that alterations in thylakoid membrane proteins and/or their structural reorganization in wheat DELLA mutant (*Rht-B1c*) significantly contribute to the alleviation of salt-induced damage of the photosynthetic apparatus. Molecular mechanisms involved in the photosynthetic responses of wheat DELLA mutants to salt stress are discussed.

Kartseva T, Börner A, Misheva S: Wheat semi-dwarfing genes affect plant response to drought-induced oxidative stress in a genotype dependent matter. Genet. Plant Physiol. 8 (2018) 38-50.

The genotype-specific impact of three gibberellin-insensitive height reducing genes (*Rht* genes) on wheat plant response to oxidative stress provoked by water deficit was investigated. Seedlings (6-day-old) of six near-isogenic lines (NILs) (*Rht-B1a+-D1a (rht), Rht- B1b, Rht-B1c, Rht-D1b, Rht-B1b+ -D1b* and *Rht-B1c+-D1b*) in four cultivar backgrounds were exposed to 15% polyethylene glycole-induced osmotic stress for 8 days. Main growth parameters and leaf content of free proline, hydrogen peroxide (H_2O_2) and malondialdehyde (MDA) were measured to assess plant stress tolerance and the corresponding level of oxidative stress. All factors (treatment, *Rht* allele and cultivar) and their interactions had significant effects on the growth parameters and stress indicators. Tolerance index of root length on average over the four cultivar backgrounds decreased in the order: *Rht-B1c+-D1b* (63%) > *Rht-B1b+-D1b* ≈ *Rht-B1c* ≈ *Rht-D1b* ≈ *Rht-B1b* > *rht* (53%), while tolerance index of shoot length insignificantly depended on the *Rht* allele. However, the percentage reduction in root and shoot length in *Rht* NILs varied appreciably among the cultivars. On average over the genetic backgrounds, the stress markers assay of dehydrated plants showed the lowest H_2O_2 content in lines carrying the

allele *Rht-D1b* in the background of the cultivars 'April Bearded', 'Bersée' and 'Maris Huntsman'. This allele in the 'Maris Widgeon' background had the opposite effect on the H_2O_2 contents under simulated drought. No difference in the leaf MDA content was observed between the *Rht* NILs both in control and stressed plants on average over cultivars. The highest accumulation of free proline in controls was measured in plants carrying the combinations *Rht-B1b+-D1b* and *Rht-B1c+- D1b*; however, under stress, the *Rht-B1b* plants accumulated proline to the highest degree. The observed general effect of individual *Rht* alleles varied depending on the genotypic background. This information accentuates the need for an accurate choice of an *Rht* allele when introducing them into a specific genetic background to develop a drought tolerant cultivar.

Shchukina L V, Pshenichnikova T A, Khlestkina E K, Misheva S, Kartseva T, Abugalieva A, Börner A: Chromosomal location and mapping of quantitative trait locus determining technological parameters of grain and flour in strong-flour bread wheat cultivar Saratovskaya 29. Cer. Res. Commun. 46 (2018) 628-638.

Bread wheat is the primary bread crop in the majority of countries in the world. The most important product that is manufactured from its grain and flour is yeast bread. In order to obtain an excellent bread, grain with high physical properties is needed for flour and dough. The Russian spring wheat cultivar Saratovskaya 29 is characterized by its exclusively high physical properties of flour and dough. The purpose of this work was to identify the chromosomes carrying the main loci for these traits in Saratovskaya 29 and to map them using recombinant substitution lines genotyped with molecular markers. A set of inter-varietal substitution lines Saratovskaya 29 (Yanetzkis Probat) was used to identify the "critical" chromosomes. The donor of individual chromosomes is a spring cultivar with average dough strength and tenacity. Substitution of 1D and 4D*7A chromosomes in the genetic background of Saratovskaya 29 resulted in a significant decrease in the physical properties of the dough. Such a deterioration in the case of 1D chromosome might be related to the variability of gluten protein composition. With the help of recombinant substitution double haploid lines obtained from a Saratovskaya 29 (Yanetzkis Probat 4D*7A) substitution line the region on the 4D chromosome was revealed in the strong-flour cultivar Saratovskaya 29, with the microsatellite locus *Xgwm0165* to be associated with the unique physical properties of flour and dough. The detected locus is not related to the composition gluten proteins. This locus may be recommended to breeders for the selection of strong-flour cultivars. Additionally, a QTL associated with vitreousness of grain was mapped in the short arm of chromosome 7A.

Aspects of wheat cytogenetics and aneuploidies at NARDI- Fundulea

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Wheat cytogenetics and aneuploid researches started at NARDI-Fundulea 5 decades ago when first haploid forms were identified among pairs of twins and triplets coleoptiles from germinated polyembrionated seeds (Figure 1). On the average of three year tests, the highest frequency of polyembrionated seeds was found in a breeding line F.147-63 (1 pair of twins to 2,074 seeds) and the lowest one (1 to 9,325 seeds) in the cultivar Dacia. In an attempt to classify twin coleoptiles by theirs size, from a total of 228 twins, 38.8% were equal well developed, 60.1% were of different size and 3.1% presented outstanding differences (Giura, 1969). The frequency of haploid was greater among twins having a market different size, the smallest one being, as a rule, haploid.

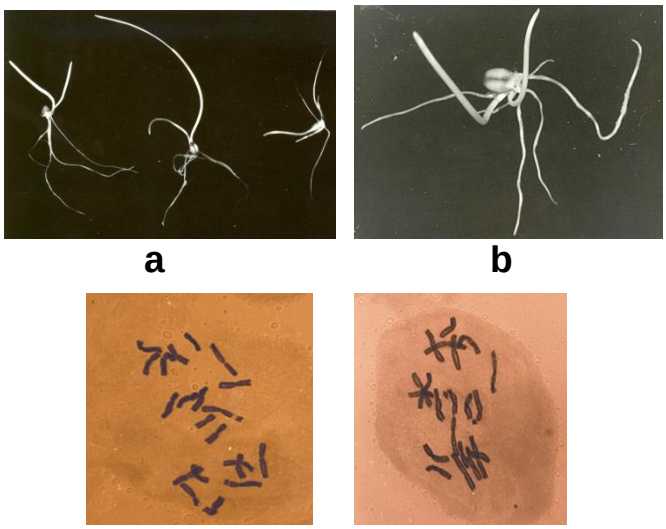


Fig. 1: Twins coleoptiles of different size (a), equal size (b) and haploid somatic metaphase (n=21).

At that time, there was also the intention to develop chromosome deficient lines following the same procedure used by Ernie Sears i.e. to cross the haploids by normal wheat and sorting in progeny for heteroploid and aneuploid types.

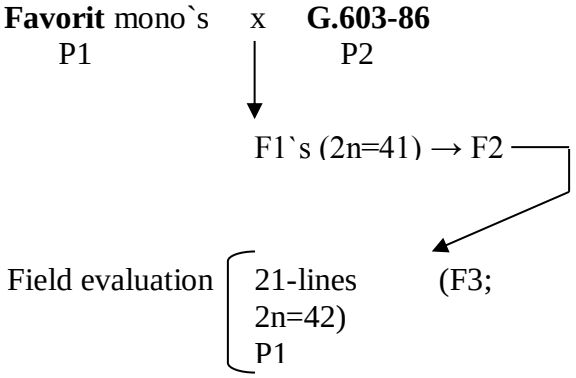
After these preliminary explorations we proceeded to develop a monosomic serie in the variety Bezostaya-1 under EWAC-cooperative project using intervarietal transfers of aneuploid condition from Chinese Spring (CS), monosomic set developed by E. Sears and provided by PBI- Centre, Cambridge, UK. Complete monosomics set of Bezostaia-1 with 2-3 sub lines per each chromosome was finished (Bc.10), in 1981.

In the same time, but under a COMMECON agreement, another monosomic complete set with 2-3 sub-lines per each chromosome on Romanian cultivar Favorit was finished in 1983, based on winter wheat Cheyenne monosomics, kindly provided by Rosalind Morris, Univ.Nebraska.

It is worthy to mention that during the series of backcross generations, both monosomics sets were periodically checked for "*univalent shift*" by test crosses with CS- telo, ditelo or double ditelo lines, provided also by PBI-Centre.

Monosomics set of the Romanian cultivar Favorit has been prevalently used in genetic analyses to elucidate the genetic control for some traits of agronomic interest, to identify new gene and evaluate their function in a genetic background represented by cultivars and advanced breeding lines developed by our institute. Thus, in case of Fundulea 133 cultivar that in 1988 occupied around 18% of the cultivated area with wheat in Romania and was used as genitor in many hybrid combinations, the genetic determinism study of adult plant resistance to brown rust was considered appropriate at that period. By using monosomic F2 analyses, it was found that resistance to brown rust (APR race 77-73) of F.133 genotype is controlled by two dominant complementary genes, one located on chromosome 1B (*Lr26*) and the other one (*Lr16*?) on chromosome 4B (Giura, 1993) Concomitantly it was found that the ear reddish-brown coloration in F.133 cultivar is controlled by *Rg* gene ('brown ear'), located on 1B chromosome in the same linkage block with Lr26 gene; the recessive allele being responsible for the white color of ears.

Genetic analysis by using Favorit monosomics was also applied, in same instances, even for the study of quantitative traits determined by the action of several genes. In such situation the disomics F3 for each chromosome were cytologically extracted from monosomic F2 populations. In the case of G.603-86 wheat line with large grain and a grain length that vary between 8.5 and 9.0 mm, genetic analysis on F3 disomic populations revealed that the large grain trait is controlled by genes located on several chromosomes (Figure 2) with positive effect for grain weight: 4A and 6D for grain length: 4A, 1B, 4D and for grain width: 1A and 1B (Giura and Săulescu, 1996). It is interesting to mention that none of the chromosomes associated with thousand kernel weight (TKW) positive variation in our study have been previously described as carrying genes that control the grain size.



Positive influence		
Grain weight	Grain length	Grain width
4A	4A	1A
6D	1B	1B
	4B	

Fig. 2: Genetic analysis of grain size in wheat line G.603-86 (Cologna x F.6-75) by using disomics F3 (2n=42) extracted from monosomic F2 populations.

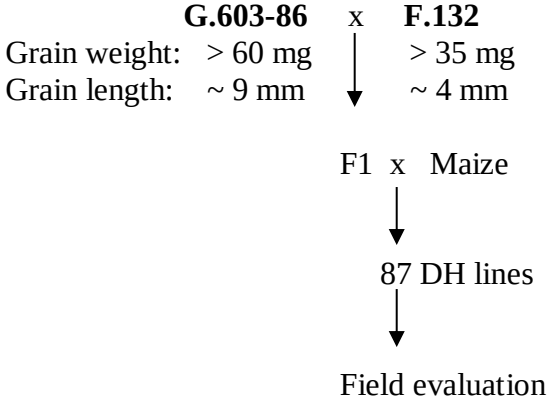


Fig. 3: Development of DH lines for genetic analysis at molecular level.

In order to advance with the genetic analysis we developed using the *Zea* system a mapping population of 87 DH (doubled haploid) lines taking as hybridization partner the breeding line F.132 with small grains (fig.3). After field evaluations, data collected will be corroborated with those resulted from the analyses at molecular level, in order to establish markers/QTL`s associated to pursued traits.

Another mapping population of sixty two DH lines from the cross between cultivar Izvor (high osmotic adjustement) and Jiana (medium osmotic adjustement) was used to study the relationships between genetic differences in capacity of osmotic adjustment and other measures of drought resistance (Bănică et al.2008) and the association between the capacity for osmotic adjustement, as estimated by the pollen grain test developed by Morgan (1999) and several SSR markers located on chromosome 7A. SSR markers Xwmc9, Xwmc596 and Xwmc603 were significantly associated with pollen grain response to immersion in solution of 55%PEG +KCL, being located at approximately 9.1 cM estimated distance from the *Or* gene (Ciucă et al., 2010).

As a result of special mutagenic protocol application consisting of recurrent irradiation (two cycles), hybridization of M1`s and DH technology, a new and different mapping population of 441 mutated/recombinant DH lines was generated (Giura, 2013) and studied in field conditions and several, at molecular level too (Dobre, et al., 2014; 2016 a, 2016 b, 2018).

The Favorit monosomics were also used to develop complete set or partial sets of intervarietal substitution lines and also for transferring individual chromosomes carrying useful genes in cultivated gene pools for breeding purposes (substitution with limited recombination).

In the case of breeding line F.27-70 noticed at national and international level for its high grain protein content and superior productivity compared to the American wheat variety Atlass-66 (Ceapoiu et al.1977), a study of the genetic control of grain protein control and baking quality traits was initiated at that time. By using substitution lines Favorit/F.26-70 it was found that genetic determinism for pursued traits is more complex, being involved 4 chromosomes: 4B, 4D, 5B and 5D in comparison with Atlas 66 where only two chromosomes 5A and 5D were involved in high grain protein level (Morris et all., 1978). The involvement of chromosome 7B in controlling the protein content and rheological properties of the dough (high gluten tenacity) and a 3 days earlier flowering compared to Favorit parent were also emphasized (Giura and Ittu, 1986; Giura et all., 1986). The main differences between 7B substitution line and recipient parent Favorit for same traits are presented in table 1.

Table 1: Differences for several traits between substitution line Favorit/F.26-70(7B) and the recipient parent Favorit.

	Donor parent F.26-70	Recipient parent Favorit	Substitution line Favorit/F.26-70 (7B)	Difference
Date of heading (no days after 1 May)	26	29	26	-3 days
TKW	40.83	43.94	43.70	- 0.24
Protein (%N x 5.7)	16.26	14.41	15.19	+0.78*
Protein (mg/grain)	5.89	5.46	5.77	+0.31**
Protein yield (q/ha)	5.64	5.61	5.90	+0.29*
Yield (q/ha)	40.06	45.43	45.58	+0.15
Some dough reological characteristcs:				
Farinograf development time (min)	2.5	4.0	5.5	+1.5**
Farinograf mixing (min)	1.0	2.5	4.5	+2.0**
Extensograme/ action (135`)	0.75	1.04	2.11	+1.7**
Loaf volume (cm^3)	591	544	548	+4.0

*) P 0.05-0.01; **) P <0.01

A more detailed analysis for chromosome 7B was attained using substitution recombinant lines Favorit/F.26-70(7B) produced by using both classic procedure (development of recombinant disomics) and *Zea* system (development of recombinant DHL`s via recombinant haploids). The later procedure is quicker and more reliable. The study was carried out within EWAC cooperation between Germany, Russia and Romania. It was found that earlier flowering under long days is controlled by a new identified gene *Bpd-B2*, located on the short arm of the chromosome 7B at 8.8 cM distal and 20.7 cM proximal to microsatellite markers Xgwm0537 and Xgwm 0255, respectively. The flowering acceleration induced by *Ppd-B2* gene was significantly correlated with higher protein content and a major gene for this character *Gpc-B2* (Grain protein content-B2) was mapped at 4.4 cM proximal to Ppd-B2 (Khlestkina et al. 2009). It was remarked that this gene does not affect grain size and there was not noted a negative correlation between yield and protein content (Giura et al. 2008). Therefore, this gene is probably involved in nitrogen uptake and/or translocation and the carriers need to be farther analyzed.

Chromosome engineering procedure of intervarietal substitution has been also applied to develop new genetic stocks carrying the *kr1* recessive allele for promoting intergeneric crossability and allele *ph1b* of allosindetic pairing induction.

In crossability tests of wheat genotipes including the substitution lines for Chinese Spring (CS)-5B chromosome (*kr1kr1*) with several rye genotypes and breeding lines it was noticed an increased crossability from 2.58% in the case of Favorit cultivar to 19.30% for substitution line Favorit/CS-5B. Similarly, the substitution line Bezostaia-1/CS-5B presented 12.47% crossability in test-crosses with rye against only 3.77% recorded for the cultivar Bezostaia-1.

However, in the meantime, a new opportunity has emerged by casually identification of an advanced semi-dwarf (*RhtB1*) breeding line F.132 showing a good but variable cross compatibility with rye genotypes. This line carries also *Lr34* and *Lr67* genes that confer good

resistance to leaf rust and has in ascendance Pecking-8 genotype of Chinese origin from which probably inherited crossability feature. Separating the component biotypes by DH technology, it was possible to select several DH lines with crossability ranging from 25.5 to 29.2% (Giura, 2002). Seven lines retested in two seasons presented seed set values of 25.4-35.4%. One of the lines, DH F.132-1-30 (carrying probably *kr1*) was then selected as donors in a program aiming to gather both kr1 and kr2 alleles in a genetic background more adapted to local conditions. As partners were used two sub-lines of Martonvasari-9 genotype both carrying *kr1kr1* and *kr2kr2* alleles (kindly provided by Marta Molnar-Lang, Martonvasar Institute, Hungary). The F1`s hybrids were then crossed with maize and 151 DH line produced. These lines were crossed with rye genotype Harkovskaia. Finally some DH lines with higher percent crossability ranging from 57.34 to 77.60 were selected (Giura, 2016). These winter wheat DH lines with superior crossability and agronomic traits, well adapted to local conditions represent a new challenge to extent donor spectra of related species for alien genes introgressions.

Because intergeneric hybridization is only the preliminary step for accessing new genetic variability, the induction of meiotic allosyndetic recombination becomes decisive step to broadening the genetic bases by alien gene introgression from distant related species into cultivated gene pools. In practice this could be realize by using either a genotype deficient for 5B chromosome (monosomic or nullisomic) or a substitution lines carrying *ph1* mutant gene.

In an attempt to exploit genetic variability of *Aegilops.crassa*(6x), and to increase the chances of recombinations between the chromosomes of D genomes and even of M^{cr} genome with wheat homoeologous chromosomes, in the first cross were used the Favorit monosomics 5B as female parent. Deficiency for 5B was reflected in significant increase of multivalent meiotic associations. Meiotic metaphase analysis in F1`s and in Bc1 showed an increase of allosydetic recombination highlighted by tetravalents and pentavalents presences (Table 2 and Figure 4). After repeated cycles of backcrosses and phenotype selections, most of the introgression lines obtained was distinguished by high grain protein content between 14.6 to 18.9% and high quality parameters (Figure 5). It is assumed that both high protein and especially quality indices are traits controlled by genes transferred from *Ae. crassa*(6x). Previous study for quality parameters during backcross generations have reveled the presence of gliadin fractions specific for D genomes of *Ae. crassa*(6x) (Giura, A., 1982).

Table 2: Average of chromosomal association in meiotic methaphase (MI) in intergeneric hybrids Favorit/Aegilops crassa (6x) in presence/absence of 5B chromosome and in Bc1 generation.

	2n=	No. cels	I	II stick	II ring	Total	III	IV	V
Favorit/Ae.crassa(6x)	42	45	34,98± 0.31	3.07± 0.19	0.24± 0.10	3.31± 0.15	0.13± 0.05	-	-
Mono5B Favorit/ Ae.crassa(6x)	41	35	20.94± 0.54	5.51± 0.35	2.63± 0.29	8.14± 0.34	0.97± 0.21	0.14± 0.06	0.06± 0.04
Bc1 Mono5B Favorit/ Ae. crassa(6x)//Bezostaia1	41	29	12.62± 0.85	8.48± 0.47	5.76± 0.37	14.24± 0.44	0.17± 0.09	0.17± 0.09	-

*)I=univalents; II=bivalents; III=trivalents; IV=tetravalents; V=pentavalents

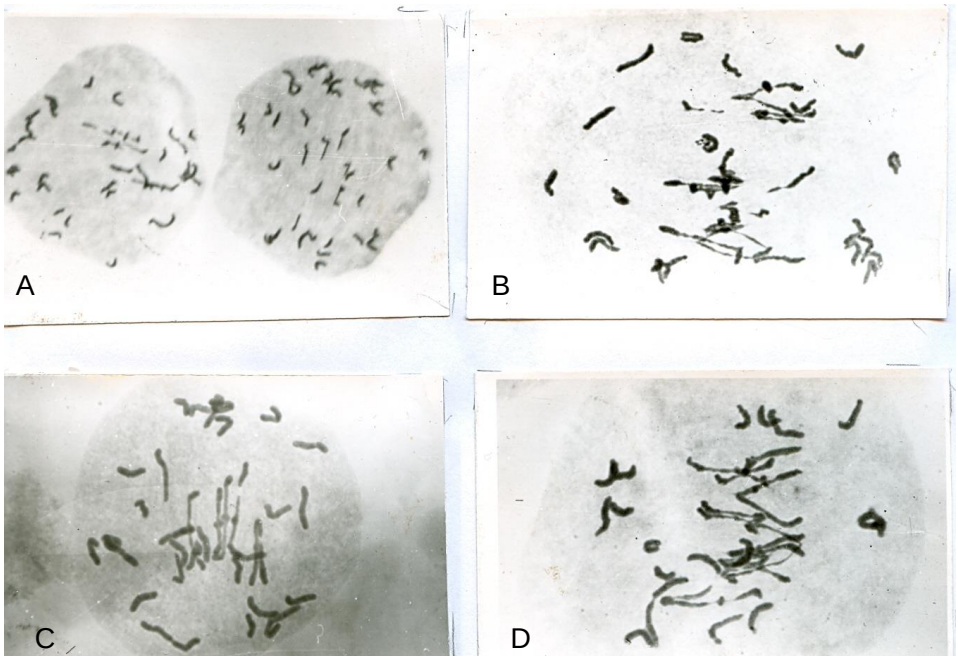
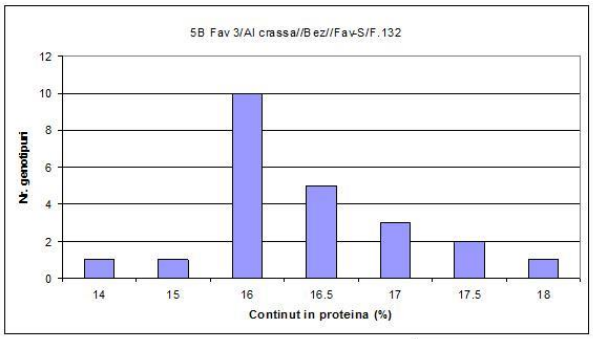
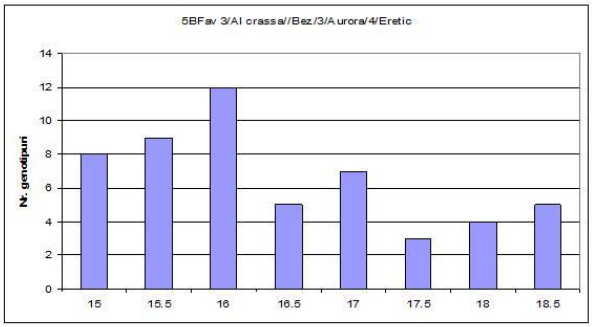


Fig. 4: Meiotic methaphase (MI) in intergeneric F1 hybrids Favorit / Ae. crassa (6x)
A – Reduced meiotic pairing: chromosome 5B present
B, C, D – High levels of chromosome pairing (allosyndetic pairing) in absence of chromosome 5B: bivalents (II); trivalents (III); tetravalents (IV); pentavalents (V).



Dispersion of introgression lines for protein content
5BFav.3/Ae.crassa(6x)//Bez./3/Fav./4/F.132



Dispersion of introgression lines for protein content
5B Fav.3/Ae.crassa(6x)//Bez./3/Aurora/4/Eretic

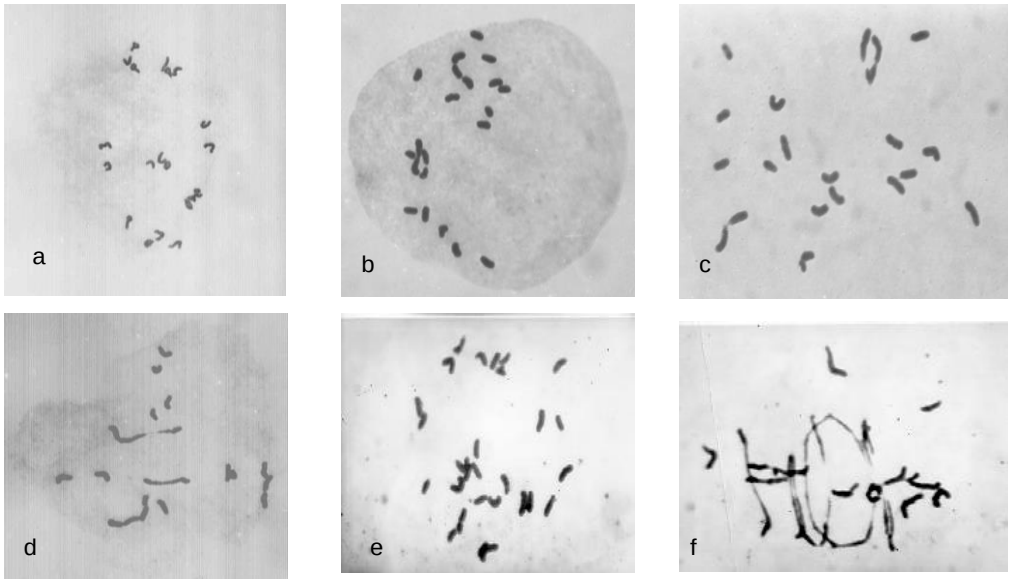
Fig. 5: Protein content in several wheat introgression lines.

A comparative analyses regarding meiotic allosyndetic pairing in F1`s hybrids wheat x rye involving Favorit genotype (Fav), Favorit- mono 5B (2n=41), Chinese Spring 5B (*ph1b ph1b*), and substitution lines Fav.(CS *ph1ph1*) showed that the deficiency for chromosome 5B in Favorit might induce the highest rate of meiotic pairing between wheat and rye chromosomes (Table 3 and Figure 6).

Table 3: Methaphase MI analysis in F1 wheat / rye hybrids with different alleles at *Ph1* locus.

Wheat genotypes	Rye genotypes	F1 2n=	No.analized cells	I	II	III	IV	Y	Chiasmata
Favorit (*Ph1Ph1*)	S. 145	28	91	27,5± 0.13 (22-28	0.2± 0.06 (0-3)	-	-	-	0.2±0.06 (0-3)
Favorit mono 5B (*Ph1* -)	Mallis	27	88	12.5± 0.04 (5-23)	4.5± 0.19 (1-9)	1.8± 0.13 (0-4)	0.02± 0.01 (0-1)	0.01± 0.01 (0-1)	10.8±0.36 (5-17)
Chinese Spring (CS) (*ph1bph1b*)	Sv.145	28	101	13.9± 0.26 (5-22)	4.5± 0.15 (1-8)	1.6± 0.10 (0-4)	0.07± 0.02 (0-1)	-	10.0±0.22 (6-15)
Favorit / CS-5B (*ph1b ph1b*)	Sv. 145	28	100	14.4± 0.30 (5-20)	4.6± 0.15 (1-9)	1.4± 0.11 (0-4)	0.02± 0.01 (0-1)	-	8.9±0.25 (4-15)

*) I= univalents; II= bivalents; III= trivalents; IV= tetravalents; V= pentavalents



Metaphase I in wheat haploids (n=21)

a) 21^{I}; b)$15^{I} + 3^{II}$ stick; c) $16^{I} + 1^{II}$ stick + 1^{III}; d) $12^{I} + 3^{II}$ stick+1^{III}

Metaphase I in intergenerici F1 hybrids wheat-rye (2n=28 and 2n=27)

e) 2n=28; 28^{I}

f) 2n=27 (deficiency for 5B): $10^{I} + 4^{II}$ stick + $1^{IV} + 1^{V}$

Fig. 6: Meiotic M_I configuration in wheat haploids and in F1`s wheat x rye hybrids in presence/absence of 5B chromosome.

In the same context, species related to wheat have been and are still used to enrich genetic diversity of cultivated germplasm by applying several cytogenetic procedures. It is argued that for long-term sustainability of wheat production, new genes or allelic variants are imperatively needed. Consequently, the interest for gene transfers from wild related species into cultivated wheat gene pool has been renewed. Depending of the degree of phylogenetic relationships, classical methods of direct hybridization, with the use of modern durum and common wheat varieties as maternal forms, selection and incomplete backcrossing, as well as processed based on the development of special genetic stocks such as synthetic hexaploids (some with tetraploid level for homologues genomes AAAA and BBB`B`, octoploids and decaploids were used to evaluate the components of related species genetic variability. Other amphiploids with higher ploidy levels were created using either wheat varieties or breeding lines carrying recessive crossability alleles, as maternal forms and diploid and hexaploid related species, as paternal form. Derived genetic materials as addition lines, substitution lines or lines of translocation were used to obtain new introgression and prebreeding lines carrying useful genes (Giura and Marinescu, 1988). Among these we can mention: a introgression line resulted from the cross Mono 5B Favorit/spontaneous substitution lines Favorit/*Aegilops variabilis* with resistance to prevalent races of brown rust; introgression lines with "stay green" trait from Favorit/*Ae. comosa* cross and a new introgression line with resistance to main foliar pathogens derived from *Triticum Timopheevi* x *Triticum monococcum* hybridization.

However, the time needed to transfer desired alien gene that implies specific kinds of genetic manipulations, greatly depends on the gene source and the evolutionary distance of the source to the recipient wheat genome. If the gene sources is a related species sharing at least one genome in common with the bread wheat, then the gene transfers may be achieved in 6-8 years

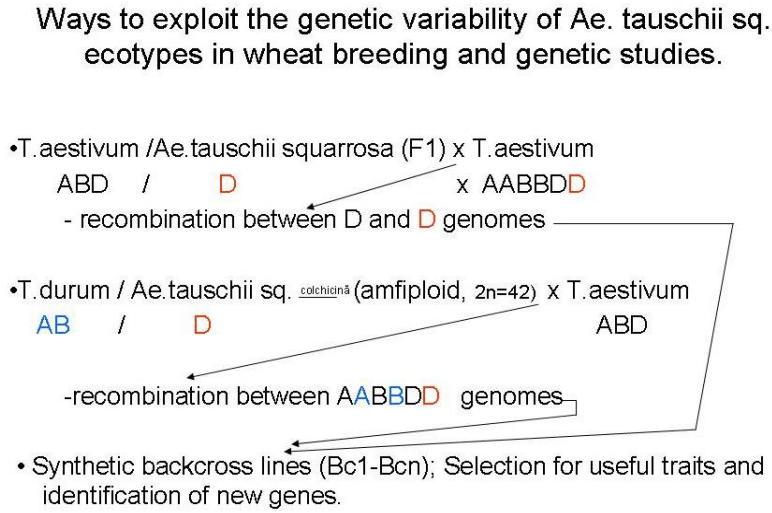
by using classical procedures or, in shorten time by using the DH technology- a faster way to attain homozygosity. In this respect, interspecific hybridization between wheat and its progenitors offers in addition many advantages due to genomic similarity and great potential of gene or polygene transfers by direct recombination of chromosomes of homoeologous genomes.

Among the common wheat progenitors, the diploid D genome progenitor, *Aegilops tauschii* has the widest geographic distribution from Turkey to China. (Ogbonnaya et al. 2005). Adaptive evolutionary processes operating on *Ae.tauschii* populations in thouse contrasting edaphic and climatic conditions, let to emerge a wide range of morphological and genetic variants at every trait.

Wheat genetic stocks development using wild germplasm at NARDI-Fundulea

- **Synthetic amphyploids:** - 38 hexa-amphyploids (AABBDD)
  (7 T.durum genotypes / 38 Ae.tauschii ecotypes)
  - 4 hexa-amphyploids (AAAABB)
    (T.durum / T.monococcum; T.boeoticum; T.urartum)
  - 1 hexa-amphyploids (AAB`B`DD)
    (T.durum / T.speltoides)
- **Synthetic amphyploids at octo and decaploid levels:** ex. T.aestivum / Ae.ovata (2n=70)
  T.aestivum / Ae.variabilis (2n=70)
- **Alien addition and alien substitution lines:**
  - T.aestivum / several relates species as donors:
    Secale cereale,T.triaristata(4x), T.triuncialis, T.variabilis, T. comosa, T.caudata.
- **Introgression lines:** - T.aestivum / several related species as donors:
  T.urartum, T. ventricosa, Ae.crassa(6x), T.comosa, T.caudata,T.dicoccoides, T.charlicum.
  - T.durum / T.charlicum, T.dicoccoides.
- **Translocation lines (mutagenesis):** T.aestivum cv.Glosa / Ae. ventricosa.

The gene transfers from *Ae. tauschii* could be realized by using two main procedures: a) direct crosses between wheat and Ae.tauschii ecotypes followed by subsequent backrosses and b) via synthetics hexa-amphyploids crosses and backrosses into modern wheat cultivars to obtain synthetic derived backcrossed bread lines. In this case, the novel genotypes could recombine genetic variability components of AB durum genomes and D *Ae. tauschii* genome from synthetics with homoeologous ABD genome of bread wheat and generate transgressive variation by positive interactions.



Out of 46 new synthetics hexa-amphiploids derived from *Triticum durum/ Aegilops tauschii squarrosa* crosses (Giura, 2010), twenty-three were investigated up to now under field conditions. Parents of these synthetics are represented by 7 winter durum genotypes and 17 Ae.tauschii biotypes. The synthetics displayed various growth habits, specific growth rhythms and distinctive morphological characteristics. The genotype interaction of AB durum genomes with D tauschii genome could also have positively influenced several traits especially those involved in spike morphology and seed dimensions. All synthetics developed long spikes that varied from 8.9 cm in E-19A to 14.5 cm in E-28A (table 4). When analyzing the number of spikelet per main spike significant positive differences were found for five synthetics. Several synthetics also set significantly more grain/spike compared to the average of experiment. Regarding 1000 kernel weight (TKW), nine synthetics were noted for their significantly higher values. As expected, general relationships between analyzed parameters were found (table 5). These correlations denote a high interdependence and at least one of these parameters could be used to select the synthetics as donors.

Reduced spikelet fertility and lower number of grain/spike in some synthetics could have been resulted from daily/nightly temperature variation during flowering period that likely caused meiotic abnormalities.

Spikes of parental forms, synthetic hexa-amphyploid (b) and of derived synthetic recombinant line (e)

Table 4: Morphometric analysis on spike productivity components in synthetic hexa-amphyploids (*T.durum/Ae.tauschii*).

Code	Genealogy	Spike length	Spikelets/ spike (no)	Seeds/ spike (no)	Grain length (mm)	Grain width (mm)	TKW (g)
E 1A	Pandur/Ae.tauschii 2472	13.1	**21.2**	**48.4**	7.9	3.3	42.3
E 2A	`` / `` 2377	13.5	**20.1**	**46.6**	**8.6**	**3.8**	55.4
E 3A	Agedur/ Ae.tauschii 2470	11.6	**20.6**	**50.6**	7.6	**3.8**	47.4
E 5A	`` / `` 2475	10.5	13.1	19.5	**8.9**	3.6	**53.1**
E 6A	`` / `` 2530	13.3	**20.1**	**50.1**	7.9	**4.0**	**49.9**
E 14A	`` / `` 2451	11.0	13.8	22.9	8.2	3.6	33.7
E 15A	`` / `` 2454	12.3	18.1	27.6	8.5	3.6	38.3
E 16A	`` / `` 2468	12.7	19.0	52.0	8.2	3.7	46.5
E 7A	Elidur / Ae. tauschii 2380	10,8	14.5	**54.5**	8.1	3.7	**51.1**
E 10A	`` / `` 2454	10.5	14.5	25.0	8.3	3.4	46.1
E 17A	Amadur/ Ae.tauschii 2472	12.9	17.5	24.4	**8.6**	3.6	**51.8**
E 18A	Grandur/ Ae.squarrosa str.39-1	**14.3**	19.8	63.7	8.1	**3.8**	**50.7**
E 19A	`` / Ae.tauschii 2377	8.9	12.8	16.7	8.5	3.5	48.2
E 35A	`` / `` 2569	12.9	19.4	41.4	8.6	3.4	39.8
E 20A	Condur/ Ae.tauschii 2390	12.1	19.3	25.3	7.3	3.4	30.2
E 21A	`` / `` 2412	**14.0**	**21.4**	28.2	**8.6**	3.5	40.4
E 22A	`` / `` 2417	13.7	**20.9**	**56.2**	8.0	3.6	44.7
E 24A	`` / `` 2464	13.5	19.1	**42.1**	**8.9**	3.6	**53.5**
E 25A	`` / `` 2472	13.1	19.2	**42.9**	8.4	3.7	**49.7**
E 26A	`` / `` 2474	13.3	19.7	**44.1**	**8.7**	3.6	**52.1**
E 28A	`` / `` 2477	**14.5**	**21,1**	**55.3**	7.8	3.7	42.4
E 29A	DDU-297/Ae.tauschii 2464	13.5	19.0	**41.3**	**8.8**	**3.8**	**58.7**
E 32A	`` / `` 2569	12.9	19.5	33.3	8.3	3.5	43.6
	Average ± standard deviation	**12,6±0.3**	**18.4±0.6**	**39.7±2.8**	**8.3±0.1**	**3.6±0.3**	**46.5±15**

Table 5: Correlation between some parameters describing spike productivity in synthetic hexa-amphyploids.

	Spikelets /spike	Grains/spike	TKW
Spike length	0.875**	0.602**	
Spikelet/spike		0.607**	
Grain length			0.561**

**)Significant at P<=0.001; **) P<=)0.01

Conclusions

In Romania, as well as in other European countries, the sets of aneuploid lines created under EWAC coordination have opened the way for modern genetic analysis in wheat and made possible the development of chromosomal genetic engineering methods at intraspecific, interspecific and intergeneric levels with benefits of both theoretical and practical interest.

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Past and present of wheat breeding at N.A.R.D.I. Fundulea – Romania

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Wheat is the second most important crop in Romania and the first among cereals used in human consumption. The area occupied by wheat has varied in time from 1.5 million to 3 million hectares. In 2017 wheat was sown on 2.11 million hectares. Yields have been very variable depending mostly on limiting climatic factors, but also on inputs used. In 1950 average country yield was less than 1 t per ha, and in 2017 it reached over 4.8 t/ha. The last 5 years average was 3.92 t/ha.

The climate in Romania is classified as temperate continental, with summers generally warm to hot, the average maxima being around 29 °C, and frequent temperatures over 35 °C, but weather conditions are very different, from region to region and from one year to another. Absolute minimum temperatures can reach -36 °C in the East and only -25 °C near seaside, absolute maximum temperatures can go up to 41 °C in the South and only 38 °C in Transylvania, and average annual rainfall vary from 375 in the East to 636 mm in the West. This variable and difficult climate, further aggravated by the present and future climate changes, represents a considerable challenge to wheat breeding.

Wheat breeding began in Romania at the beginning of 20th century, first selections from local wheat being made in 1900, and first crosses between local and western European wheat being obtained in 1914. However, larger scale and systematic work was only organized by the Agronomic Research Institute of Romania (ICAR) founded in 1927. Breeding centers were then organized in Bucharest, Cluj, Cenad, Iaşi, Câmpia Turzii etc. First successful wheat cultivar, **American 15 (A15)**, selected in Romania from the Kansas cultivar Tenmarq and released in 1933, was the most widely grown wheat for about 30 years, reaching 2 million hectares.

Unfortunately, World War II and the following isolation imposed by the communist regime, slowed down genetic progress, and in 1958, it was realized that wheat breeding results in Romania lagged behind those obtained in other countries. The Fundulea Institute newly founded in 1957 recommended the introduction of several foreign cultivars (Triumph, Concho, but mainly Bezostaya 1 and later Avrora and Kawkaz), which significantly overyielded and soon replaced the old Romanian cultivars. At the same time, the Fundulea Institute developed the largest wheat breeding program in the country, cooperating with regional breeding centers in Turda, Lovrin, Şimnic, Albota, Podu-Iloaie, Suceava, Oradea etc.

First results from the Fundulea breeding center, appeared during the period 1971-1979, and included the release of 9 standard-height cultivars with better adaptation to Romanian conditions, from **Dacia, Excelsior** and **Favorit**, released in 1971, to **Fundulea 29**, which was grown on 40% of the wheat area in Romania. After having outstanding results in the International Winter Wheat Performance Nursery in 1980 and 1981, Fundulea 29 was introduced in Canada.

The next period (1984-1993), included the release of the first 6 semidwarf winter wheat cultivars, well adapted to the conditions of Southern Romania, based on the introduction of the

gene *Rht-B1b* from the Mexican cultivar Nadadores 63. Cultivars **Flamura 85, Fundulea 4** and **Dropia** were particularly successful, each being grown on more than 20% of the wheat area.

The following period (1998-2017) is illustrated by the release of 14 new semidwarf cultivars, which brought further genetic progress and diversification. Out of these, **Boema, Glosa**, **Izvor**, **Miranda, Otilia** have occupied significant acreages, Glosa being the leading cultivar with more than 30% for many years.

Genetic progress for yield was estimated at about 50 kg/ha/year, or about 1%/year. This was accompanied by an improvement in bread-making quality and in yield stability, due to improved lodging resistance, earliness, disease and drought resistance. From 1977 on, wheat cultivars released from Fundulea have been grown on more than half of the wheat acreage in Romania. Several cultivars created at Fundulea were registered and were grown in countries like Canada, Hungary, Turkey, Kyrgyz Republic and Bulgaria.

Genetic progress has been accelerated in recent years, by routinely using the *Zea* system of producing doubled haploids from most interesting crosses. So far, 5 cultivars obtained using this system have been released, namely Faur F (2004), Glosa (2005), Litera (2010), Miranda (2011) and Pitar (2015). In the case of Glosa, using the DH system allowed registration 3 years earlier and cultivation on cumulated 1.2 million additional hectares, in comparison with what could have been possible using the classical pedigree breeding scheme.

Presently the main breeding objective is to make further progress in combining high yielding potential with bread making quality, yield stability and resilience to limiting environmental factors, for a sustainable agriculture, having in mind the future climate changes. Our hopes are based on:

- breeding for higher yielding potential by combining higher biomass with good harvest index. This involves better early growth (NDVI), without sacrificing winter hardiness, and improving lodging resistance, without further reducing height;
- increasing genetic diversity in the breeding program by:
    - combining the yield potential of best West European cultivars with better winter hardiness, drought and heat tolerance, as well as improved bread making quality;
    - taking advantage of the genetic progress made in spring wheat breeding in large and efficient programs (CIMMYT, Australia etc.) by transferring it to winter wheat adapted to continental climate;
    - using results of the pre-breeding program developed at Fundulea for introgression of useful genes from related species, mainly *Secale* (using *Triticale* as a bridge) and *Aegilops.*
- exploiting the genetic deviations from the regression between grain yield and protein concentration. We continue to work with the *Gpc 1* gene, but mainly concentrate on using a recently identified *Aegilops tauschii* derivative, that showed an average deviation from the Y-P regression of more than 1%, in 25 yield trials performed in very diverse conditions;

- maintaining the necessary level of sprouting resistance, by continuing routine testing and selection;
- taking advantage of the generally hot environment during grain filling in Fundulea, and using additional tests for tolerance to high temperatures, to improve yields in the predicted climate conditions;
- minimizing the response to drought by optimizing earliness and by using the osmo-regulation (*or*) gene and other traits related to performance under water stress;
- maintaining the necessary level of winterhardiness, by continuing routine testing and selection;
- breeding for resistance and/or tolerance to the main diseases, in the order of priority *Septoria tritici* (mainly by using, introgressions from *Aegilops* sp., produced in Fundulea and other centers), *Fusarium* head blight (by using the *Fhb-1* gene, but mainly own lines obtained as result of cumulating minor genes), leaf rust (mainly by using the adult resistance genes *Lr34. Lr46, Lr67* etc.), yellow rust (by using Western European cultivars, *Yr5, Yr15* etc.), barley yellow dwarf virus (BYDV).
- breeding for adaptation to reduced fertilizer inputs. Results of testing a number of cultivars at two levels of Nitrogen availability in several locations suggested that the genetic variability in N use efficiency among the modern Romanian cultivars exists, but is limited. More efforts in this respect are envisaged.

To support the breeding effort, intense cooperation takes place with the teams involved in genetics (DH production, prebreeding), physiology (abiotic resistance tests) and molecular genetics. Routine molecular tests are performed, for presence of the genes *Lr34, Lr37, Lr68, Stb16, Fhb1, Or, Bdv2, Gpc-1, Glu-A1, Glu-D1,* translocation 1R, etc., but on a relatively small scale. The wheat breeding program has also benefited from many international cooperation programs, involving reciprocal germplasm exchange and/or testing. We have learned from our own experience that international cooperation is of a paramount importance for genetic progress and became absolutely necessary in order to face the considerable challenges that expect us in the future.

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Phenotypic and molecular variability of Serbian and Austrian winter wheat varieties

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Summary

Genetic variability of locally adapted Serbian and Austrian winter wheat varieties was evaluated in order to assess their potential as a genetic material that can be exploited in crosses between two different European breeding pools. A field trial with 20 elite wheat varieties from each country was set at the Institute of Field and Vegetable Crops, Novi Sad, Serbia in a row-column design with three replications during the 2016/2017 season. The genotypes were phenotyped for tillering, heading and flowering time, plant height, ear length, number of spikelets per spike, number of grains per spike, thousand-kernel weight, chlorophyll content and resistance to prevalent wheat diseases. Additionally, the varieties were genotyped with 30 microsatellites. The varieties from two geographic regions and different release periods were clearly differentiated with population structure obtained from marker data. A significant phenotypic variation was found for most of the traits. Coefficients of variation were the largest for chlorophyll content (16.5%) and plant height (10.1%). Generally, the early genotypes were more susceptible to leaf rust (r = -0.6), while the late maturing genotypes produced more grains per spike (r = 0.4). The Serbian varieties had earlier tillering ($p < 0.03$), heading ($p < 0.00$) and flowering ($p < 0.00$) dates, shorter plant stems ($p < 0.00$), higher chlorophyll content ($p < 0.00$) and were more susceptible to leaf rust ($p < 0.00$) than the Austrian ones. The principal component analysis indicated general properties of the groups that would facilitate the choice of parent combinations for crossings.

Introduction

The effects of climate change, such as extremely high temperatures, low relative humidity, uneven rainfall distributions, strong insolation and droughts, are becoming very frequent in Serbia (Gocic & Trajkovic 2013). Similarly, warmer and dry summers are observed in plain terrains of the western parts of Austria (Alexandrov et al. 2002). A natural mechanism of plants to respond to changing environments, known as phenotypic plasticity, can be used to tackle climate change. Phenotypic plasticity is the ability of a genotype to express different variations of a trait when environmental conditions change or when a genotype is grown in a range of differing environments (Fusco & Minelli 2010). It is believed that the genotypes with high phenotypic plasticity can better adapt to different conditions and expand to diverse environments. Estimating plasticity and genetic variability of important wheat agronomic traits may help breeders to identify cultivars that are more suitable for production in less favourable, risk-prone environments and to distinguish them from cultivars suitable for production in optimal, non-stressed environments, as well as to define breeding strategies to broaden genetic diversity of elite wheat varieties from different regions. The geographical specificities of Serbia and Austria make them distinct and characteristic testing environments for field trials. The aims of this study were: 1) to assess genetic variability of locally adapted elite Serbian and Austrian winter wheat varieties, 2) to evaluate the most important morphological, phenological and agronomic characteristics of the selected varieties, and 3) to assess their potential that can be

exploited in crosses between two different European breeding pools.

Materials and methods

The field trial was set at the Institute of Field and Vegetable Crops, Rimski sancevi (45°20' N, 19°51' E, 84 m a.s.l.), Serbia, in a row-column design with three replications during the season 2016/2017. In total, 40 elite winter wheat varieties were selected, 20 from Austria and 20 from Serbia together with two check varieties, Bezostaya 1 and Amadeus. The phenotypic evaluation was performed for ten morphological, phenological and agronomic yield-related traits, namely tillering time (days from sowing), heading time (days from sowing), flowering time (days from sowing), plant height (cm), ear length (cm), number of spikelets per spike, number of grains per spike, thousand-kernel weight (g), chlorophyll content (CCI = % transmittance at 931nm. / % transmittance at 653 nm) at flowering and resistance to prevalent diseases. Genomic DNA was extracted from the seedlings using CTAB protocol. The genotyping was done with 30 microsatellite markers (Table 1). Total PCR mix contained 25 ng genomic DNA, 0.2 mM dNTP, 1×Taq buffer with KCl, 2 mM MgCl2, 1 U Taq polymerase, 0.5 pmol of fluorescently labelled forward primer and 0.5 pmol of reverse primer. PCR began with DNA denaturation at 94 °C for 5 min, followed by 38 cycles at 94 °C for 30 s, 52-62 °C for 45 s, 72 °C for 45 s and the final extension for 7 min at 72 °C. The 10 μL reaction volume for fragment analysis contained: 2 μL of differently labelled PCR products mixture, 0.2 μL GeneScan500 LIZ size standard and 7.8 μL Hi-Di formamide. The PCR products were separated by capillary electrophoresis on ABI Prism 3130 and their sizes were determined with Gene Mapper Software Version 4.0 (Applied Biosystems).

Table 1: Names, chromosome positions and repeat motifs of 30 analysed SSR markers.

No	SSR	Chr.	Repeat	No	SSR	Chr.	Repeat
1	*barc102*	3B	(TAA)20	16	*gwm413*	1B	(GA)18
2	*barc1096*	4B	(CT)10	17	*gwm458*	1D	(CA)13
3	*barc110*	5B	(ATT)28	18	*gwm495*	4B	(GA)20
4	*barc187*	1B	(CT)26	19	*gwm513*	4B	(CA)12
5	*barc3*	6A	(CCT)17	20	*gwm577*	7B	(CA)14(TA)6
6	*cfa2149*	4B, 5A	(TG)20	21	*gwm636*	2A	(GA)28imp
7	*gdm63*	5D	(CT)20	22	*gwm639*	5A, 5B, 5D	(GA)19
8	*gpw3017*	4B	(GA)33	23	*wmc125*	4B	(GT)11(GT)20
9	*gwm261*	2D	(CT)21	24	*wmc14*	7D	(CT) (CA)
10	*gwm291*	5A	(CA)35	25	*wmc25*	2B, 2D	(GT)26
11	*gwm296*	2A, 2D, 7D	(CT)28	26	*wmc262*	4A	(GA)29
12	*gwm325*	6D	(CT)16	27	*wmc317*	2B	(GT)23
13	*gwm350*	7A, 7D	(GT)14	28	*wmc410*	5A	(CA)26
14	*gwm371*	5B, 5D	(CA)10(GA)32	29	*wmc601*	2D	-
15	*gwm408*	5B	(CA)22(TA)(CA)7(TA)9	30	*wmc617*	4A, 4B, 4D	-

The molecular diversity parameters were analysed in GenAlEx 6.5. Population structure was calculated using model-based clustering method based on parametric model of frequency distribution with unknown number of subpopulations integrated into STRUCTURE software. The analysis of molecular variance (AMOVA) was applied to partition genetic variation among

groups based on the codominant allelic distance matrix. The phenotypic data were used to perform analysis of variance and principal component analysis.

Results and discussions

In total, 209 alleles were detected in 30 SSR loci with the mean number of 6.4 alleles per locus. The proportion of heterozygous individuals was 1%. The average PIC value was 0.61, while twelve microsatellites had PIC values above 0.70, indicating relatively high discriminatory power of microsatellite markers. In previous studies, similar PIC values of 0.64 were obtained with 39 SSRs (Roussel et al. 2005) and 0.67 with 19 SSRs (Röder et al. 2002) for a larger number of European wheat varieties. The smaller number of detected alleles and the average number of alleles per locus obtained in our study comparing to the previous ones, was due to much smaller sample size and narrower time span of variety release periods.

Population structure analysis performed with the programme Structure divided the genotypes distinctly by the country and the average year of release (Figure 1). Although the two Austrian groups were closer in terms of their average release periods (8 years difference), than the two Serbian (16 years difference), the groups were denoted as Austrian older with 11 varieties, Austrian new with 9 varieties, Serbian older with 14 varieties, and Serbian new with 6 varieties. El-Esawi et al. (2018) also showed an importance of geographic origin in a wheat diversity study, finding significant difference between western and central European winter wheat variety groups.

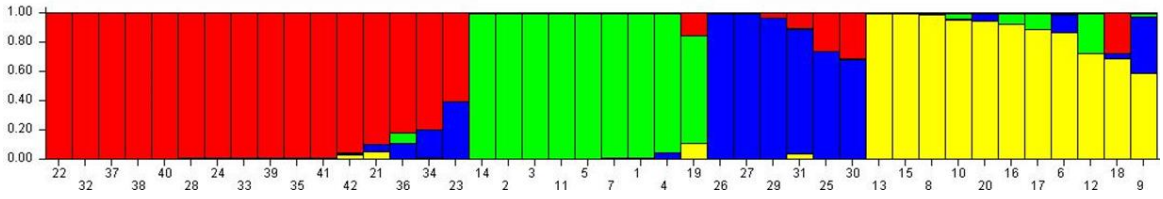

Fig. 1: Population structure of 42 wheat varieties estimated with SSRs. Red - Serbian older, green - Austrian new, blue - Serbian new, yellow - Austrian older varieties.

Analysis of molecular variance showed that genetic variation was much higher within the groups, accounting for 80%, than the variance among the groups (20%). Similar distribution of variance, where the majority of the diversity was attributed to differences among varieties within populations was obtained in Austrian and Belgian breeding pools (El-Esawi et al. 2018). The varieties were more differentiated by their geographical origin than by their release period, which is in accordance with the finding of Roussel et al. (2005), who demonstrated significant geographical variation between the wheat varieties from western and south-eastern European countries and, to less extent, temporal variation among the wheat varieties from different breeding periods.

A significant phenotypic variation was found for most of the traits (Table 2). Coefficients of variation were the largest for chlorophyll content (16.5%) and plant height (10.1%). Tukey's honest significant difference tests were used for multiple comparisons of means. The Serbian varieties had earlier tillering, heading and flowering dates than the Austrian. It is worth noting that while there were no differences in heading and flowering between the old and new Serbian varieties, old and new Austrian varieties significantly differed. Heading and flowering dates of the new Austrian varieties were recorded earlier than in old Austrian varieties, indicating a shift

in breeding towards earlier genotypes. This shift in wheat breeding has been observed in countries with frequent terminal drought as a strategy to minimize of the risk of drought stress (Shavrukov et al. 2017).

Table 2: Analysis of variance and comparison of means for nine phenotypic traits of four groups of winter wheat.

Group	A1	A2	S1	S2	Mean	CV (%)
Tillering	122.4 a	122.4 a	119.9 b	119.8 b	121.1	1.1
Heading	183.9 a	180.8 b	176.5 c	177.6 c	179.5	2.0
Flowering	186.2 a	183.6 b	180.6 c	181.2 c	182.7	1.5
Ear length	10.5 a	10.2 a	9.7 a	10.5 a	10.1	9.7
Plant height	77.2 a	76.4 a	67.5 b	67.2 b	72.4	10.1
Chlorophyll index	28.8 b	32.1 b	38.2 a	31.4 b	33.4	16.5
Grains per spike	51.0 a	51.8 a	49.0 a	53.0 a	51.2	6.2
Spikelets per spike	19.3 a	19.4 a	19.4 a	20.2 a	19.6	8.4
Thousand-kernel weight	48.7 a	48.5 a	48.2 a	48.8 a	48.5	5.9

A1 - Austrian older, A2 - Austrian new, S1 Serbian older, S2- Serbian new varieties

No significant differences among the groups were found for the average values of ear length, number of grains per spike, number of spikelets per spike and thousand-kernel weight. On average, the Austrian genotypes were higher than the Serbian ones, but there were no differences between the older and new varieties. The old Serbian varieties had significantly higher chlorophyll content index at the flowering then the other groups. This is the only trait showing differences between two groups of Serbian varieties.

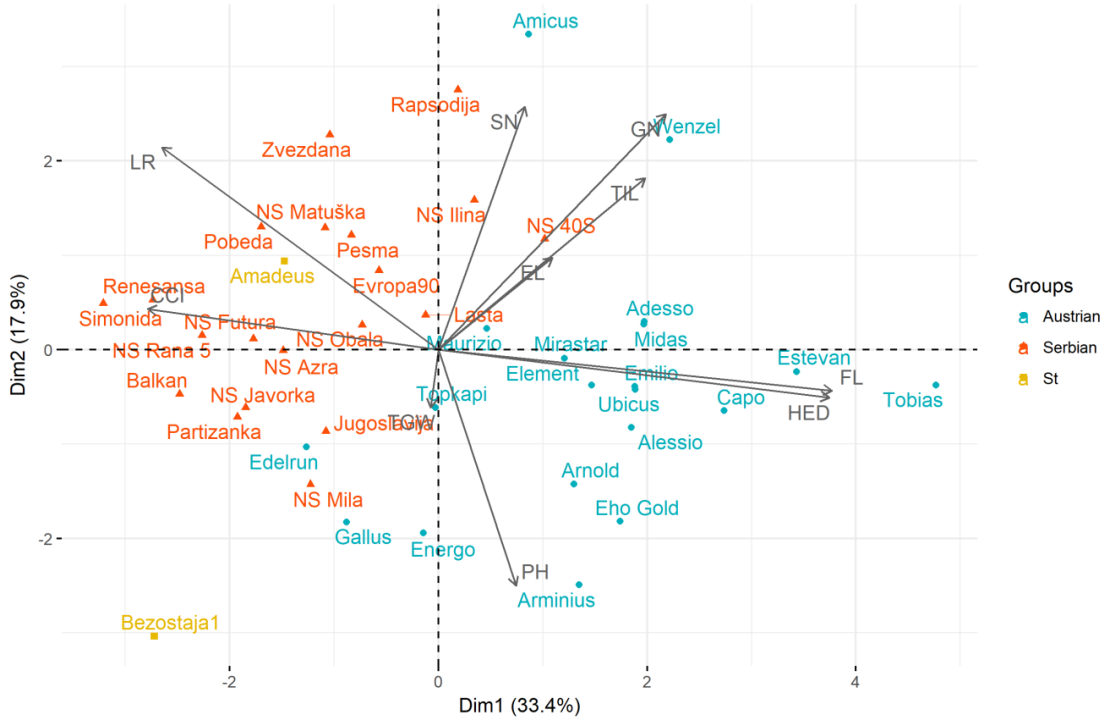

Fig. 2: Principal component analysis biplot of Austrian and Serbian winter wheat varieties.

The principal component analysis differentiated the varieties from Serbia and Austria (Fig. 2). The Austrian varieties were in general later maturing and higher than the Serbian, grouping around the vector for flowering and heading. On the other hand, Serbian varieties were more susceptible to rust and had higher chlorophyll content. The Austrian and Serbian varieties did not differ in the number of spikelets, number of grains per spike or thousand-kernel weight. The PCA biplot showed positive correlations between flowering date and number of grains per spike, as corroborated with the Pearson's coefficient ($r = 0.4$; $p < 0.01$). Pearson's correlations among the traits showed that the early genotypes were more susceptible to leaf rust ($r = -0.6$; $p < 0.0001$) and had higher chlorophyll content ($r = -0.5$; $p < 0.001$) than the late ones. In other studies, a positive significant correlation (Neumann et al. 2011) and no correlation between leaf rust and flowering time were found (Gao et al. 2016). Since rust infections before or at the flowering time of cereals are most damaging (Agrios 2005), it seems that early seasonal occurrence of the disease in 2017 concurred with flowering stage of early genotypes, causing severer symptoms in early that in late genotypes. The integration of data from the trial in Austrian environment and from two experimental years is expected to give a more detailed insight into phenotypic and molecular variability of the analysed wheat varieties and facilitate the choice of parent combinations for crossings.

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Leaf hairiness in wheat: genetic, evolutional and physiological aspects

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Leaf hairiness in plants is a part of leaf architecture and is widely spread among dicots and monocots. It is of great importance for adaptation to environmental factors and determines microclimate on leaf surface. It was found to improve photosynthetic efficiency and to regulate the temperature on leaf surface (Ehleringer and Mooney 1978; Schuepp 1993). This morphological trait is known to be a protection from pests (Roberts et al. 1979). A wide variability for leaf pubescence is known in the tribe Triticeae. The largest variation was found among hexaploid species (Vavilov 1987). Bread wheat forms unicellular unbranched leaf trichomes, the density and length of which are characteristic of a cultivar. Hairiness is characteristic of a number of drought resistant wheat cultivars referred to the steppe ecological group. Two major genes *Hl1* and *Hl2,* controlling leaf hairiness are mapped on 4B and 7B chromosomes (Dobrovolskaya et al., 2007). The diversity and adaptation value in various environmental conditions are poorly understood among cereals. For a long time, the development of research was hampered by the lack of an adequate method of quantitative assessment of leaf trichome density and length. The new method of computer image processing technique has been elaborated for measurements of this trait (Genaev et al. 2012). From the other hand, for such investigations, the special genetic material is necessary in the form of isogenic lines - carriers of certain genes for leaf pubescence that will help to explore the inheritance, mode of expression and the physiological significance of this trait. The aim of this work was to investigate the genetic variability of the trait in bread wheat and among its relatives, to obtain the isogenic lines with certain leaf pubescence genes and to assess their participation in adaptation to drought conditions.

The study of diversity among bread wheat cultivars has showed that the genotypes with different pubescence formed the separate groups (Fig.1). This indicates about discontinues variability for the trait determined by a small number of genes. Spring Siberian and Chinese cultivars grouped together with trichome numbers from 20 to 50 and trichome lengths from 80 to 200 μk (Fig. 1 a, d). The densest trichome layer was found in cv. Saratovskaya 29 (S29). Introgression of the pubescence gene $Hl2^{aesp}$ from *Aegilops speltoides* into glabrous leaf cultivar Rodina (the line 102/00^{i}) resulted in the increase of trichome length comparing to the donor (Fig.1 d, e). Introgression of the pubescence gene from *Triticum timopheevii* into the cultivar S29 led to the formation of a new phenotype with rather rare trichomes, shorter than that of the donor species (Fig. 1 c, f).

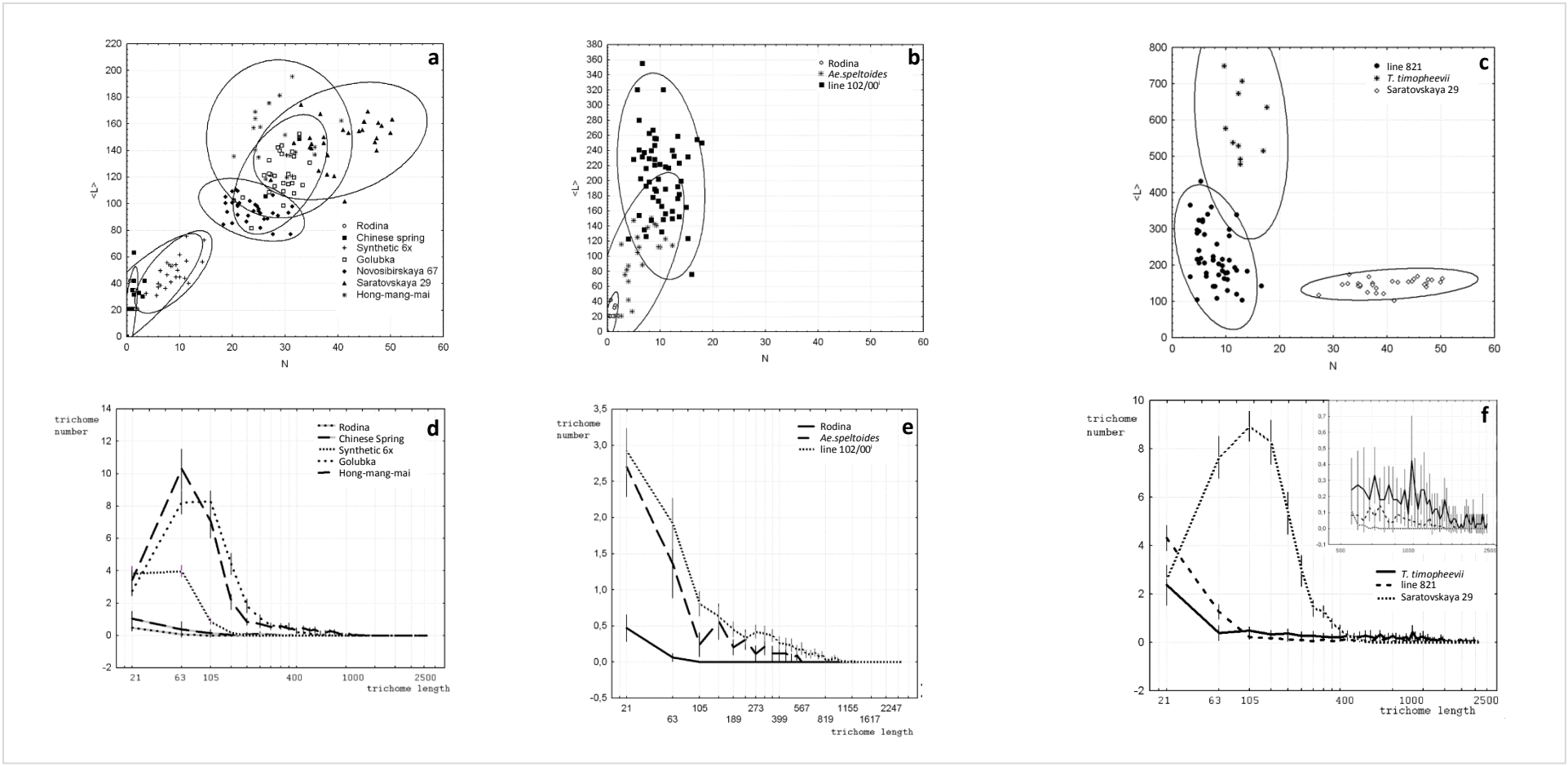

Fig. 1: Variety of quantitative characteristics of pubescence between wheat cultivars, lines with introgressions from *Aegilops speltoides*, *Triticum timopheevii* and donor species. Distribution of trichome number N and average trichome length <L> among spring bread wheat cultivars (a), in spring wheat cultivar Rodina, *Aegilops speltoides* and introgression line 102/00^{i} (b) and in spring wheat cultivar Saratovskaya 29, *Triticum timopheevii* and introgression line 821; the average trichome length (μm) distribution profiles among spring bread wheat cultivars (d), in spring wheat cultivar Rodina, *Aegilops speltoides* and introgression line 102/00^{i} (e) and in spring wheat cultivar Saratovskaya 29, *Triticum timopheevii* and introgression line 821 (f).

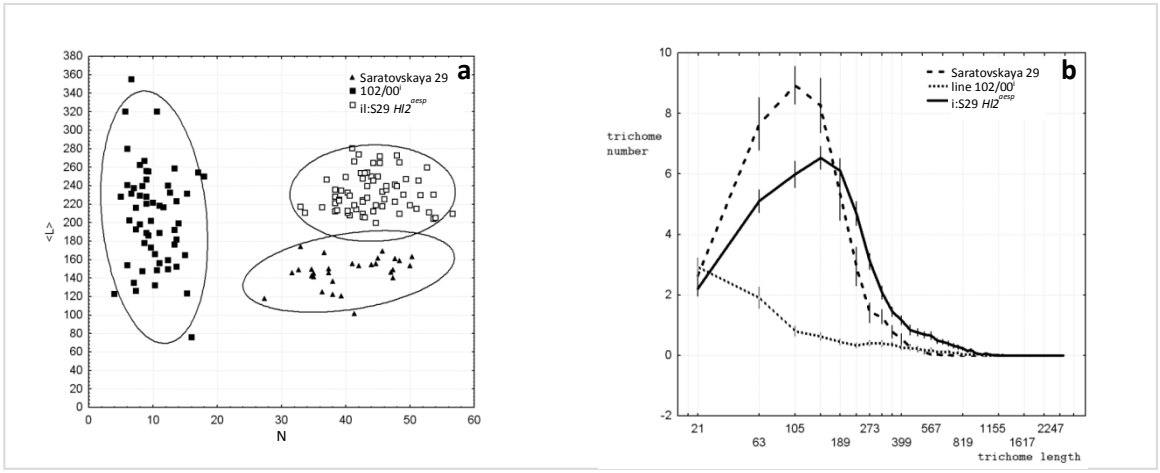

Fig. 2: Quantitative characteristics of leaf pubescence in the introgression line 102/00^{i} and its parents. Distribution of trichome number N and average trichome length <L> (a); the average trichome length (µm) distribution profiles (b).

Near-isogenic line was developed using the above mentioned gene *Hl2^{aesp}* from the introgression line 102/00^{i}. The recipient genotype was a densely pubescent cultivar S29 characterized by a high resistance to drought and carrying two genes for leaf pubescence. The line i: S29 *Hl2^{aesp}* was obtained through successive backcrosses (Fig.3) using the qualitative selection by trait. Quantitative characteristics of the line (trichome density and length) has been characterized in detail at the end by means of the computer image-processing program LHDetect2 (Genaev et al. 2012). Lately, the microsatellite marker *Xgwm400* was found linked to the target gene. As could be seen from Figure 2 the pubescence characteristics in the line are similar to S29 for trichome density but their average length is higher. This line carries three genes for the trait. Another near-isogenic line with complete absence of pubescence was obtained for S29 (Fig. 3) using the recessive alleles of pubescence genes from cv. Rodina. The phenotypic manifestation of pubescence in the new lines is presented on Figure 4.

Another kind of leaf pubescence from the species *T. timopheevii* was introduced into S29 and the poorly haired cultivar Diamant 2 (Dm2). The donor of the gene was the introgression line 821 with *timopheevii* introgressions in 2A, 2B and 5A chromosomes (Fig. 6, a). The gene position was supposed to be in 5A chromosome because earlier it was shown that A genome of *T. monococcum* carries the QTL for this trait near the microsatellite locus *Xcfd39* (Jing et al. 2007). Using the monosomic lines for 5A chromosome the gene was introduced into the genotypes of two above mentioned cultivars (Fig. 6, b). Molecular investigations have showed that the allelism for microsatellite marker *Xgwm126* is associated with variability for introgressed leaf pubescence.

In many species, leaf pubescence was showed to be responsible for the important physiological processes, such as gas exchange and light perception (Ehleringer and Mooney 1978; Galmés et al. 2007). For this reason, the lines were investigated for photosynthetic efficiency and antioxidant enzymes activity under contrasting watering. On Figure 7 all measurements of the traits in the isogenic lines are presented in percent to the recipients which are taken as 100%/. The line i: S29 *hl1 hl3* with recessive alleles for leaf pubescence shoed a lower biomass, especially on drought (Fig. 7 A). The same was observed for the line i: S29 *Hl2^{aesp}* on drought and for tolerance index of biomass (IT). The glabrous line had a high photosynthetic and transpiration rates (Fig. 7 B, C).

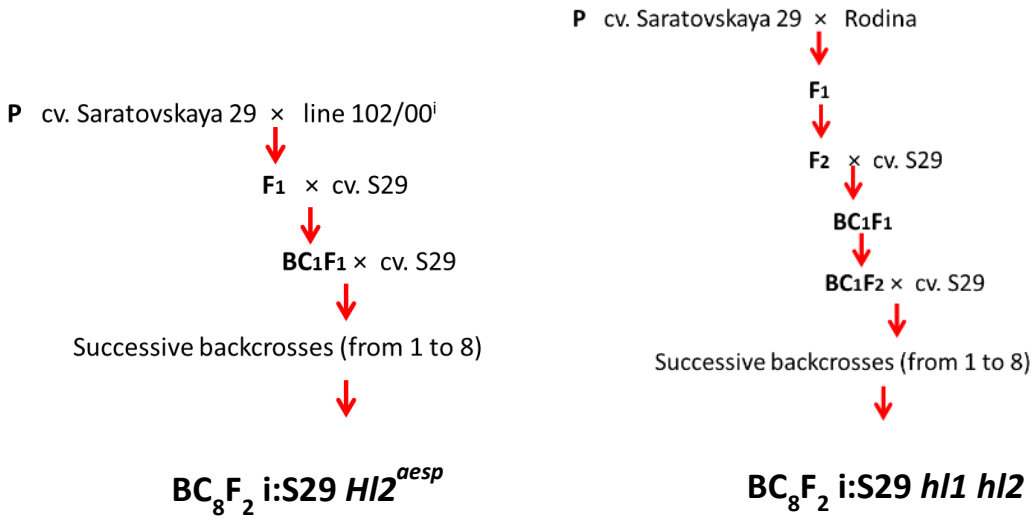

Fig. 3: Crossing scheme used to develop the near-isogenic lines i: S29 *Hl2*^{aesp} (a), and i: S29 *hl1 hl3* (b).

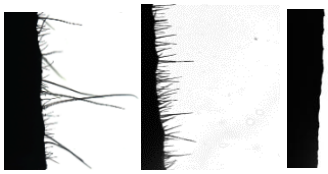

Fig. 4: Phenotypes of leaf surface of the isogenic lines and their recipients. From left to right: i:S29 *Hl2*^{aesp}; S29; i:S29 *hl1 hl3*.

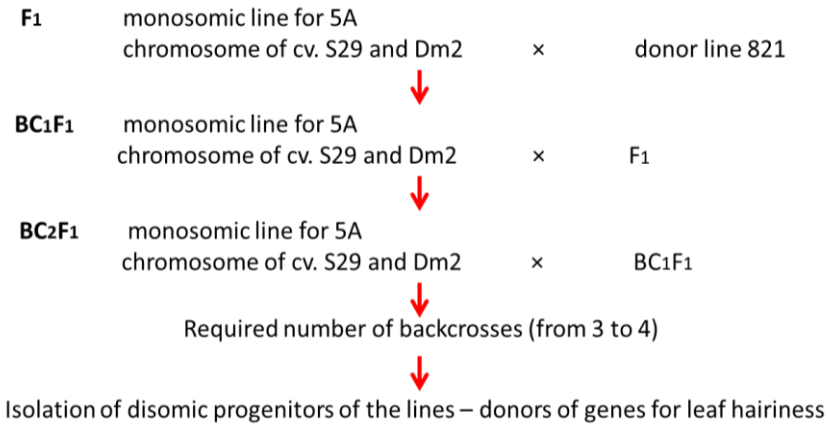

Fig. 5: Transfer of 5A chromosome from the introgression line 821 into cvs. S29 and Dm2.

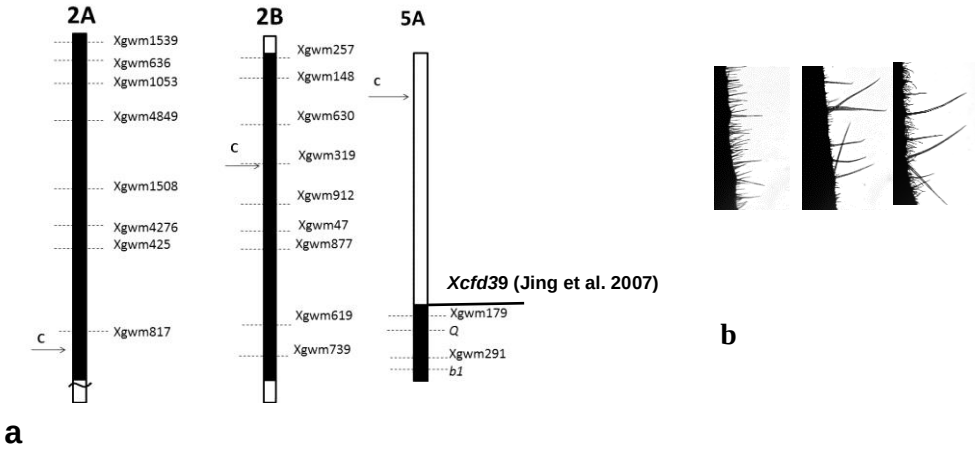

Fig. 6: Chromosomal introgressions in the line 821 (a) and phenotypic manifestation of leaf pubescence in the substitution line S29 (821 5A) (b). From left to right: S29; line 821; S29 (821 5A).

Another line showed a significant depression of both traits under both conditions. Introducing of the gene from *T. timopheevii* in two unrelated cultivars gave different effects. While the photosynthesis was not significantly affected in the line S29 (821 5A) (Fig.7 D) another line Dm2 (821 5A) showed a dramatic decrease in all photosynthetic parameters. This, in turn, resulted in very high water use efficiency (WUE). Under limited water supply the S29 line demonstrated a decrease of transpiration rate and stomatal conductance. Both introgression lines had a high WUE under drought.

This genetic material will be further used to identify the genes for leaf pubescence in wheat genome, to establish there belong to the gene families, to study their structure and expression. It is also allow the associating the different types of pubescence with physiological reactions of the leaf during adaptation to environmental factors.

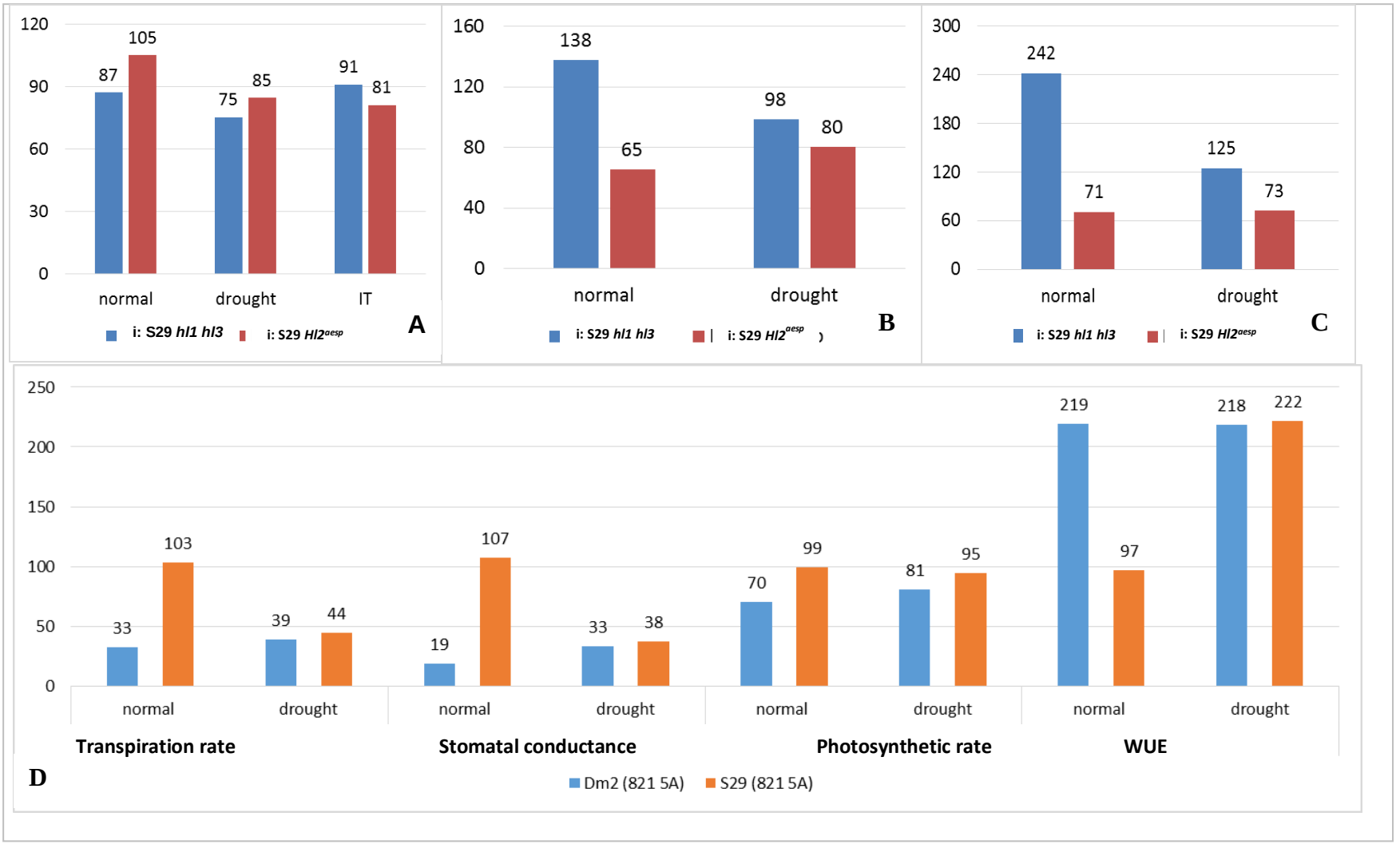

Fig.7: Physiological traits in the lines with introgressions from *Ae. speltoides* and *T. timopheevii* under contrasting water supply. S29 and Dm2 (recipients) are taken as 100%. Explanation in the text.

Acknowledgements

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Effects of *Ppd* alleles on heading and flowering time of wheat in climatic conditions of South-Eastern Europe

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Summary

Photoperiod response (*Ppd*) genes play a key role in fine-tuning of heading and flowering time and adaptation of wheat to different agro-climatic conditions, which could increase crop yield. The aim of this study was to analyse genotypes carrying different *Ppd-1* alleles and their influence on wheat phenology in growing conditions of the South-Eastern Europe. The experiment was conducted with 10 well-adapted Serbian wheat varieties and 54 NILs of cv. Paragon with single, double and triple doses of *Ppd-1* alleles. The genotypes were sown in the plot size of 2 m^2 at the location of Rimski Sancevi (45°20`N, 19°51`E), in randomized complete block design with 3 replications. Heading and flowering time were recorded during three growing seasons (2014, 2015 and 2017). The results showed that the genotype, growing season and their interactions had significant effects on both analysed traits. The Serbian varieties (set 1) were significantly earlier regarding the heading and flowering time than cv. Paragon, and most of the NILs. The NILs with introgressed single (set 2) insensitivity *Ppd-1* alleles were earlier than the original cv. Paragon in 2014 and 2015, but not in 2017. When the same alleles were introgressed in a double dose (set 3), they significantly reduced heading and flowering time in all seasons. The NILs with introgressed *Ppd-1* null alleles and knock-outs (set 4) were significantly later than cv. Paragon, and all other sets of genotypes. All indicated changes caused by different *Ppd-1* alleles were variable expressed in different growing seasons.

Introduction

In a global climate change scenario, with increasing probability of extreme climate episodes (IPCC, 2014), a further improvement of wheat production could be achieved by fine-tuning of plant development cycles in order to avoid or escape from extreme drought or heat events during the most sensitive phases of yield formation. To achieve this goal, breeding programs should create varieties with more efficient and precise phenology, maximizing yield in the prevalent environmental conditions (IPCC, 2014; Hunter et al., 2017). Furthermore, for each particular environment, a balance must be found between late flowering time that would allow unhindered grain development and increase grain number, and early flowering that would avoid severe heat and drought stress during the most vulnerable wheat stages, namely flowering and grain filling (Arjona et al., 2018).

Adaptation genes play a major role in plant response to environmental signals (Bentley et al., 2013). Flowering time in wheat is controlled by at least 20 genes dispersed over the wheat genome (Sanna et al., 2014). However, most of the genetic variation for this important trait accounts for three genes: vernalisation requirement (*VRN* genes), photoperiod sensitivity (*PPD* genes), and earliness per se (Eps genes) (Distelfeld and Dubcovsky, 2009). This complex of genes can help breeders to realize optimal adaptation to local eco-geographic region (Novoselović et al. 2015).

The aim of this study was to use specific genetic material (near isogenic lines (NILs) of cv. Paragon) in order to determine the effect of different *Ppd-1* alleles on heading and flowering time of wheat genotypes in environmental conditions of the South-eastern Europe.

Materials and methods

The experiment was conducted at the experimental field of the Institute of Field and Vegetable Crops in Novi Sad (IFVCNS), the location of Rimski Šancevi, Serbia (45°20`N, 19°51`E). The material contained the following sets of the genotypes:

Set 1 - 10 modern Serbian wheat cultivars
Set 2 - 15 NILs of cv. Paragon with introgressed single insensitivity *Ppd-1* alleles (early alleles)
Set 3 - 21 NILs with introgressed double insensitivity *Ppd-1* alleles (early alleles),
Set 4 - 18 NILs with single, double or triple introgressed *Ppd-1* null alleles and knock-outs (late alleles).

The near isogenic lines (NILs) were produced at the John Innes Centre, UK and obtained during the project FP7-KBBE-2011-5: ADAPTAWHEAT. The introgressed *Ppd-1* alleles originated from different donors and different wheat genomes (A, B and D). In order to evaluate the effect of each *Ppd-1* allele, the original cultivar Paragon, from which the NILs were produced, was used in the experiment as a control.

The genotypes were sown in the plot size of 2 m^2 with 6 rows per plot in 3 replications. Standard agronomical practice for wheat production was applied. Heading (GS 55 after Zadoks et al. 1974) and flowering time (GS 61 after Zadoks et al. 1974) as a number of days from the date of sawing were recorded during three growing seasons (2014, 2015 and 2017), representing variable climatic conditions of the South-Eastern Europe.

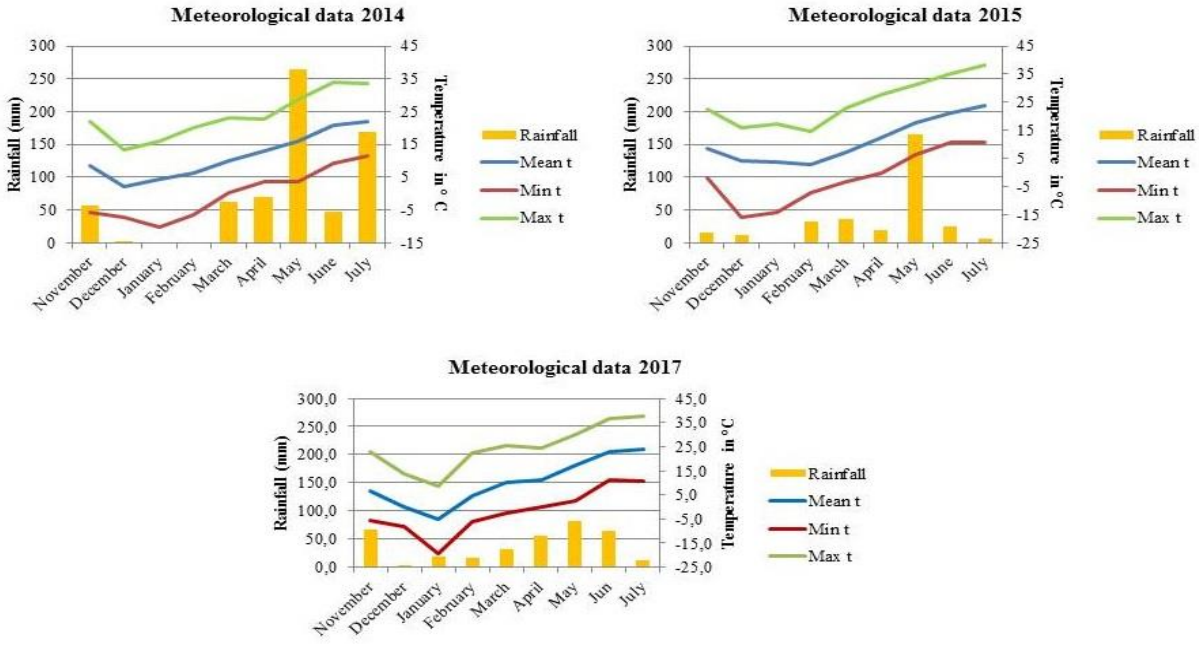

Fig. 1: Meteorological data for the three growing seasons.

Statistical data analysis (ANOVA, comparison of means) was carried out in STAR- Statistical Tool for Agricultural Research v. 2.0.1.program.

Results and discussions

The results have shown significant variations in heading time among different sets of the wheat genotypes, as well as among the growing seasons (Fig. 2.). The variations among the genotypes were the largest in the season 2014 and the smallest in the season 2015. Also, the heading time was the shortest in the season 2014 and the longest in the season 2017.

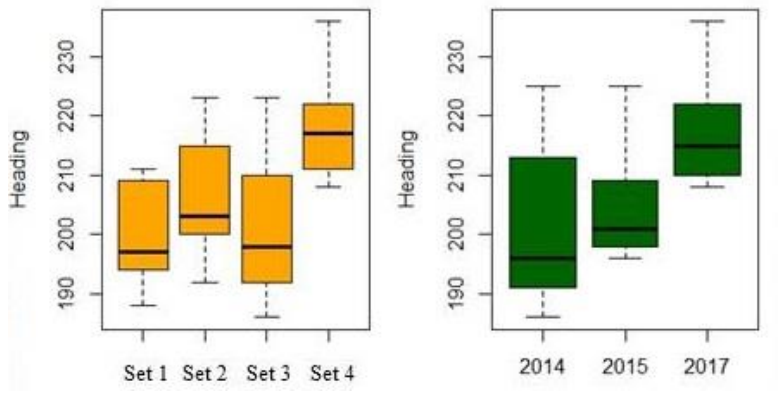

Fig. 2: Variations in heading time among the sets of wheat genotypes and the growing seasons.

Similar results were obtained for the flowering time too, but with smaller differences among the sets 1, 2 and 3, and no difference between the seasons 2014 and 2015 (Fig. 3).

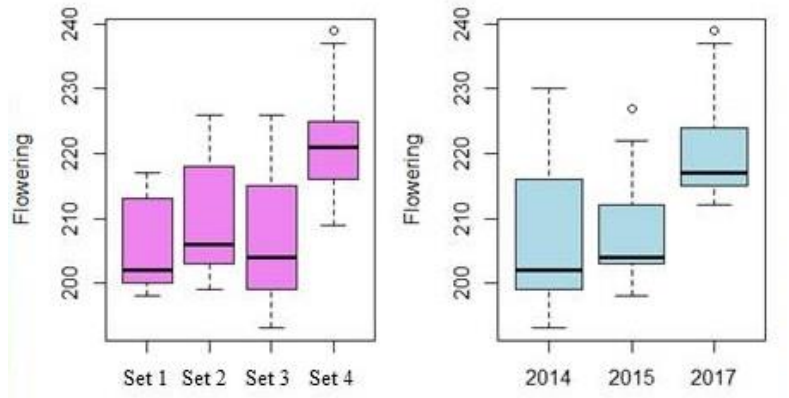

Fig. 3: Variations in flowering time among the sets of wheat genotypes and the growing seasons.

The first growing season was characterised with a huge amount of precipitation during the spring, especially in May, when heading and flowering occur in Serbian agro-climatic conditions. The second and the third seasons had similar amount of precipitation during the spring and the early summer but with better distribution in the season 2017. It can be conditionally concluded that among these three seasons, the 2014 was the worst, while the 2017 was the most favourable for wheat production (Fig. 1).

It means that the heading and flowering time were the most variable and with the lowest average values in the less favourable conditions, trying to find and use the best possible moment for these important developmental events. In better growing conditions during the season 2017 these traits were less variable and with significantly higher values. These results are in agreement with the studies of Trevaskis (2010) and Cockram et al. (2007), stated that in temperate regions, time of flowering normally coincides with favourable climatic condition because developmental switch from vegetative to reproductive growth is critical for enabling wheat plants to flower at optimum time for pollination, seed development and dispersal as well as for adjusting a wheat life cycle for maximum yields.

Table 1: The average values for heading and flowering time of cv. Paragon, the Serbian cultivars (set 1) and different sets of NILs (sets 2, 3 and 4).

	Heading				**Flowering**			
	2014	**2015**	**2017**	**Mean**	**2014**	**2015**	**2017**	**Mean**
Paragon	214.0	208.0	214.0	212.0[a]	218.0	212.0	217.0	215.7[a]
Set1 Serbian varieties	192.6	197.2	209.4	199.7[b]	200.5	200.6	213.6	204.9[b]
Set 2 *Ppd-1* early alleles	198.3	201.9	216.7	205.6[c]	203.9	205.1	219.5	209.5[c]
Set 3 *Ppd-1* early alleles (double doses)	188.9	198.4	212.2	199.8[b]	197.3	202.6	216.9	205.6[b]
Set 4 *Ppd-1* late alleles	215.9	213.4	222.9	214.7[d]	220.2	218.0	226.3	221.5[d]
Mean	201.9[a]	203.7[b]	215.0[c]		208.0[a]	207.7[a]	218.7[b]	

Analyses of the effects of different *Ppd-1* alleles in comparison with the cultivar Paragon (Tab. 1) have shown that *Ppd-1* early alleles significantly decreased, while *Ppd-1* late alleles significantly increased heading and flowering time, especially when present in double and triple doses. In the season 2014, *Ppd-1* early alleles decreased heading time for 16 and 25 days in average in the sets 2 and 3, respectively, while flowering time was decreased for 14 and 21 days, respectively. At the same time, *Ppd-1* late alleles had very small effects on heading and flowering time (increased for 2 days only). For the second growing season (2015) the both *Ppd-1* early and late alleles had significant effects on heading and flowering time. Finally, in the season 2017, the effect of *Ppd-1* early alleles was significantly smaller than the effect of *Ppd-1* late alleles.

Serbian varieties (set 1) were significantly earlier regarding the heading and flowering time than cv. Paragon and the NILs from the sets 2 and 4. The NILs from the set 3 were the most similar to Serbian cultivars.

Matching the appropriate photoperiod response to eco-geographic region is of a great importance. Novoselović et al. (2015) reported that in general, "early" flowering group had higher yield than "late" group, suggesting the advantage of "early" over "late" alleles under conditions of eastern Croatia, which are very similar to Serbian agro-climatic conditions. More specifically, they also reported that among homoeologous loci *Ppd-A1* locus had the highest effect on grain yield. This could be a useful strategy for wheat breeders in a region to introduce

such alleles and combine it with omni-present *Ppd-D1a* alleles in Southern European wheat germplasm (Worland 1996) to preserve or increase genetic yield potential.

Conclusions

The results have shown variable effects of different *Ppd-1* alleles during the three growing seasons. *Ppd-1* early alleles significantly decreased, while *Ppd-1* late alleles significantly increased heading and flowering time in comparison to the original cv. Paragon, especially when present in double and triple doses. In unfavourable growing conditions *Ppd-1* early alleles were more expressed then in optimal conditions, while for *Ppd-1* late alleles the situation was opposite. In general, all genotypes were significantly earlier in less productive and later in more productive growing conditions.

Acknowledgement

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TaGW2-6A gene association with kernel length and TKW in some European winter wheat cultivars.

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Summary

Grain size is one of the most important traits contributing to grain yield, which is also related to higher flour recovery and milling quality of grain. Grain size in wheat has been one of the targets for selection both during domestication and modern wheat breeding. The complexity of genetic control for grain size and weigh set in motion many studies that lead to the discovery of important QTLs, on almost all chromosomes (Wu et al., 2012; Patil et al., 2013).

TaGW2 is an orthologue of rice gene *OsGW2*, which encodes E3 RING ubiquitin ligase and controls the grain size in rice. In wheat, three copies of *TaGW2* have been identified and mapped on wheat homoeologous group 6 viz. *TaGW2-6A*, *TaGW2-6B* and *TaGW2-6D*.

In this study we focused on the genetic diversity of *TaGW2-6A* locus and the association of haplotypes with TKW and/or TKW components. 34 European winter wheat cultivars were analyzed and the results revealed that the main haplotype was Hap-6A-G, present in 25 cultivars, Hap-6A-A being present only in 7 cultivars.

Association analysis showed that *TaGW2-6A* gene, viz. the favorable haplotype Hap-6A-G, is associated with kernel length and TKW ($p<0.05$).

Introduction

Climate changes, soil availability and accessibility, soil degradation, increase of the world population and other factors, influences the food production and food security. This factors lead to new challenges for farmers, breeders and scientist worldwide. Estimations show that wheat demand will increase by a further 40% before 2020, as a result of world population increase (Rajaram, 2005; Dixon et al., 2009). Since wheat is one of the most important cereals along with rice and maize, annual increase of 1.6%-2% in grain yield is required in the coming years in order to fulfil the global demand (Patil, 2013; Faris, 2014).

One of the most important traits contributing to grain yield is grain size, which is also related to higher flour recovery and milling quality of grain. Grain size in wheat has been one of the targets for selection both during domestication and modern wheat breeding. The complexity of genetic control for grain size and weigh set in motion many studies that lead to the discovery of important QTLs, on almost all chromosomes (Wu et al., 2012; Patil et al., 2012).

Thousand-kernel weight (TKW), mainly determined by grain width, grain length and grain thickness, but also by grain shape and density, is a complex trait and a more detailed knowledge of its genetic control is useful for breeding programs and breeding efficiency worldwide.

TaGW2 is an orthologue of rice gene *OsGW2*, which encodes E3 RING ubiquitin ligase and controls the grain size in rice. The gene *OsGW2* negatively regulates grain width through

control of cell division in the spikelet hull. Loss-of-function mutations in the coding sequence, or interference with the expression level of *OsGW2*, resulted in enhanced grain width, grain weight and grain yield. In wheat, three copies of *TaGW2* have been identified and mapped on wheat homoeologous group 6 viz. *TaGW2-6A*, *TaGW2-6B* and *TaGW2-6D*. Studies showed that *TaGW2-6A* gene is also a negative regulator of grain-width and grain-weight (Su et al., 2011; Jaiswal et al., 2015).

Materials and methods

Plant material was obtained from NARDI Fundulea, Romania and consisted of 34 European winter wheat cultivars, tested in the experimental Fundulea field.

Phenotypic data contain the length and width of kernels, TKW and FFD (factor form-density = TKW/length * width; Giura and Săulescu, 1996), average from three years (2013, 2014 and 2015).

DNA extraction was performed on two dry seeds using SDS3 method (Cristina et al., 2017).

DNA amplification was performed with "MyTaq Red DNA Polymerase" PCR kit from Bioline in an ABI ProFlex™ 3 x 32-well PCR System. PCR parameters for the amplification were as follows:

- Hap-6A-P1 primer: 15 µL final reaction volume containing 1X reaction buffer, 0.5 mM primers, 0.6U DNA polymerase and 60-80 ng DNA sample. The PCR product was diluted with 75 µL autoclaved H20 and used as template for the second round of amplification;

- Hap-6A-P2 primer: 10 µl final reaction volume containing 1X reaction buffer, 0.4 mM primers, 0.3U DNA polymerase and 1 µl sample from previous amplification (P1+75 µL H2O).

PCR programme: initial denaturation at 95 °C for 1 min, followed by 35 cycles: 95 °C – 15 s, 60 °C/57 °C – 15 s, 72 °C – 10 s, and a final extension at 72 °C for 5 min.

The PCR products obtained with Hap-6A-P2 primer were subjected to digestion with 5U of TaqI restriction enzyme at 65 °C for 30min.

Gel electrophoresis for the separation of digested PCR products was carried out with 2% routine use agarose gels, stained with ethidium bromide and visualized on UV light.

Statistical analysis (t-test) was performed using the online tools available at: http://www.socscistatistics.com/tests/studentttest/Default2.aspx.

Results and discussions

Improving wheat production through exploitation and utilization of superior genes and allelic variations represents a powerful approach used by modern breeding programs. The identification of superior allelic variations has been one of the main targets of scientists and breeders for overcoming current and future limiting factors that affect the wheat production.

Highlighting the haplotypes of *TaGW2-6A* gene was carried out with two rounds of amplification (Nested PCR) and a digestion of the final PCR product. Thereby, the PCR product obtained with Hap-6A-P1 was diluted (1:5) and used as template for the second PCR with Hap-

6A-P2. The 418bp PCR product from the second round of amplification had been subjected to digestion with *TaqI* restriction enzyme resulting specific fragments of 167bp for Hap-6A-A haplotype and 218bp in case of Hap-6A-G haplotype (Figure 1). Heterozygous/heterogenic genotypes were also present (cultivars Alex and Fundulea 29) and noted as "H". These two results will be revised with new DNA extractions and modified digestion protocol.

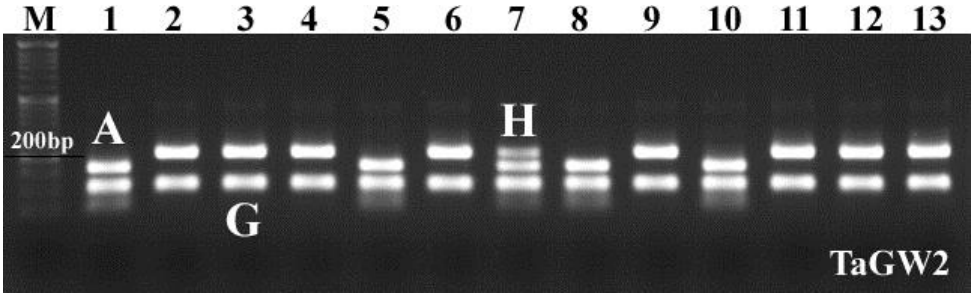

Fig.1: Haplotypes of *TaGW2-6A*.

Molecular results are presented in table 1, sorted by haplotypes (A=Hap-6A-A, G=Hap-6A-G and H=heterozygous/heterogenic). Results showed that haplotype Hap-6A-A was present only in 7 cultivars out of 34 analyzed while Hap-6A-G was present in 25 cultivars. Contrary to the Su et al. (2011) results, we observed that the favorable haplotype was Hap-6A-G, with an average TKW of 39.5g, while Hap-6A-A had an average TKW of 36.2g. Similar results were shown in Qin et al. paper (2014), where Hap-6A-G was shown as preferred/selected in European cultivars.

Table 1: Phenotypic data and molecular results.

	Cultivar	**Length (mm)**	**Width (mm)**	**FFD**	**TKW (g)**	***TaGW2-6A***
1.	Apache	6.293	3.223	1.671	33.80	A
2.	CS	5.23	2.87	2.072	31.10	A
3.	F132	6.329	3.024	1.988	38.00	A
4.	Izvor	6.260	3.337	1.855	38.87	A
5.	Jagger	6.22	2.95	1.924	35.30	A
6.	Otilia	6.008	3.262	1.876	36.77	A
7.	Pajura	6.510	3.368	1.803	39.68	A
8.	A15	6.46	3.04	1.777	34.90	G
9.	Aerobic	6.04	3.33	1.584	31.85	G
10.	Ariesan	7.87	3.34	1.720	45.20	G
11.	Bezostaia 1	6.646	3.127	1.891	39.37	G
12.	Boema 1	6.605	3.366	1.877	41.83	G
13.	Capo	6.069	3.144	1.910	36.43	G
14.	Ceres	6.688	3.285	1.863	40.83	G
15.	Dacia	6.056	3.290	1.972	39.40	G
16.	Diana	7.145	3.204	1.997	45.67	G
17.	Doina	6.214	3.229	1.680	33.73	G
18.	Dropia	6.649	3.396	1.981	44.73	G
19.	Exotic	6.279	3.326	1.805	37.77	G

	Cultivar	Length (mm)	Width (mm)	FFD	TKW (g)	*TaGW2-6A*
20.	F628	6.813	3.367	1.613	37.00	G
21.	Flamura 85	6.538	3.273	1.963	41.90	G
22.	Fundulea 133	5.967	3.003	1.934	34.67	G
23.	Fundulea 4	6.601	3.192	1.889	39.93	G
24.	Glosa	6.722	3.379	1.867	42.50	G
25.	Iulia	6.578	3.132	1.890	39.07	G
26.	Litera	6.613	3.352	1.734	38.37	G
27.	Lovrin 231	7.305	3.301	1.864	44.83	G
28.	Miranda	6.451	3.145	1.837	37.43	G
29.	Odeskaia 51	6.225	3.164	1.793	35.20	G
30.	Pitar	6.337	3.328	1.941	40.68	G
31.	Renesansa	6.661	3.396	1.821	41.27	G
32.	Transilvania 1	7.361	3.218	1.833	43.03	G
33.	Alex	6.857	3.260	1.639	36.90	H?
34.	Fundulea 29	6.354	3.147	1.692	33.90	H?

Association analysis (*t*-test) showed that *TaGW2-6A* gene, viz. favorable haplotype Hap-6A-G is associated with kernel length, p-value of 0.0182 ($p<0.05$) with an average difference of 0.47mm between haplotypes. Also, favorable haplotype showed association with TKW, p-value of 0.0438 ($p<0.05$) with a difference of 3.29g between haplotypes. No association has been found with kernel width or FFD (Table 2).

Table 2: Mean phenotypic data values and the association analysis results.

TaGW2-6A	Length (mm)	Width (mm)	FFD	TKW (g)
Hap G	6.60	3.25	1.84	39.50
Hap A	6.12	3.15	1.88	36.22
Difference	0.47	0.11	-0.04	3.29
p – value	**0.018269**	0.074723	0.387102	**0.043812**

Kernel length of cultivars with Hap-6A-A ranged between 5,23mm and 6,51mm, while the length of kernel for cultivars with Hap-6A-G ranged between 5,97mm and 7,87mm, most cultivars beeing in 6,2-6,8 mm length group. Frequency and normal distribution of kernel length for *TaGW2-6A* haplotypes are presented in figure 2.

Also, TKW of cultivars with Hap-6A-A haplotype ranged between 31,1g and 39,7g, while TKW for cultivars with Hap-6A-G ranged between 31,85g and 45,67g, most cultivars being in 38-46g TKW group. Frequency and normal distribution of TKW for *TaGW2-6A* haplotypes are presented in figure 3.

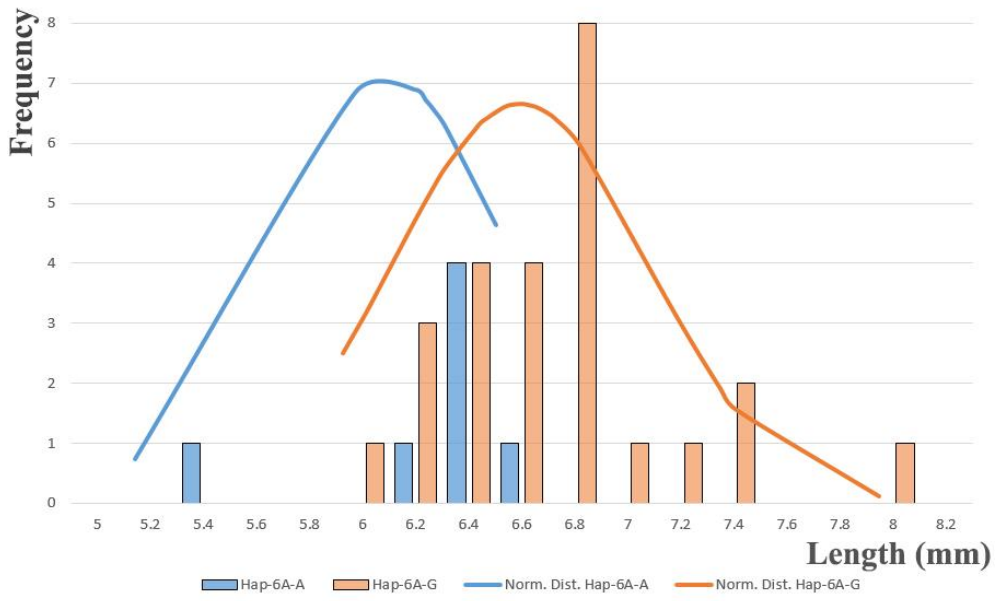

Fig. 2: Frequency and normal distribution of kernel length for *TaGW2-6A* haplotypes.

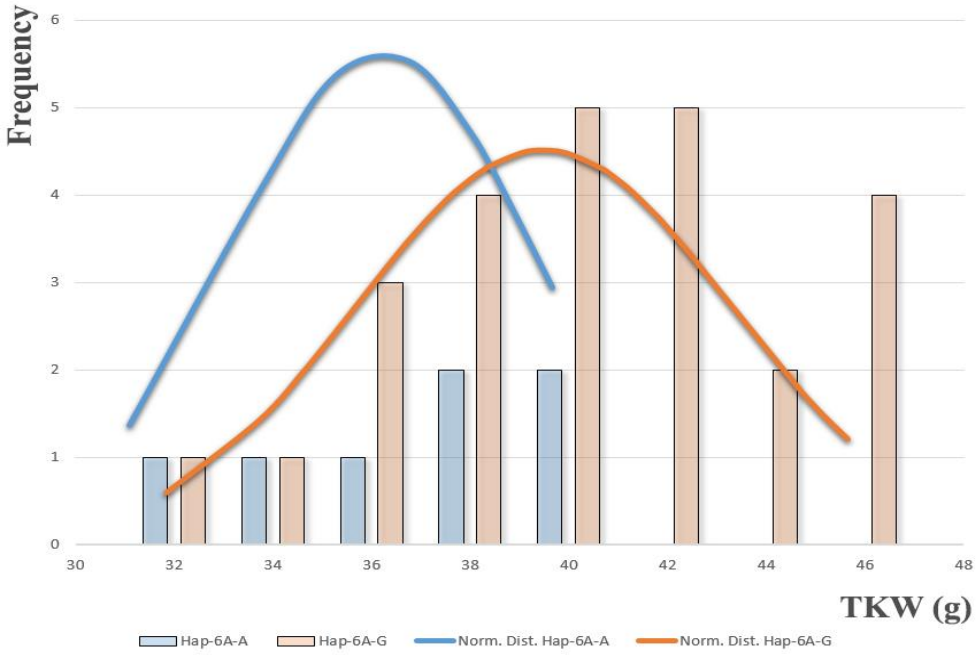

Fig. 3: Frequency and normal distribution of TKW for *TaGW2-6A* haplotypes.

Genetic diversity analysis of 34 European winter wheat cultivars, at *TaGW2-6A* locus, revealed the presence of Hap-6A-A haplotype only in 7 cultivars, with an average kernel length of 6.12mm and an average TKW of 36.22g. The presence of Hap-6A-G haplotype was revealed in 25 cultivars, with an average kernel length of 6.60mm and TKW of 39.50g.

TKW is a complex trait that requires genetic dissection of QTL's and genes that influence this trait or individual components of it. Our results show that *TaGW2-6A* gene (favorable haplotype Hap-6A-G) had a positive influence on kernel length and TKW over the years 2014-2016. Further studies are required for identification and validation of other important genetic factors

leading to the improvement of TKW and/or TKW components.

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Genetic dissection of drought tolerance by analysis of a recombinant chromosome substitution double haploid mapping population of bread wheat for 2A chromosome

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Drought is one of the most damaging environmental stresses affecting grain yield of bread wheat throughout the geographic regions of the crop's production. The genetic enhancement of drought tolerance probably represents the most sustainable approach of alleviating the loss in economic yield caused by drought. The plant response to moisture deficit is physiologically and genetically complex. Great efforts are made to separate this polygenic trait into individual genetic factors that can be used for improvement of drought tolerance. Earlier, it was found that 2A chromosome is critical for drought tolerance in spring cv. S29. The line with 2A substitution for this chromosome showed the lowest level of cumulative activity of antioxidant enzymes under drought (Osipova et al. 2013). The aim of this work was to map the responsible QTLs and to identify the candidate genes in the certain regions of 2A chromosome.

Genetic material and growing conditions

A set of substitution recombinant double haploid lines (SRDHL) S29 (YP 2A) was used where the recipient is a drought tolerant cultivar and the donor is drought sensitive. The plants were grown in three environments: i) walk-in growing chambers CLF PlantMaster (Irkutsk), where the plants were grown in the soil (humus:sand:peat mixture, 1:1: and drought was generated by water withheld, starting at the three leaf stage and continuing until the soil water content had fallen to 30 % of saturation (Osipova et al. 2011); ii) hydroponic green-house (Novosibirsk) with expanded clay and with the Knop solution for plant nutrition where drought was created on the same stage by full stop of watering; iii) field natural conditions (Novosibirsk), sowing from May to September.

Phenotyping, genotyping and QTL analysis

The lines were studied for 35 phenotypical traits including different photosynthetic parameters, antioxidant enzymes activity, rate of development, yield components under normal and water stress conditions (Table 1). The response of each trait to water deficient conditions was additionally assessed in the form of a tolerance index (IT), given by the expression Td/Tc x 100, where Td was the mean performance of the stressed plants of a given line and Tc that of the well-watered ones. For each line, the mean values, standard deviation, variation limits, and the ratio of the maximum to the minimum value were calculated. The mean values were used

for analysis using the methods of multivariate statistics — principal component analysis and cluster analysis in the PAST program (Hammer et al. 2001).

For the molecular marking of the lines, Illumina 15K array of wheat (TraitGenetics GmbH) was used ((Wang et al. 2014). This allowed the development of a high-density chromosome map 2A (135 SNP markers). Analysis of the genetic structure of the SCSLs population was carried out using the STRUCTURE 2.3.4 program. (Pritchard et al. 2000). According to the results of genotyping the population was divided into 9 sub-clusters. Results on the genetic structure were used to search for marker-trait associations (MTA). Associative mapping was performed using the TASSEL 3.1 program.

The donor of 2A chromosome YP was later in ripening and shorter than S29. It was more productive in normal conditions. On drought, it gave more grains from plant than the recipient but TGW was low. The largest difference for antioxidant enzymes activity between two cultivars was found for LOX in doth conditions. In the set of SRDHL, the traits mostly demonstrated a transgressive variation that indicates the polygenic control. The largest differences between minimal and maximal values among the lines were detected for photosynthetic parameters under both watering conditions. For example, E and G_S levels differed 10 times; the activity of DHAR, GR, LOX differed 5-9 times. Among the yield components, the twofold differences between the maximum and minimum values showed the productivity components of secondary tillers and the overall yield of the plant. Three principal components described the variability of the recombinant population in each of the two irrigation conditions (Table 2). In the first variant they accounted for 76, 4% of variability, in the second – 81,3%. The first component, contributing more than 40% of the total variability were represented by different traits under different watering. In the control, G_S and WUE contributed the most to the diversity between the lines, whereas in drought their significance decreased. LOX activity was the main source of variability under drought; however, this feature was also significant under irrigation. For PC2 on irrigation, besides the mentioned traits, the chlorophyll fluorescence indicators (ETR and F_0) were of great importance. Under water deficit conditions photosynthetic parameters and earliness were the main sources of variability. PC3 in both conditions was determined by the number of days before earing, the different components of the crop and the activity of LOX and SOD. Phenotypic variability between the lines was studied using cluster analysis. The lines were grouped into four main clusters under both irrigation conditions (Table 3). Mean values of the traits of the entire population of SRDHL were compared with the means of the individual clusters. Under irrigation, the grouping of lines was largely associated with the variability of photosynthetic parameters. Variability in LOX activity was significant for two of the four clusters. On drought, the main grouping trait was LOX activity; photosynthetic parameters were as follows in importance. Both under irrigation and drought a decrease in yield components in a cluster occurred simultaneously with a decrease in the level of LOX activity and vice versa. The decrease was mainly observed for the grain number and weight of the secondary tillers. At the same time, the variability in photosynthetic parameters was not related to the variability in the yield components.

The molecular linkage map was used for mapping of genetic loci determining phenotypic traits. Two main QTL clusters were found with the help of association mapping approach in the positions 101,97 cM and 108,5-109,2 cM (Table 4).

A total of 107 marker-trait associations (MTAs) were identified with significance levels from 0,05 to 0,001. Given the complexity of the genetic control of most traits, as well as the particularity of the mapping population, an acceptable level of significance of the associations

in this experiment was considered as P <0.01. Fifteen associations on watering and 22 associations on drought corresponded to this and higher levels of significance (Table 4). The other remaining associations were suggestive. The highest reliable associations were obtained for the number of days before flowering on watering and drought, the height of the plant on watering and the tolerance index of the rate photosynthetic rate on drought (Table 4). In both irrigation conditions, it was possible to map the traits attributed to all five studied groups (Table 1), as well as ITs.

The highest number of QTLs for two watering conditions was found in the position 108.5 109.2 cM. On irrigation, this area was associated with 9 traits determining chlorophyll fluorescence (ETR and Yield), weight of the main shoot, development rate (DT), as well as such components of the crop as TGW and the total number of grains from the plant. During the drought, the number of mapped QTLs increased to 16. The loci associated with the activity of antioxidant enzymes DHAR, SOD and LOX were mapped here, ITs for ETR and Yield, photosynthetic parameters A and E, and carotenoid content. This cluster included four of the nine MTAs found under irrigation (DF, plant height, TGW, and the total number of grains from the plant). The QTL cluster in the position 101.97 cM was associated on watering with five traits: two of them were related to the dynamics of PSII, one to the weight of the green shoot, the rest were associated with yield components. On drought, the range of traits has changed. It included ITs for stability of photosynthesis, chlorophyll fluorescence and phenological traits.

Using the bioinformatic analysis of the revealed regions of 2A chromosome the gene sequences were found which may be considered as candidate genes responsible to water stress. At the position 108.5 - 109.2 cM the cluster of 29 genes was found. Of these, 20 genes encode proteins or important regulatory subunits whose functions are associated with response to external signals. They were attributed to eight functional groups, such as, regulation of transcription; regulation of gene expression; post-translational modification of proteins; ubiquitin-proteasome system; lipid metabolism; redox status regulation; AAA-type ATPase family protein; deubiquitinization. Fourteen genes were identified in the position 101.97 cM. Of these, 10 genes encode proteins with important functions for adaptation of plants relevant to post-translational modifications of proteins, redox regulations, binding of Ca^{2+}.

Acknowledgements

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Table 1: List and abbreviation of the traits studied in SRDHL S29 (YP 2A) under normal and drought conditions.

Photosynthetic parameters	Chlorophyll fluorescence	Activity of antioxidant enzymes	Plant development	Yield components
Transpiration rate (E), stomatal conductance (G_S), rate of photosynthesis (A), water use efficience (WUE), content of pigments in leaves (ChA, ChB, Car), weight of green shoot	Basic chlorophyll fluorescence yield (F_0); maximum electron transport rate (ETR); effective photochemical quantum yield of PSII (Fv/Fm); non-photochemical quenching (NPQ)	Superoxide dismutase SOD); dehydroascorbate reductase(DHAR); ascorbate peroxidase (APX); glutathione reductase)GR); catalase (CAT); lipoxygenase (LOX)	Number of day till tillering (DT), flowering (DF) and wax ripening (DWR)	Tillers number (NT); spike, stem and peduncle length (SL, PH, PL); spikelets number (SpkN); number and weight of grain of the main (GN, GWmain), secondary spikes (GN, GWsecondary) and of the whole plant ((GN, GWtotal); TGW

Table 2: Principal Component analysis of the variance in trait performance under the two water regimes.

Principal components	Well-watered condition		Water deficit conditions	
	Contribution to the total variation, %	Input of certain traits into principal component	Contribution to the total variation, %	Input of certain traits into principal component
PC1	43,1	G_S (-0,92); LOX (0,22); DT (0,22); WUE (0,11)	41,2	LOX (0,95); TGW (-0,16); G_S (-0,12); SpkN (-0,12); DT (-0,12)
PC2	17,2	LOX (0,66); ETR (0,51); F_0 (-0,34); G_S (0,27); DT (0,26)	24,6	G_S (0,68); DT (0,66); WUE (0,16); LOX (0,16); SpkN (0.12)
PC3	16,1	DT (0,65); GNsecond (-0,35); GNtotal (-0,31); LOX (-0,28); GNmain (-0,20); F_0 (0,16); SOD (0,14); G_S (0,16)	15,5	TGW (0,60); GNsecond (0,40); G_S (-0,38); DT (0,26); LOX (0,24); GNmain (0,18); SOD (0,11)

Table 3: Variability of average values of studied traits in the groups obtained during cluster analysis. The average value of every trait was compared with the average value of the same trait in the full set of SRDHL S29 (YP 2A) under every watering condition.

Growing conditions	CLUSTERS			
	1	2	3	4
WATERING	High photosynthetic parameters (E - 164%; Gs - 170%, A - 139%), decreased WUE (77%)	Decreased productivity (85-86%), decreased photosynthetic parameters (E - 40%; Gs - 37%; A - 44%), increased F_0 (133%), decreased Yield, ETR (73%), LOX (66%)	Increased chlorophyll A and B content (115%), NPQ (113), LOX (200%)	Decreased photosynthetic parameters (E - 74%, Gs - 72%, A - 83%), diverse variability for productivity
DROUGHT	Earliness, high photosynthetic parameters (E - 145%, Gs - 155%, A - 135%), decreased WUE (67%); decreased productivity (77-80%) and increased TGW (116%), low LOX (68%)	Low E and Gs (73%), decreased LOX (63%)	Low E and Gs (74 and 75%), increased WUE (124%), high LOX (146%)	Increased productivity (about 115%), increased Gs (130%), increased LOX (112%)

Table 4: Marker-trait associations in the population of 92 SRDHL in normal and drought conditions (summarized from five environments).

Position on 2A, cM	WATERING	Position on 2A, cM	DROUGHT
108,5-109,2	Shoot weight**; ETR**; Yield**; Days till flowering***; Plant height**; Peduncle length**; Grain number of secondary spikes**; TGW**; Total number of grains per plant**	108,5-109,2	IT-A**; IT-E**; IT-ETR**; IT-Yield**; Carotenoids content**; DHAR**; SOD**; LOX**; Days till flowering***; Days till wax ripening**; Plant height**; TGW**; Total number of grains per plant**; IT-Grain number of secondary spikes**
101,97	Shoot weight**; ETR**; Yield**; Peduncle length**; Grain number of secondary spikes**; Total number of grains per plant **	101,97	IT- A***; IT-E**; IT-Gs **; IT-ETR**; Yield**; Days till flowering**; Days till wax ripening***; Plant height**

** - P<0,1; *** - P< 0,001

Analysis of recombinations between 1RS and 1BS chromosomes by using PCR and GLI/GLU markers

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The 1RS.1BL translocation (where the short arm of chromosome 1B (1BS) is replaced by 1RS from winter rye cv. Petkus) is extensively used in wheat breeding programs for introgression of resistance genes for leaf (*Lr26*), stem (*St31*), striped (*Yr9*) rusts, powdery mildew (*Pm8*) (Graybosch et al., 2001; Merker et al., 2000; McIntosh et al., 2013; Singh et al., 2015) as well as for increasing the wheat yield and tolerance to adverse weather conditions (Zarco-Hernandez et al., 2005; Howell et al., 2014). With the positive effects, that 1RS chromosome brings to wheat, it has simultaneous negative effects on flour quality because of the introduction of the *Sec-1* gene cluster that encodes rye storage proteins (α and *ω*-secalins) and the concomitant loss of gliadins/glutenins loci on wheat chromosome 1BS (Dhaliwal, MacRitchie, 1990). Breaking these negative relationships are thus of main interest for 1RS.1BL translocated line improvement.

Usually there is not recombination between the rye and wheat homoeologous short arm chromosomes. However, by crossing Chinese Spring *ph1b* mutant with 1RS.1BL translocation lines of cv. Pavon 76 (Lukaszewski, 2000) recombinant lines were created with 1RS chromosome that had two intercalary segments of 1BS wheat chromatin, one carrying *Gli-1/Glu-3* loci and another replacing S*ec-1* loci that have been removed from translocated 1RS chromosome. Unfortunately, these materials have not appeared so far in commercial wheat cultivars (Lelley et al., 2004) mostly because of a dissection of the rye QTL effects for increased root biomass, grain yield and drought tolerance (Sharma et al., 2011; Howell et al., 2014).

Our aim was to find new combinations of loci/genes which occur as a result of homeologus recombination between the 1RS of rye and 1BS of wheat, among the 63 winter wheat lines (BC_1F_8 CS*ph1b*/E125-03//CS*ph1b* self[7]), which were derived from a cross between Erytrospermum 125/03 (that has 1RS.1BL translocation from rye cv. Aurora) and Chinese Spring *ph1b*-mutant (CS*ph1b*).;.

Materials and methods

Sixty-three original wheat lines (BC_1F_8 CS*ph1b*/E125-03//CS*ph1b* self[7]) were analyzed. They derived from the cross between the highly rust-resistant introgression line Erythrospermum 125-03 (E125-03), with 1RS.1BL translocation from cv. Aurora (Motsnyi et al., 2008) and wheat cv. Chinese Spring *ph1b*-mutant (CS*ph1b*). The parental lines CSph1b and E125/03 and it's F_1 and BC_1 grew up under the insulators. For receiving the recombinant lines several ears of E125/03 were emasculated and each of them was pollinated by 2-3 plants of CSph1b and 36 kernels were received. Next year 4 ears of F_1 were backcrossed by CSph1b and 58 grains were received. Each ear was pollinated by several ears of CSph1b, because CSph1b has small amount of pollen. 10 plants were received and tested for conjugation ability. Two plants were selected

and sown by ears. According to this way that I.I. Motsnyy had received 92 plants from the first BC_1 and 107 plants from second one. From progeny only most resistant plants (from one to ten) were selected from each line up to F_8. These lines are resulted from individual selection during different years for resistance to leaf and stem rusts under permanent artificial infectious pressure.

With the aim to detect the presence of 1RS.1BL translocation and recombination events between chromosomes 1BS and 1RS, 1-3 seeds of each line were analyzed by molecular markers. Among the markers there were either specific to rye 1RS chromosome (*Sec1Gene, Sec1Pro, AF1/AF4, IB-267, NOR, PAW161, Rye F3/R3, RIS*) or to wheat 1BS chromosome (*XTaglgap, Xgwm 18, Xwmc798, Xwmc619, Xwmc406, Xwmc31, Xwmc128, Xwmc419, Xgwm273, Xbarc137, Xgdm136, Xgwm11, Xgdm36, Xwmc626, Xwmc694*); and allele-specific markers – *Gli-A1, Gli-B1, Gli-D1*. The PCR products were analyzed in 2% agarose gel and by using Applied Biosystems 3730xl DNA analyzer.

Allelic composition of storage proteins, the genes codding which are located on short arm's 1st and 6th homeological group of chromosomes in *Gli-1, Gli-2* loci, was detected by Acid (A-PAGE) according to Poperelya (1996). Allele names are given as recommended by Metakovsky et al. (1991) and McIntosh (2003). Long arms of 1st homeological group of chromosomes were controlled by glutenin analysis in SDS-PAGE according to Laemmly procedure modified by Rybalka (2007). Glutenin allele names were given according to Payne et al., (1984). For analysis 4-8 seeds were used, each kernel has been cut on two parts and one part with germ have been used for DNA analysis with molecular markers and the other part – for analysis of storage proteins. Last one was ground individually, gliadins were extracted by 70% ethanol, and insoluble residues became the base for glutenin analysis. At the same time for E125/03 and CSph1b were analyzed 15-17 seeds.

Results and discussions

Allelic composition of the two parents was assessed using storage protein loci. Er.125/03 has alleles *Gli-A1b, Gli-B1l, Gli-D1b*, and *Glu-A1 2*, Glu-B1 7+9, Glu-D1 2+12*. All seeds of E125/03 have the same profile of gliadins and glutenins (so they were identical). It was homozygous and had all 1RS rye markers, no 1BS markers, all 1AS and 1DS wheat markers. On the contrary for cv. CS*ph1b* we revealed alleles *Gli-A1 a, b, c, s; Gli-Ba wide, a narrow, b, new*; *Gli-D1null, b, g* or *j*; *Glu-A1null, 1, 2*, Glu-B1 7+8, 7+9, Glu-D1 5+10, 2+12, null*. We observed that in CS*ph1b* due to the presence *ph1b* deletion there is inducing instability in genome, the results of which there are forming of multivalents and appearing null alleles at *Gli-D1* (1DS) or *Glu-A1* (1AL) or at locus *Gli-A2* (6AS) (data does not present). According to Sanches-Moran et al. (2001) *ph1b* plants showed different numbers and types of chromosomal rearrangements (terminal and interstitial translocations) and aneuploids, which mostly involved the A and D genomes. These intergenomic exchanges are produced in every generation in plants with *ph1b*.

The 63 original lines were analyzed by using PCR-markers specific to rye and wheat chromosomes there were shown that 14 wheat lines are without translocations, all of them have wheat gliadin alleles in electrophoregramms of proteins and 11 lines have classic 1RS.1BL translocation. These lines are characterized by the presence of rye markers and absence of the amplification fragments with markers specific to 1BS of wheat. These 11 lines have not wheat gliadin alleles, only *Gli-B1l*, as mother line Er.125/03 has. 17 wheat lines according to PCR-analysis and storage protein electrophoresis performed on several seeds, were characterized as

heterogenic material, the results were differ from seed to seed.

We have revealed three wheat lines (526PH16, 530PH16, 580PH16) in which, 1RS could be transferred to 1A and had replace 1AS. These lines had amplified fragments from both wheat 1BS and rye 1RS specific molecular markers. The argument to proof that 1AS have been replaced by 1RS, was that there have no specific hybridization sites for primers to *Gli-A1* locus. By electrophoresis of storage proteins of these lines we detected variant of gliadin blocks that are peculiar for 1BS and spectrum of fragments specific for rye storage proteins

Wheat line 542PH16 is recombinant, because we have detected fragments of amplification for this line with markers to 1RS but not with *IB-267*, and we did not detect any PCR products with microsatellite markers to 1BS. By analysis of storage proteins we have shown new composition of gliadin bands: spectrum of gliadins from 1DS has not 2 protein bands, for 1BS or 1RS we have seen two new bands, one of them is look like band which is described by Kozub et al. (2014) and this component is visible as one new band in spectrum of LMW SDS-glutenins. Some components of storage proteins look like allele *Gli-B1o*.

Wheat lines 569PH16, 570PH16, are recombinants, which have 1RS segment from centromere up to *Sec-1* locus, though we have revealed fragments of ampliphications with markers specific to rye, but we also have detected *Gli-B1.2* and *Taglgap* allele specific to CS*ph1b*. We assume that these lines have recombinant short arm of 1B chromosome, which consists from two parts, one part chromosome from centromere up to *Sec-1* locus from rye and the second part has smaller size – terminal part of 1BS that contains *Gli-B1* and *Taglgap* loci. This hypothesis is confirmed by the absence of PCR products for numbers of microsatellite loci located on 1BS in the interval from centromere up to middle of 1BS.sat18-0.50-1.00 region of chromosome.

Wheat line 583PH16 is recombinant but without 1RS, there were not detected PCR fragments specific for marker loci from 1RS and at the same time were revealed double fragments for *Taglgap, Gli-B1.1* and *Gli-B1.2*. As result of analysis of gliadins, we revealed presence of two allelic blocks of components specific for CS*ph1b* 1BS *Gli-B1a* and *Gli-B1b* and absence of gliadin blocks specific of 1DS chromosome. We assume that there was recombination between chromosomes 1B and 1D and was formed 1BS.1DL chromosome addition to the normal 1BS.1BL chromosome.

For wheat line 578.2PH16, 590.2PH16 we detected broken 1BS chromosome these lines have not *Gli-B1* alleles but all the microsatellite markers for loci 1BS have been detected except microsatellite locus *Taglgap,* which are located on distal part of 1BS in region of gliadin genes.

The new lines that have been analyzed and described are interesting for the further study of agronomically important traits including those determining the bread making quality. But at the first step we are planning to check if there is "conditions for genome stability" in the lines by using molecular markers for *ph1b* mutation. The stable lines, in which there are not homeological recombinations, must not have *ph1b*/*ph1b* genotype, such genotype permit to save new composition of loci/genes 1RS:1BS.

Conclusion

As a result of involving CS*ph1b* mutant in cross with the line E125-03 (carrier 1RS.1BL) different types of translocations and recombinations have been developed: lines with two type of recombinations between 1RS and 1BS, lines with relocation of 1RS instead of 1AS arm and with restoration of 1BS, lines with presence of second 1BS arm except 1DS (lines with duplication of 1BS arm), lines with deletion of the terminal part of 1BS arm.

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Useful genetic variability generated in wheat by using a specific mutagenic protocol

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Introduction

Wheat is considered to be the second crop plant in terms of the agricultural importance providing 30-40% of the human population's food needs.

Wheat also provides more calories, fibers and proteins than any other crop, and is indispensable in the diet of the population (Abdel-Aal and Huel, 2002). In addition, the uniqueness of wheat consists in the fact that its seeds contain glutenin proteins, which along with other components that confer quality, make possible to get a various bakery products (Kronstad, 1997). Even if classically method of breeding will remains the main used tool for wheat breeding, the genetic basis of variability on which it operates becomes more and more limited. Besides spontaneous mutation that constantly sustained crop plant evolution before, during and after domestication, artificial mutagenesis is the nearest way to induce new useful genetic variability today.

Mutagenesis is the process in which heritable changes occur in the genetic information of the organism that are not caused by natural genetic segregation or recombination, being induced by chemical, physical or biological agents (Kharkwal el al., 2012; Roychowdhury et al., 2013).

For useful results of mutagenesis application in any breeding programs it is necessary to select biological material that carries genes of interest, and also for mutagenic agent and the dose of the mutagen that can achieves the optimum mutation frequency with the least possible unintended damage (Mba et al., 2010).

Globally, as results of many breeding programs that include mutagenesis, more than 3200 mutants were released as new cultivars, in around 200 cultivated species; in wheat around 255 mutant cultivars have been registered (http://mvgs.iaea.org).

After applying the mutagen factors the Ml derived mutant plants are heterozygous because only one allele per locus is affected by one mutation during treatment. By applying DH-technology it became possible to attain a complete homozygosity at every locus and to make a rapid and easier selection for specific traits, especially for those controlled through recessive alleles.

The objective of this study was to evaluate the variability attained by applying both, mutagenesis and recombination, followed by rapid homozigosity in a set of 307 mutant and mutant/recombinant DH-lines.

Materials and methods

The mutant and mutant/recombinant wheat genotypes were obtained at NARDI Fundulea by using a specific mutagenic protocol including two modern wheat genotypes, two irradiation cycles applications, hybridization of M1`s and DH technology (Giura, 2011, 2013).

The lines were sown each year in the field, in October, along with parental genotypes, in pairs of 2 rows of 1 m in length with a distance of 25 cm between the rows and 50 cm between the pairs of rows. The different climatic changes from the all three experimental seasons, with variations for temperature and recorded precipitations, might have influenced the genotypes response and plant development.

The plant height was measured with a linear graduated meter, at maturity stage, for identifying the semi-dwarf lines that can be considered an important breeding material to prevent plant lodging.

Since the protein content together with Zeleny sedimentation values and wet gluten content are very important quality indices in bakery, lines with a higher quality and productivity are ideal materials for sustaining food needs.

With FOSS INFRATEC 1241, analyzer using near-infrared transmittance technology, were determined protein content and also Zeleny and wet gluten, the latter being estimated according to the standard calibration set on the device.

Thousand kernel weight (TKW) was measured in steps, the counting was done with Contador Seed Counter and the mass was determinate with electronic balance. For test weight (TW) we used a graduated cylinder of 100 ml volume and for volume weighing it was used an electronic balance.

Results and discussions

Multi annual field evaluations have pointed out an extensive variability for the analyzed parameters. Same aspects regarding morphological and productivity traits have also been the subjects of several other studies (Giura, 2008, 2011, 2013; Şerban et al., 2012, Dobre et al., 2014, 2016a, 2016b, Dobre, 2016; Barbu et al., 2017, 2018).

In the set of mutated/recombinant DH lines, plant height proved to be a very variable character in each seasons, with variations of about 50 cm (Figure 1). Although the range in which most of the lines were ranked was differently in all three years, the majority of them being situated in the height interval of 70 -110 cm. The interaction between the genotype and the conditions of the year 2015 resulted in a shorter plant height for all genotypes, about 80% of them measuring between 76 and 90 cm. The situation recorded in 2016 was relatively similar, over 78% of the lines registering a height between 81 and 95 cm. The environmental conditions for 2017 determined the elongation of the plant's height so that over 72% of the genotypes were situated within the interval of 91-105 cm.

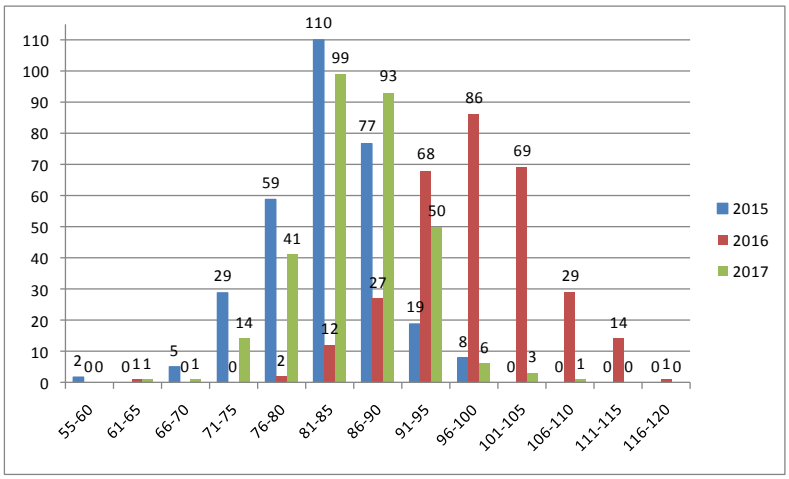

Fig. 1: Variation of plant height in 2015-2017 period.

Regarding TKW distribution (Figure 2), it was registered a very large variation between the three seasons in which values were closer, but for each years the range of variation was over 20 g. It is known that grain size and their form are the component of production with the highest phenotypic stability and consequently these attributes remain constant concerns of wheat breeding programs. In 2017, the values recorded for TKW were situated in the range of 40 - 52 g (76% of total genotypes), similar but still lower values than in 2015, when more than 87% of the genotypes recorded a TKW between 43 to 55 g. The 2016 year conditions were unfavorable for high yield productions so, in these terms, 76% of the genotypes registered a lower TKW around 34 - 49 g.

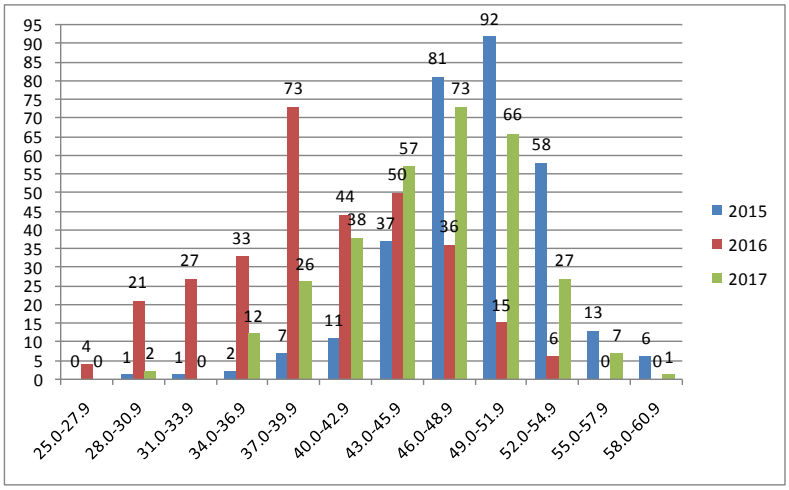

Fig. 2: Distribution of thousand kernel weight (TKW) in 2015-2017 period.

As regard volumetric mass values (TW) it was registered a variations between 180 and 200 g/0.1dm^3 (Figure 3), the highest variability occurring in 2015 and 2017, years when the majority of the lines (93% in 2015 and 90% in 2017) registered a volumetric mass between 820-880 g/0.1dm^3 in 2015 and 760-850 g/0.1 dm^3 in 2017. The year 2016 favored a slightly lower variability of the analyzed parameter, only 82% of the lines having a TW between 760

and 850 g/0.1 dm^3. Evaluating the two charts showing the status of productivity indicators, it could be concluded that 2015 was a favorable year for both MMBs and TWs.

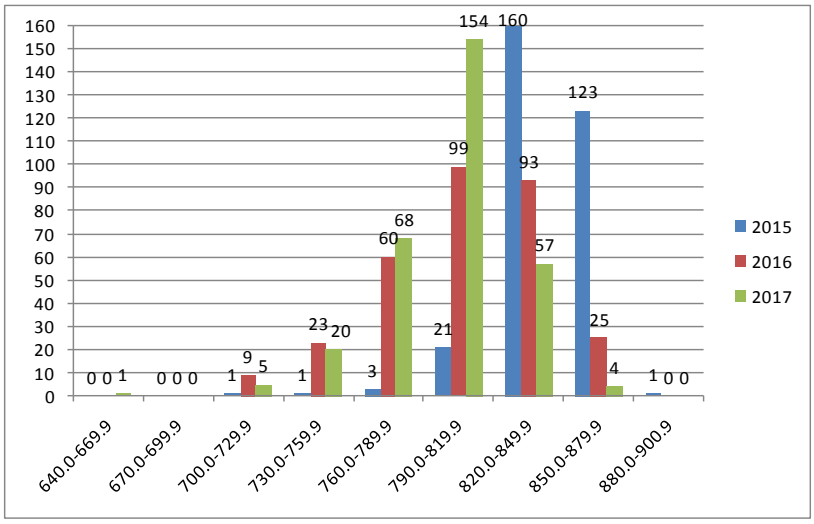

Fig. 3: Variation of volumetric mass (TW) in 2015-2017 period.

As regard protein content variability the difference between lowest content and higher content was in average 8-9% (Figure 4). In 2015, 81% of the lines have had a protein concentration of 13-16%, better than both 2016 and 2017, years when majority of lines (84%) registered 11-14% and 12-15% protein content respectively.

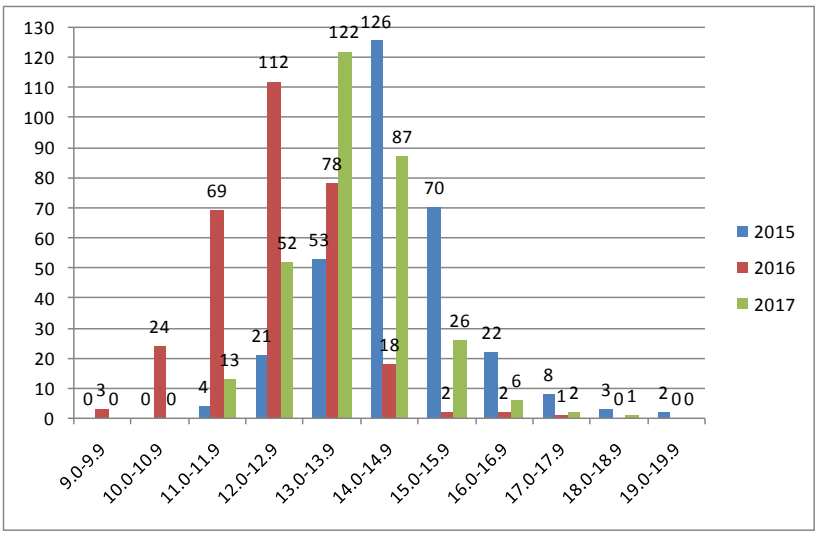

Fig. 4: Variation of protein content in 2015-2017 period.

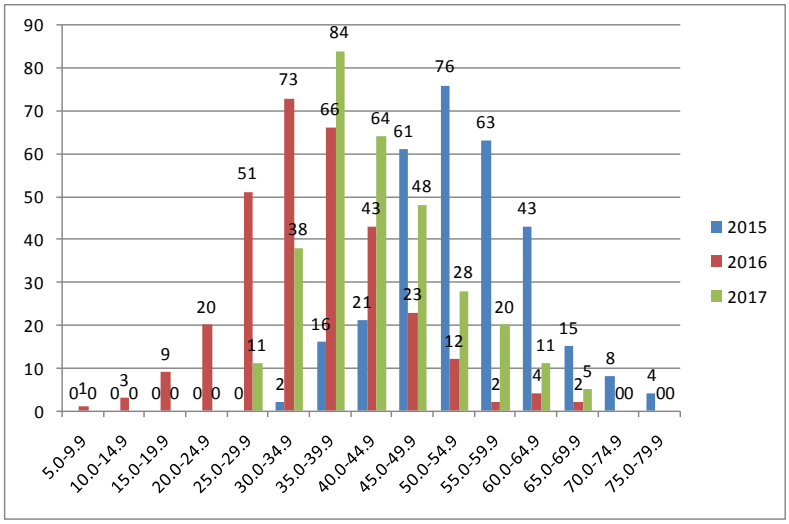

Fig. 5: Variation of Zeleny sedimantation values in 2015-2017 period.

Zeleny sedimentation index had a large variation within the set, as can be seen in figure 5. The highest variability for this character in 2016 was registered, with differences of over 40 ml in comparison to 2015 and 2017. Although the variability is relatively large, with a significant variation (≥40 ml), most of the analized lines recorded values in narrower ranges such as 45-65 ml in 2015 (79% of the lines), 25-45 ml in 2016 (75% of lines) and 30-50 ml in 2017 (76% of lines). Dispersion for wet gluten showed a similar trend to the other two quality indexes analyzed, with which it is often correlated. However, the variability for this character (figure 6.) was expressed by significant differences between lines with the lowest content in wet gluten and the line with the highest content (20-30%). As for each analyzed parameters, with the exception of "outliers" there is a range of values where most genotypes are found, namely: 80% of the genotypes have a wet gluten content between 30 and 40% in 2015 ; 79% of the genotypes registered wet gluten values between 25-35% in 2016 and 78% of the lines had a wet gluten content in the range of 30-40% in 2017.

Conclusions

The large variability recorded individually for each analyzed parameter highlights the benefits of applying a complex mutagenic protocol that included recurrent irradiation (gamma rays), M1`s hybridization and DH technology to produce a large number of homozygous DH lines. By their evaluation it became possible to select the better lines as a new and valuable source of variability either for quality parameters or productivity indices or even for phenotypic characteristics superior to parental genotypes in some cases.

The useful variability generated and described in the present paper evidenced a possible way for broadening the genetic bases for wheat breeding.

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Improvement of resistance to powdery mildew in triticale by transfer of *Pm4b* and *Pm6* genes from common wheat cultivars

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Introduction

Triticale (×*Triticosecale* Wittmack) is the intergeneric hybrid between the female parent wheat (*Triticum* ssp.) and the male parent rye (*Secale* spp.). This artificial cereal combines the rye characteristics of cold and disease tolerance, and adaptation to unfavorable soils and climates with the productivity and nutritional qualities of wheat (Walker et al. 2011; Troch et al. 2013). Therefore, triticale is widely grown in areas that are not suitable for wheat production due to abiotic stress conditions. Triticale, particularly modern cultivars with *Ddw1* dwarfing gene are popular in filed production in many countries in Europe (Poland, Germany, Belarus, France, Hungary, Austria, Spain) and in China, Unites States or Mexico.

In recent years, an increase in triticale infection by powdery mildew has been observed in Poland.

The spread of powdery mildew was predominantly caused by the cultivation of triticale cultivars with identical or related resistance types. Rapid spread of new virulent powdery mildew genotypes is an effect of genetic uniformity of cultivars, long-distance wind transfer, use of pesticides, high adaptability of powdery mildew as well as mutations and recombinations that cause genetic changes in the powdery mildew population. Alterations in *Blumeria graminis* physiological specialization can be noticed. Genetic variation of powdery mildew population results in the loss of initially high effectiveness of powdery mildew resistance genes and poses a constant threat to resistance sources that are used in breeding (Bayles 1997; Zeng et al. 2007).

The most efficient way of controlling and limiting the crop damage caused by this pathogen is the introduction of resistant cultivars to cultivation. Therefore it is necessary to look for new resistance sources with the purpose of introducing new resistance genes or their combinations that will be effective against existing pathogen populations (Kowalczyk 2001). In order to improve the triticale resistance to powdery mildew, to susceptible triticale cultivars, the *Pm4b* resistance gene (that originates from *Triticum carthlicum*) was transferred from common wheat. The *Pm4b* gene is located on the long arm of the 2A chromosome (Rong, et al. 2000), hence it can be easily introduced into triticale. In 1989-1992 *Pm4b* was one of the most effective genes conferring resistance to all *Blumeria graminis* f.sp. *tritici* populations in Central Europe (Švec et al. 1998). The *Pm6*, *Triticum timopheevii* - derived powdery mildew resistance gene, was introduced into common wheat by recombination between *T. aestivum* B genome and *T. timopheevii* G genome. This gene was mapped on the long arm of the 2B chromosome. It was widely used in European wheat breeding programs, especially in combination with the *Pm2* gene.

The aim of the study was the evaluation of triticale hybrids resistance to powdery mildew and the assessment of selected yield components of these hybrids. Triticale hybrids analyzed in this study carried *Pm4b* and *Pm6* genes introduced from common wheat cultivars.

Materials and methods

Research material consisted of the F1 - F_5 triticale hybrids carrying *Pm4b* and *Pm6* genes introduced from common wheat, that were obtained in the Institute of Plant Genetics, Breeding and Biotechnology of the University of Life Sciences in Lublin, and their parental forms. The hybrids were obtained through crosses between the triticale cultivars Fidelio, Magnat and Lamberto with common wheat cultivars Meridien, Nowalis, Clever, Finezja and Tonacja. The Meridien and Nowalis wheat cultivars contain the *Pm4b* resistance gene, while the Clever, Finezja and Tonacja carry the *Pm2* and *Pm6* genes combination.

Field trails were conducted at Czesławice Experimental Station. Hybrid kernels were sown in four-rowed plots, in three replicates. Control forms consisted of triticale and common wheat cultivars. During the vegetation period the powdery mildew infection level was evaluated twice according to COBORU recommendations on a 9-degree scale, where 1 refers to severe infection covering over 50% of leaf area, while 9 means lack of powdery mildew infection. A total of 30 plants from each cross combination and parental triticale forms were analyzed. After harvesting, plants height, number of kernels per plant, number of kernels per spike and 1000 kernel weight was determined. The obtained data was analyzed with post-hoc Tukey's HSD test ($p < 0.05$) with SAS 9.2 software.

DNA markers were used to confirm the presence of powdery mildew resistance genes in triticale hybrids. The extraction of DNA was performed according to modified CTAB-based method (Aldrich and Cullis 1993). The concentration of DNA was assessed with NanoDrop 2000 spectrophotometer (Thermo Scientific). The DNA integrity was analyzed by the means of agarose gel electrophoresis. Identification of *Pm4b* and *Pm6* genes was performed using primers developed by Yi et al. (2008) and Ji et al. (2008) (Table 1). PCR reactions were carried out on TProfessional Basic (Biometra) thermocycler. PCR products were separated on 1.5% agarose gel.

Table 1: Primer sequences used for *Pm4b* and *Pm6* genes identification.

Primer	Sequence 5'-3'	Reference
Pm4F	CTCATTCTTGTTTTACTTCCTTCAGT	Yi et al. (2008)
Pm4R	GTCTCGTCTTCAGCATCCTATACA	
$NAU/STS_{BCD135\text{-}2L}$	GCTCCCAACCAAGAGAAGAA	Ji et al. (2008)
$NAU/STS_{BCD135\text{-}2R}$	TCTGTCGGTCCTCTGATGTG	

Results and discussions

Yi et al. (2008) developed STS marker (STS_{-241}) linked to the powdery mildew resistance gene *Pm4b* at a distance of 4.9 cM. Primer sequences designed by them were used in order to identify the *Pm4b* presence in triticale hybrid plants obtained in presented study. The specific PCR product of 241 bp obtained with Pm4F and Pm4R primers confirmed the presence of *Pm4b* gene among tested plant material. The gene was identified in a number of F2 hybrids of Fidelio, Magnat and Lamberto triticale cultivars with Meridien and Novalis wheat cultivars.

Ji et al. (2008) in their study focused on the development of *Pm6* gene marker that could be easily applied in marker-assisted selection in wheat breeding programs for powdery mildew

resistance. Primer pair NAU/$STS_{BCD135-2}$ developed by researchers amplified a 230-bp PCR fragment associated with *Pm6* gene. Obtained STS marker was linked to the *Pm6* powdery mildew resistance gene at a distance of 0.8 cM. This marker was tested in presented study and its presence was confirmed in a number of F2 hybrids of Fidelio and Lamberto triticale cultivars with Clever, Finezja and Tonacja common wheat cultivars, thus confirming the presence of the *Pm6* gene in these forms.

Analyzed resistance to powdery mildew of F1 hybrids obtained in the Institute of Plant Genetics, Breeding and Biotechnology was significantly higher than the resistance of their triticale and wheat parental forms. The highest level of infection was noticed in tested triticale cultivars. Detailed results of hybrids resistance assessment to powdery mildew infection are presented in Kowalczyk et al. (2011).

According to Kowalczyk et al. (2011), the infection of F2 hybrids ant their parental forms by powdery mildew in 2009 was not very high, partially due to dry and warm weather in April and May. Obtained results showed that triticale hybrids were characterized by significantly lower powdery mildew infection level than triticale cultivars. Most of analyzed hybrid plants were not infected at all. Out of all hybrids tested that carried *Pm4b* gene, highest resistance was noticed in Fidelio × Novalis cross combination (8.20° on COBORU scale), whereas among hybrids with *Pm6* gene, in Magnat × Finezja cross combination (8.25° on COBORU scale). On the other hand, highest infection level among hybrids carrying *Pm4b* gene was observed in Fidelio × Meridien (7.84° on COBORU scale), while among *Pm6* hybrids in Fidelio × Clever (7.16° on COBORU scale).

Subsequent years of research showed that triticale hybrids containing *Pm4b* and *Pm6* resistance genes were significantly more resistant to powdery mildew in comparison to their parental triticale (Figure 1) and common wheat forms. In following years, starting from F2 generation, plants underwent selection - only those carrying powdery mildew resistance genes and of reduced plant height and beneficial yield components (the number of kernels per spike and 1000 kernel weight) were chosen. As a result, forms with reduced plant height were obtained, however, hybrids were higher than analyzed triticale cultivars. F3 and F4 hybrids were characterized by the highest number of kernels per spike and highest 1000 kernel weight. Slightly lower values of these components were found in the F5 generation (Figure 2). Gruszecka and Kowalczyk (2000) and Kowalczyk and Gruszecka (2000) state, that in triticale breeding crosses with other species and genera are used with the purpose of introducing resistance to powdery mildew. Nonetheless, hybrids of triticale and wheat, especially in the first generations, are characterized by a reduced value of yield components. In presented study, hybrid forms were higher than parental triticale cultivars. However, as a result of selection carried out in earlier generations, they were characterized by a similar number of kernels per spike as their parental forms. Even so, selected hybrids had slightly lower weight of 1000 kernels when compared to the parental triticale cultivars.

Conclusions

1. The introduction of *Pm4b* and *Pm6* genes into triticale cultivars significantly increases resistance to powdery mildew.
2. Pyramidation of resistance genes is a good strategy that should be used in resistance breeding program of triticale.

3. The use of DNA markers allows the selection of hybrids (forms) carrying *Pm4b* and *Pm6* powdery mildew resistance genes.

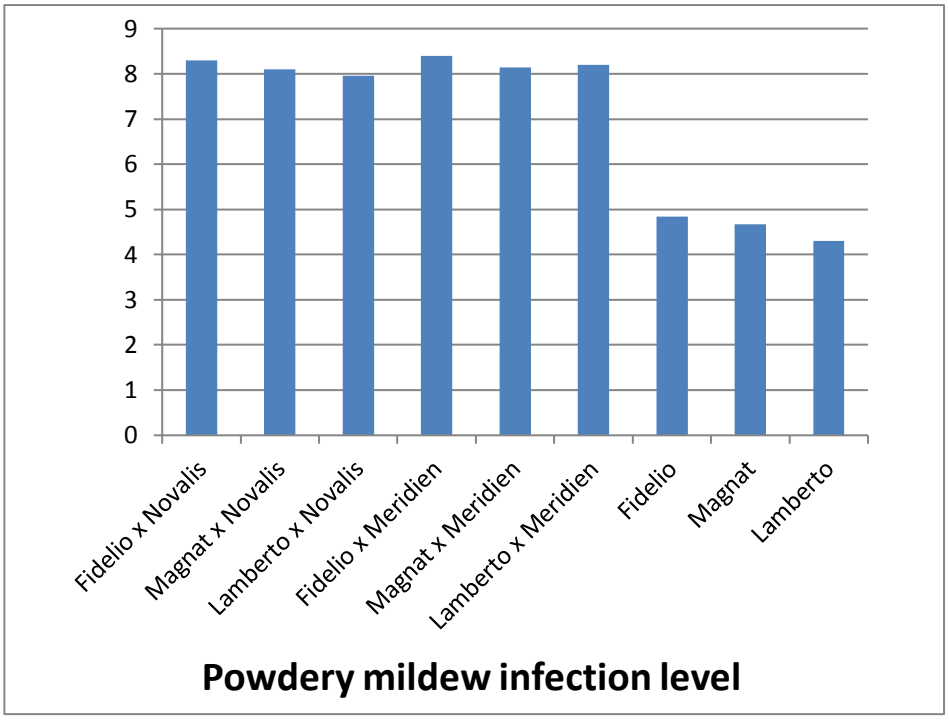

Fig. 1: Powdery mildew infection level according to COBORU 9-degree scale of F5 triticale hybrids and parental triticale cultivars.

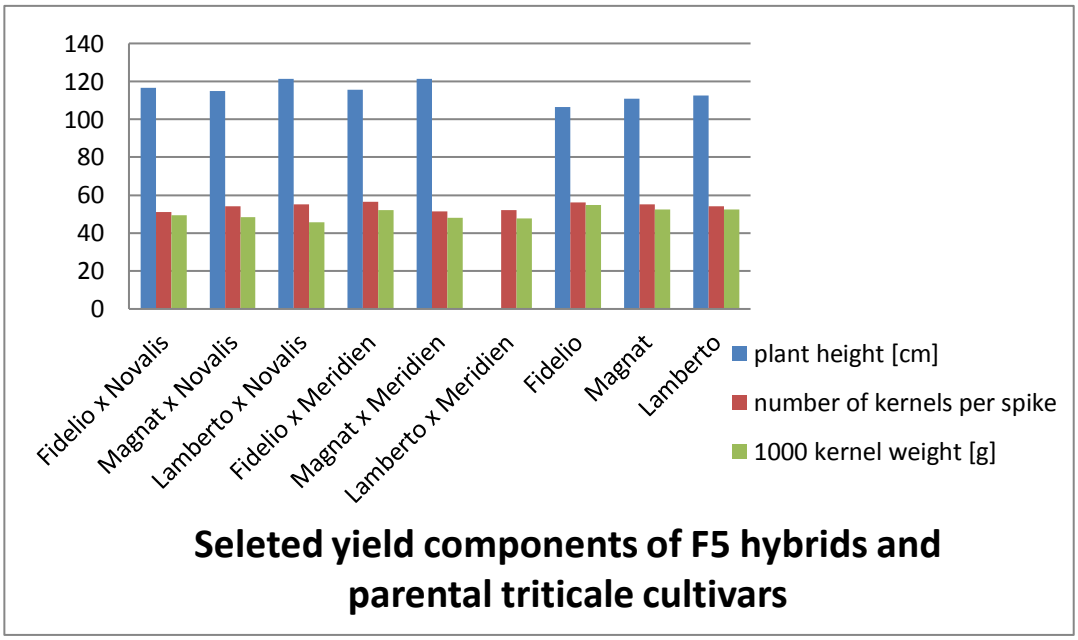

Fig. 2: Selected yield components of F5 hybrids and parental triticale cultivars.

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Oat powdery mildew – identification and characterization of new sources of resistance

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Oat is a plant grown all over the world. The largest producers of oats, according to FAO are: Russia, Canada, Poland, Australia, Finland, USA, Spain and Brazil (http://www.fao.org). Oat is widely used primarily as animal feed, however, it is also utilized in human nutrition, in the pharmaceutical and cosmetics industry (Bartnikowska et al., 2000; Petkov et al., 1999; Jasińska 1999). The possibility of using oat for energetic purposes is one of numerous oat applications (Janowicz 2006, Kwaśniewski 2010). Moreover, oat deserves attention as a phytosanitary plant in crop rotation, as it releases specific organic substances with fungistatic activity towards pathogens present in soil (Pawłowska et al. 1999).

Oat is a cereal susceptible to many diseases, one of the most dangerous is powdery mildew, caused by the parasitic fungus *Blumeria graminis* DC. f.sp. *avenae* Em. Marchal. This pathogen is common in Europe and North America where causes large losses in yield size and quality (Schwarzbach and Smith 1988, Aung et al., 1977, Clifford 1995; Hsam et al., 1997).

Losses in cereal production caused by powdery mildew can be limited by cultivation genotypes with effective resistant genes (Feuillet and Keller 1998). It is necessary to conduct systematic analysis of the structure and dynamics of pathogen virulence changes in order to predict the effectiveness of resistance sources used in breeding programs. Obtaining cultivars with permanent, stable and effective resistance in various environmental conditions is a complex process that requires large work expenses. Pietrusińska and Czembor (2015) indicated that the process of obtaining resistant cultivars should be preceded by an accurate characterization of the level of resistance of cultivars grown in a given area and analysis of pathogen population virulence.

Due to the low number of studies concerning powdery mildew in oat, the aims of our work taken in recent years were:

- Analysis of changes in the virulence structure of *Blumeria graminis* f.sp. *avenae* population present in Poland
- Analysis of the effectiveness of oat powdery mildew resistance and the identification of these, with the highest potential and application possibility in breeding programs
- Characterization of the level of resistance against powdery mildew of Polish oat cultivars
- Identification of new, effective sources of powdery mildew resistance among wild species belonging to the genus *Avena* and the identification of species most useful for improving oat resistance

Determination of *Blumeria graminis* f.sp. *avenae* population virulence in Poland

Monitoring the dynamics of changes in the virulence of the *Blumeria graminis* population in a given area is of great importance for better utilization of available sources of resistance to this pathogen (Heun 1987). Analysis of pathogen virulence variability allows to determine the dynamics of changes occurring in the population related to the possibility of overcoming the resistance conditioned by dominant genes. Data on the level of pathogen virulence may be useful in the selection of resistance genes that provide a high level of protection over many years under different environmental conditions and in the selection of effective genes to create gene pyramids. In the available literature, there are no reports on changes in oat powdery mildew virulence, therefore, in our work we determine the level of virulence of the *Blumeria graminis* f.sp. *avenae* population in Poland (Okoń and Ociepa 2017).

The tested powdery mildew isolates almost completely overcame the resistance conditioned by *Pm1, Pm3* and *Pm6* genes. The virulence frequency for these genes ranged from 80 to 100%. The isolates of the powdery mildew collected in the country began to overcome the resistance conditioned by the *Pm7* gene, however, the virulence frequency against this gene remained very low and did not exceed 10%. This proves that currently occurring pathotypes of powdery mildew have begun to overcome the resistance conditioned by this gene. None of the tested powdery mildew isolates overcame genetic resistance conditioned by *Pm2, Pm4* and *Pm5* genes.

The study showed that powdery mildew population diversity was low in Poland, but an increase in the level of virulence could be observed in relation to the resistance genes described to date. However, the slow rate of these changes allowed to presume that pathotypes that would be able to completely overcome the resistance conditioned by the *Pm4, Pm5* and *Pm7* genes would not emerge in the country in the coming years.

Characterization of the level of resistance of cultivars grown in the country

Constant changes in the environment requires detailed characterization of cultivars in the context of their resistance to various types of fungal diseases, including powdery mildew. In our work we try to determine the level of resistance of Polish oat cultivars grown in the country in the last 25 years (Okoń 2012, Okoń et al. 2016). The subject of the analysis was cultivars entered into the National Register of cultivars in 1991-2016. The analysis of the level of resistance was performed on the basis of host-pathogen tests. Used isolates allowed to postulate resistance genes in tested cultivars. Analyses showed that out of 51 Polish oat cultivars, only two (Skrzat and Gniady) had an infection pattern identical to the cultivar Jumbo carrying the *Pm1* gene. The *Pm6* gene was identified in the cultivars Dragon and Rajtar. The tests showed that the cultivars Deresz and Grajcar had an infection pattern matching the cultivar Mostyn containing the *Pm3* gene. The cultivars Celer, Kasztan, Maczo and Nawigator have been identified with different infection patterns differing from those of the reference line set, which may indicate that these varieties have combinations of different powdery mildew resistance genes. The small number of identified cultivars with resistance genes suggests that it is necessary to introduce more genes to breeding programs that determine effective resistance.

Analysis of the effectiveness of powdery mildew resistance genes described to date

Improving cultivar resistance consists in introducing effective resistance genes into their genome that will protect plants against infection for a long period of time and in different

environmental conditions. Information on the resistance of lines with defined powdery mildew resistance genes in oat dates from 1977-1998 (Jones 1977, Jones and Roderick 1986; Sebesta et al., 1991). Herrmann and Roderick (1996) described the APR122 line as highly resistant both in laboratory conditions at the seedling stage as well as in the mature plant stage. Hsam et al. (1997; 1998) demonstrated that the APR122 line with the *Pm7* gene had a high level of resistance to powdery mildew isolates used by these authors. Hsam et al. (1997; 1998) also indicated that the *Pm4* gene was highly effective against powdery mildew in oat. However, there is no information on the effectiveness of the *Pm1, Pm3* and *Pm6* genes that have been identified in many oat cultivars (Hsam et al., 1997; 1998; Kowalczyk et al., 2004; Okoń 2012; Okoń et al., 2016). In our work we determine the effectiveness of powdery mildew resistance genes described to date in Polish conditions (Okoń 2015). The analyses showed that the *Pm1* and *Pm3* genes were ineffective against the powdery mildew pathotypes present at that time in eastern Poland. They showed a moderate level of effectiveness towards pathogen isolates collected in western Poland. The *Pm6* gene also did not determine a high level of resistance of the reference line; the resistance conditioned by this gene was overcome by all isolates collected in Poland. The studies showed that the *Pm4* and *Pm7* genes were the most effective in Polish conditions. Own observations carried out in 2016-2017 (unpublished data) showed that the *Pm5* gene, which has also not been used in breeding programs so far, also provided high level of resistance. This gene has already been introduced into the genome of hexaploid species and can be successfully used to increase the level of resistance of oat cultivars (Yu and Herrman 2005).

Searching for new, effective sources of powdery mildew resistance among wild species belonging to the genus *Avena*

Increasing the genetic variability of cultivated forms and introduction of disease-resistance genes into them is an important aspect of many breeding programs. Wild species related to crop species are a rich source of many genes that can improve many traits of cultivated forms (Reader and Miller 1991, McIntosh et al., 1998, Shi et al., 1998, Xu and Kasha 1992, Pickering et al., 1995). Wild species have been frequently used as a source of valuable genes for crops also in oat (Aung et al., 1977; Sebesta et al., 1993, Hermann and Roderick 1996; Sánchez-Martín et al., 2012; Tan and Carson 2013). It is necessary to search for new resistance sources due to the very low level of powdery mildew resistance of Polish oat cultivars and a small number of identified effective genes determining high resistance. Such necessity was already indicated by Hsam et al. (1997; 1998). To date, a number of potential sources of resistance to fungal diseases have been identified in oat both in the cultivated *A. sativa* species (Jones 1983, Hsam et al., 1997, Hsam and Zeller 1998) and wild *A. sterilis*, *A. strigosa*, *A. occidentalis*, *A. pilosa*, *A. macrostachya* and *A. barbata* species (Hoppe and Kummer 1991; Herrmann and Roderick 1996; Yu and Herrmann 2006; Thomas et al., 1980; Aung et al., 1977; Rines et al., 2007; Rines et al., 2017). However, these studies were conducted a rather long time ago, and it is necessary to search for sources that will be effective over the coming years due to continuous changes in pathogen virulence occurring in a given area. Because of this in our work we try to select genotypes belonging to wild species of the genus *Avena*, which can be potentially used to increase powdery mildew resistance (Okoń et al. 2014, 2016, 2018). The subject of the research were genotypes belonging to the wild *A. sterilis*, *A. fatua*, *A. magna*, and *A. murphyi* species, collected at the Institute of Genetics, Plant Breeding and Biotechnology. The first step in the analysis was to select species with the highest potential for powdery mildew resistance. The tests showed that genotypes belonging to the tetraploid species were characterized by the highest level of resistance. The *A. magna* and *A. murphyi* genotypes were totally resistant or showed a moderate response to powdery mildew isolates used in host-pathogen tests. Genotypes belonging to *A. sterilis* may also be the source of valuable resistance genes. The

lowest level of resistance was identified among the genotypes belonging to *A. fatua*. All the analyzed genotypes were susceptible or showed moderate levels of resistance to the powdery mildew isolates used in host-pathogen tests.

In further work we focused on *A. sterilis* genotypes. This species, like the cultivated oat, is hexaploid, which makes the transfer of new genes easier than in tetraploid or diploid species. We tested 350 genotypes belonging to *A. sterilis*, among them, 10 showed complete resistance to the powdery mildew isolates. Selected resistant genotypes were re-tested using the host-pathogen tests and 13 additional powdery mildew isolates. This allowed to more accurately determine the level of resistance of these genotypes and select those that could be used to increase cultivar resistance. The results of physiological tests have identified four most promising genotypes of *A. sterilis*: CN67383, CN113536, CN22667 and CN22664.

These genotypes can be successfully used to increase powdery mildew resistance of oat crops.

Conclusions

1. Powdery mildew populations occurring in Poland are characterized by an increase in the level of virulence in relation to the oat powdery mildew resistance genes described to date.
2. Polish oat cultivars are characterized by a low level of resistance to powdery mildew. The *Pm1*, *Pm3* and *Pm6* genes identified in Polish cultivars are ineffective against *Blumeria graminis* f.sp. *avenae* races occurring in Poland
3. The analysis of gene efficiency has shown that the *Pm4*, *Pm5* and *Pm7* genes are currently the most effective against powdery mildew in Poland.
4. Currently, the *Pm4* gene is the most promising source of resistance against powdery mildew in Poland.
5. A low number of effective powdery mildew resistance genes in oat makes it necessary to search for new effective sources of resistance among wild species related to the cultivated oat.
6. Tetraploid *A. magna* and *A. murphyi* species and hexaploid *A. sterilis* species are a valuable source of effective powdery mildew resistance genes in oat.

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Effects of the *Ppd-D1a* / *Ppd-D1b* alleles on agronomical traits of winter wheat in south Ukraine steppe region

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The dominant allele *Ppd-D1a* is a major source of photoperiod insensitivity in wheat cultivars worldwide and can promote earlier ear emergence and flowering compared to its recessive allele *Ppd-D1b* (cited by Chen et al., 2018).

The aim of our work was to investigate effects of *Ppd-D1a* / *Ppd-D1b* alleles on the agronomical traits and growth rate of analogue-lines of wheat in the south Ukraine steppe region by using discriminant and ANOVA analysis.

Materials and methods

The agronomical traits of analogue-lines (BC_7) created in PBGI on two different genetic backgrounds of the well-known old Ukrainian cultivars «Kooperatorka» and «Stepnyak» were investigated (Table 1).

Table 1: Characteristic of the analogue-lines that have been used for investigation.

Analogue-line	Pedigree	Alleles of *Ppd-1* genes
Kooperatorka (K)	selection from cv. Kooperatorka	*Ppd-A1b Ppd-B1b Ppd-D1b*
Kooperatorka rannya (Kr)	K x Kooperatorka K-90	*Ppd-A1b Ppd-B1b* ***Ppd-D1a***
Stepnyak 1 (St1)	selection from cv. Stepnyak	*Ppd-A1b Ppd-B1b Ppd-D1b*
Stepnyak 1 ranniy (St1r)	St1 x Stepnyak 2K	*Ppd-A1b Ppd-B1b* ***Ppd-D1a***

The lines were grown up in a field using one-line plots, in three replicates in 2015/2016 and 2017/2018 agronomical years. The conditions of vegetation were quite favorable for winter wheat. At the autumn period of both growing seasons the weather conditions were similar. Due to the late sowing winter wheat grew slowly and the crops in the field were in proper state. In December 2017 the average temperature (T ºC) was higher at 5º than in previous years and vegetation of winter wheat have stopped on 9 days later then in 2015. There was fixed unusual warm weather in February 2016 (the average T ºC was + 4,4 °C, the highest T ºC was + 20 °C) which led to the renewal of the vegetation of plants on 23-24 days earlier than in many years of field observations and this conditions were unlike to 2017/2018 agricultural year when resumption of the growing of plants was happened only in the second decade of March. Then due to a strong cooling, frosty weather in spring the wheat plants had stopped vegetation again at 18-19 of March. In April 2016 the resources of productive moisture reached 108-147 mm in the one meter of ploughing soil layer that was sufficient for the normal development of plants. In 2018 was observed two times lower amount of productive moisture in spring period. In June of 2016 mainly the weather conditions were favorable for vegetation of wheat (the T ºC was

21-22°C, total precipitation was 159% of the monthly norm) and in 2018 it was 23-24°C and 124%, respectively.

During the growing season, harvesting and threshing the following characteristics of plants were determined: the date of earing (DE), plant height after flowering (PH1), plant height at the time of harvesting (PH2), productive tillering (PT), the length of the main ear (l), stem length (h), the l/h ratio, the number of spikelets in the main ear (NSE), the number of fertile spikelets in the main ear (NFS), the number of sterile spikelets in the main ear (NSS), the number of kernels from the ear (NKM), number of kernels from the secondary ears (NKE), the number of kernels per plant (NKP), the weight of kernels from main ear (WKM), the weight of kernels from the secondary ears (WKE), the weight of kernels per plant (WKP), the weight of 1000 kernels from the ear (WTKM), the weight of 1000 kernels from the secondary ears (WTKE), the weight of 1000 kernels per plant (WTKP), the number of seeds per spikelet (NSSp), and ear density (D).

The data estimation was performed with three-factor analysis of variance (ANOVA) and discriminant analysis using the software package Statistica 10. The significance of differences was determined by LSD appropriate level of significance for each factor or their interaction (Rokitsky, 1973; Fukunaga, 1979). A discriminant analysis was used to determine the associations between characteristics that had the best impact in the differentiation of analogue-lines from each other by the investigated traits. It is impossible to incorporate derivative characters into discriminant complexes, which are calculated from the others as mathematical linear functions, that's why only independent traits were included in the model.

Results and discussions

In the previous study (Bakuma et al., 2018) with the help of PCR analysis with different types of molecular markers have been identified the alleles of photoperiod sensitivity genes and detected high level (95,6%) of the recurrent background recovery in the analogue-lines. However, in the genotype of line St1r was also detected *Rht8c* allele that have been inherited from the donor of *Ppd-D1a*.

For the analysis of effects of *Ppd-D1a* / *Ppd-D1b* alleles on agronomical traits and growth rate structural analysis of the yield for investigated lines have been done. Using the three-factor variance analysis was shown, that the interaction of the factors «Genotype of line for *Ppd-D1* gene» x «Genetic background» x «Year» has significant influence on the variation of the traits DE, l, h, l/h, NSE, NFS, NKM, WKM (Table 2). At the same time in period of observation 2015/2016 (Bakuma et al., 2018) and 2017/2018 agronomical years, the next factors affect significantly on the traits: «Genotype of line for *Ppd-D1* gene» on DE, PH1, PH2, l, h, l/h, NSE, NFS, NKM, WKM, WKP, WTKP; the factor «Genetic background» on DE, PH1, PH2, PT, l, h, l/h, NSS, NKE, NKP, WKE, D, NSSp and the factor «Year» on DE, PH1, PH2, PT, l, h, NSE, NSS, NFS, NKM, NKE, NKP, WKM, WKE, WKP (Table 2).

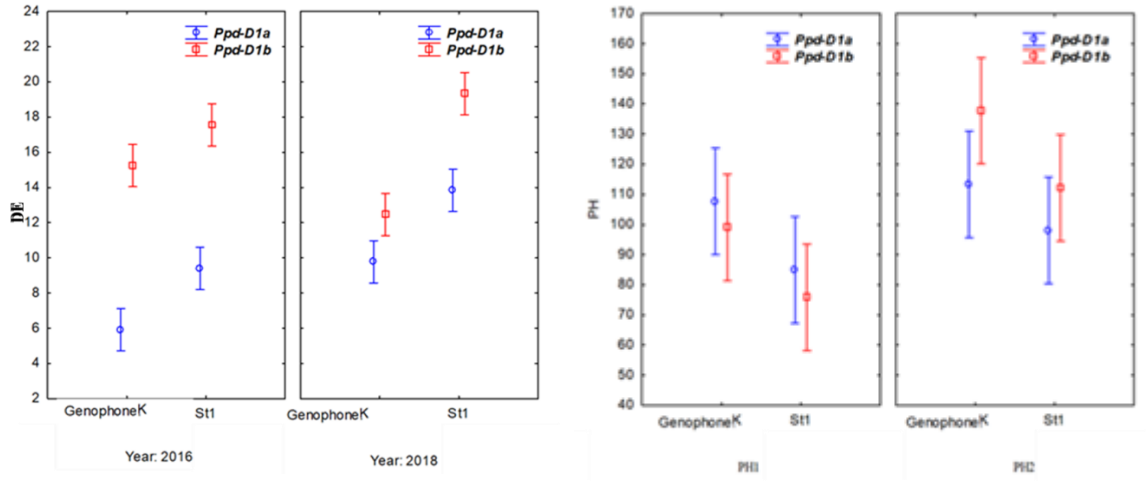
Table 2: Variance analysis of the traits for the analogue-lines grown in 2016, 2018 years.

Trait				Source of variance, mS				
	«Genotype of line for *Ppd-D1* gene» (df = 1)	«Genetic background» (df = 1)	«Year» (df = 1)	Interaction «Genotype of line for *Ppd-D1* gene » x «Genetic background » (df = 1)	Interaction «Year» x «Genetic background » (df = 1)	Interaction «Genotype of line for *Ppd-D1*» x «Year » (df = 1)	Interaction «Genotype of line for *Ppd-D1*» x «Genetic background » x «Year » (df = 1)	Error (df = 16)
DE, days[1]	246,67***	104,89***	19,83***	1,06	10,0**	32,53***	5,97*	0,96
PH1, cm	471,58***	3175,35**	9150,83***	0,31	225,76*	523,16**	21,89	45,74
PH2, cm	2220,22**	2519,05***	6718,34***	158,75	2,98	110,50	0,02	53,98
PT, pcs	0,003	22,109**	172,637***	21,769**	11,992*	0,212	2,126	2,465
l, cm	12,10***	1,19*	27,89***	0,31	0,50	0,62	1,20*	0,23
h, cm	1682,69***	3554,86***	1348,62***	400,45***	133,21*	50,48	1090,79***	16,14
l/h	0,00040**	0,00170***	0,00010	0,00011	0,00002	0,00024*	0,00056**	0,00004
NSE, pcs	26,40***	0,58	25,83***	2,05*	11,96***	0,85	3,88**	0,33
NSS, pcs	0,50	2,12***	0,62*	0,03	0,34	1,79**	0,03	0,12
NFS, pcs	34,14***	0,48	34,50***	2,61*	8,25***	0,17	3,19*	0,40
NKM, pcs	3,30*	78,58	427,52***	33,90	21,23	144,55**	128,24**	13,98
NKE, pcs	2639,19	19915,00*	266538,31***	18702,42*	34807,16**	14259,81	9101,24	3398,33
NKP, pcs	2829,16	22495,55*	288315,42***	20328,73*	36547,62**	17275,79*	11390,18	3511,48
WKM, g	0,38*	0,01	1,10**	0,01	0,75**	0,22	0,51*	0,08
WKE, g	30,72	1,96*	298,61***	7,78	127,47***	32,58*	5,36	5,20
WKP, g	37,97*	1,76	335,94***	7,31	147,75***	38,10*	9,16	5,56
WTKM, g	15,10	16,48	0,59	240,94	277,06	198,46	5,99	87,99
WTKE, g	5,18	101,80	0,02	127,89	194,51	288,11	14,40	69,84
WTKP, g	204,51**	27,93	1,21	63,02	333,19***	0,01	0,45	14,16
D	0,09	13,75***	0,691	2,78*	0,96	2,86*	1,44	0,41
NSSp, pcs	1,96	0,07*	0,01	0,49	0,54	1,12	0,12	0,31

Significant at * P = 0.05, ** P = 0.01, and *** P = 0.001; the significance was determined with the F-test; [1] – number of days to ear emergence, starting from the May 1.

The significant influence of the «Year» factor on a numbers of traits can be due to the difference in weather conditions of 2015-2016 and 2017-2018 agricultural years.

At the same time on the duration of the period of «seedlings-earing» there were revealed effects of the all factors and interaction of the factors, accept interactions «Genotype of line for *Ppd-D1* gene» x «Genetic background». We need to note that on genetic background of varieties Kooperatorka and Stepnyak in average for two years of observations the allele *Ppd-D1a* have decreased the duration of period "seedlings-earing" on 6.4 days in the environmental conditions of the South of Ukraine (Figure 1 A). It is comparable with the data of Fayt and Fedorova (2007), who revealed the effect of *Ppd-D1a* on the shortening of period «seedlings-earing» for – 3 days in southern region of Ukraine and Matsuyama et al. (2015) – 8 days in the central and south-west region of Japan.



A B

Fig. 1: A – Effects of interaction of «*Ppd-D1* genotype of line»x «Genetic background» x «Year » on date of earing (DE); B – Effects of alleles of *Ppd-D1* genes on plant height have measured just after flowering (PH1) and at the time of harvesting (PH2) average by both years.

We have shown that allele *Ppd-D1a* decreased height of plants (PH2) on 17.4 cm on the background Kooperatorka that is more strong effect than Matsuyama et al. (2015) had revealed – 10 cm. The complex of alleles *Ppd-D1a* + *Rht8c* decreased plant height in average 16% in conditions of south Ukraine (Chebotar et al., 2012) and in our investigation on 12.3%.

The tendency demonstrated by Bakuma et al. (2018), which reflect the influence of *Ppd-D1a* on the development of plants, was the same in 2016 and 2018 years. After the flowering time the height of the plants of both lines with allele *Ppd-D1a* were higher, than height of the plants of lines with recessive *Ppd-D1b* on both of genetic backgrounds, but at the harvesting time the lines with dominant allele *Ppd-D1a* were smaller, than lines with *Ppd-D1b,* since their growth have stopped after the flowering (Figure 1 B)

The quality of discriminant analysis conducted with data obtained in 2018 was high, the genotypes of all the lines differ significantly. Both discriminant functions were formed mainly by the traits of the DE, PH1, PH2, NSE and NFS. The first discriminant function, shows differentiation of lines with different alleles of the *Ppd-D1* gene, all traits positively correlated

with each other. The second discriminant function demonstrates differentiation of lines created on different genetic background, within traits PH1, PH2, NSE and NFS correlates positively, except DE that correlates negatively (Table 3).

Table 3: The discriminant analysis of the traits for the analogue-lines grown in 2018.

Trait	Partial Wilks λ	F	R^2	Roots of the discriminant function	
				Root 1	Root 2
DE	0,22	16,10 ***	0,74	-0,25	0,48
PH1	0,42	6,45**	0,84	-0,01	-0,32
PH2	0,24	14,70***	0,79	-0,28	-0,39
NSE	0,34	9,07**	0,87	-0,31	-0,27
NFS	0,39	7,16**	0,89	-0,30	-0,12
NKM	0,64	2,62	0,50	-0,17	-0,20

The square of the Mahalanobis distances between the centroids of the groups with different *Ppd-D1* genotypes was higher than among the groups with the same alleles of the *Ppd-D1* gene in 2016 (Table 4), at the same time in 2018 index D^2 Mahalanobis was higher among groups with recessive allele *Ppd-D1b.*

Table 4: The Mahalanobis distance between analogue lines that differ in the alleles of *Ppd-D1* gene.

Couples of the lines	D^2 Mahalanobis	
	2016	2018
Kooperatorka – Kooperatorka rannya	72,8***	55,7***
Stepnyak 1 – Stepnyak 1 ranniy	50,1***	43,0***
Kooperatorka – Stepnyak 1	37,3***	65,3***
Kooperatorka rannya – Stepnyak 1 ranniy	25,2***	9,8*

Conclusion

Three factor variance analysis has shown significant influence of factors and their interactions on the investigated traits. Discrimination of investigated lines by agronomic traits has shown their clear separation with high quality. Date of earing, plant height and number of spikelets in the main ear were the most informative traits for distinguishing the lines by discriminant analysis in both years.

Acknowledgments

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Genetic variability for cuticular transpiration indicators in terms of initial water content and rate of water loss of the flag leaf to an assortment of wheat tested at Simnic

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Summary

For this study, 50 common wheat varieties of different origins were used.

The detached leaves of each genotype on the plants in the experimental field were weighed to obtain the initial water content (IWC). The water loss after 4 hours by drying was calculated using the formula: WL4h = (IWC-W4h) / DW.The loss of water between 4 and 24 hours was estimated using the formula: WL4-24h = (W4h-W24h) / DW where: DW is the dry weight; IWC is the initial water content; WL4h, WL24h = water loss at 4 and 24 hours, respectively.

There were significant differences in initial water content, water loss after 4 hours and water loss after 20 hours, between years but also between varieties. There was no correlation between the initial water content and water loss in the first 4 hours, the correlation coefficient being very small (r = -0.091). Correlations between the YI yield index and the initial water content, between the YI yield index and water loss after 4 hours were poor. The rate of loss of water in the flag leaf can not be an indicator on the basis of which an appreciable tolerance of drought can be made, at least at the type of drought that occurred in Simnic in 2002-2003. In addition, the significant interaction between the effect of the varieties and the year conditions makes the method not usable for selection irrespective of climatic conditions. The results also suggest the possibility of giving up the water loss determinations within 4-20 hours, as other studies have shown.

Introduction

Clarke and McCaig (1982) and Clarke et al. (1989) proposed low water loss rate (RWL) from detached leaves as a method of identifying wheat varieties (Triticum spp.) with better drought resistance.

In a study by Patrizia Rampino et al. (2006), the authors used Relative Water Content (RWC) and Water Loss Rate (WLR) to characterize the genotypes that differ in their response to stress caused by absence of water. Varieties with RWC over 25% were considered to be resistant and those to RWC below 25% were considered sensitive to water absence. In this last category entered the commercial varieties Colloseo, Italo, Primadur and Messapia. It was observed that at sensitive genotypes RWC decreased significantly in the first 2 hours of stress induction and reached a minimum after 8 hours, while at resistant varieties RWC slowly decreased until 34-55% after 8 hours. Regarding the WLR test, it was found that in susceptible varieties it is significantly higher than in resistant varieties. After 2 hours of stress, the WLR differs significantly between sensitivity and resistence, but the difference decreases with the time and becomes insignificant after 8 hours.

The RWC values of the varieties were comparable after the first treatment except for Cappelle Desprez (sensitive), which showed a significant decrease in leaf water content this time. The

second drought cycle clearly differentiated susceptible and tolerant genotypes with tolerant varieties (especially Plainsman V) maintaining higher RWC values by the end of the second drying episode. Concerning the behavior of contrasting varieties, the results were confirmed by the data obtained by Gallé et al. (2009), which investigated the same set of wheat varieties.

Materials and methods

For this study, 50 common wheat varieties of different origins were used. Of these, 13 were the limited assortment that was tested for 14 years between 2002 and 2015 (Glosa, Gruia, Izvor, Alex, Faur, Delabrad, Crina, Dropia, Simnic 30, Bezostaia, Boema, Romulus and Lovrin 34).

Own researches are conducted at genetic variability for cuticular transpiration indicators in terms of initial water content and rate of water loss of the flag leaf, and correlation with the most adequate drought sensitivity index – YI (yield index) calculated from field yields under rainfall contrast conditions.

The detached leaves were transported to the laboratory no later than 30 minutes and weighed to obtain the initial water content (IWC). The leaves were then dried for 4 hours under laboratory conditions (20 ° C in the dark) and weighed to obtain W4h. The water loss after 4 hours by drying was calculated using the formula: WL4h = (IWC-W4h) / DW.

Next, the leaves were dried for another 20 hours at 20 ° C and reweighed to obtain W24h. Thereafter, the leaves were dried at the oven at 70 ° C to obtain the dry weight (DW).

The leaves were dried in controlled environments in climatic rooms. Parameters (temperature, In our determinations, the initial water content was on average very close in 2012 and 2013 but lower than in 2014. In 2012 it oscillated between 212.6% at Şimnic 50 and 304.9% at Capo; in 2013 between 230.2% at Glosa and 287.3% at Nathan and in 2014 between 286.8% at Mv Palma and 371.7% at Demetra. The highest amplitude was recorded in 2012, the most drought reported to the other two (Figure 1).

and 24 hours was estimated using the formula: WL4-24h = (W4h-W24h) / DW where: DW is the dry weight; IWC is the initial water content; WL4h, WL24h = water loss at 4 and 24 hours, respectively.

Correlations have been made between all categories of water loss. Correlation was also made between all categories of cuticular transpiration indicators and the yield index (YI) for the limited assortment.

Results and discussions

In our determinations, the initial water content was on average very close in 2012 and 2013 but lower than in 2014. In 2012 it oscillated between 212.6% at Şimnic 50 and 304.9% at Capo; in 2013 between 230.2% at Glosa and 287.3% at Nathan and in 2014 between 286.8% at Mv Palma and 371.7% at Demetra. The highest amplitude was recorded in 2012, the most drought reported to the other two (Figure 1).

On average, the first five varieties that were found to have low initial water content, expressed as% reported to dry matter, were: Şimnic 50, Agron, Dropia, Romansa and Bezostaia (Table 1).

Table 1: Classification of varieties for initial water content, water loss in 4 hours, 20 hours and 24 hours.

NR. VAR	SOIURI	initial water content (average) %	NR. VAR.	SOIURI	water loss in 4 hours (average) %	NR. VAR.	SOIURI	water loss in 20 hours (average) %	NR. VAR.	SOIURI	water loss in 24 hours (average) %
8	CAPO	304,8*	30	GRUIA	103,6	45	NATHAN	118,6*	8	CAPO	208,0
29	GK GOBE	302,6*	8	CAPO	103,6	11	CUBUS	116,5*	10	CRINA	199,6
45	NATHAN	301,8*	41	NIKIFOR	103,3	44	JULIUS	112,5	45	NATHAN	193,9
14	DEMETRA	300,3	33	IZVOR	101,1	10	CRINA	111,0	39	MOLDAU	193,4
10	CRINA	299,7	24	GABRIELA	96,2	29	GK GOBE	110,4	29	GK GOBE	192,1
33	IZVOR	295,8	40	MV PALMA	95,1	31	GK HATTYU	106,0	24	GABRIELA	191,8
44	JULIUS	293,5	39	MOLDAU	91,6	20	ESQUISIT	105,6	33	IZVOR	191,1
48	SHOHAM	289,1	6	BOEMA	91,4	8	CAPO	104,5	11	CUBUS	186,0
21	EXOTIC	288,4	32	MIRANDA	89,8	48	SHOHAM	102,8	42	ORATORIO	185,6
37	LITERA	287,1	38	LOVRIN 34	89,8	14	DEMETRA	102,7	21	EXOTIC	185,0
23	FLAMURA 85	285,9	49	SIMNIC 30	88,9	36	LADA	102,6	38	LOVRIN 34	182,5
18	ELIANA	285,3	10	CRINA	88,6	39	MOLDAU	101,8	32	MIRANDA	182,1
50	TRIVALE	284,7	17	DUNAI	87,8	21	EXOTIC	101,3	20	ESQUISIT	181,8
38	LOVRIN 34	284,6	46	ROMANSA	87,6	37	LITERA	99,7	37	LITERA	181,7
19	ENESCO	284,3	13	DELABRAD	87,2	42	ORATORIO	99,4	36	LADA	180,9
24	GABRIELA	283,6	2	ALEX	86,4	12	DARIEL	96,3	48	SHOHAM	180,1
6	BOEMA	282,3	42	ORATORIO	86,2	19	ENESCO	96,3	6	BOEMA	180,0
34	KARLYGASH	281,4	16	DROPIA	85,9	27	GK ELET	95,6	40	MV PALMA	178,7
36	LADA	281,1	47	ROMULUS	85,0	24	GABRIELA	95,6	14	DEMETRA	178,2
27	GK ELET	281,0	34	KARLYGASH	84,8	3	AZTEC	95,4	18	ELIANA	177,5
39	MOLDAU	280,7	50	TRIVALE	84,7	5	BITOP	95,3	2	ALEX	176,0
20	ESQUISIT	280,5	18	ELIANA	84,2	9	CAROLINA	94,9	30	GRUIA	176,0
41	NIKIFOR	279,9	1	AGRON	83,9	18	ELIANA	93,4	31	GK HATTYU	175,6
32	MIRANDA	279,8	21	EXOTIC	83,8	38	LOVRIN 34	92,7	50	TRIVALE	175,2
11	CUBUS	279,7	23	FLAMURA 85	83,0	22	FAUR	92,5	41	NIKIFOR	175,2
22	FAUR	279,5	28	GLOSA	82,9	32	MIRANDA	92,4	44	JULIUS	175,2

NR. VAR	SOIURI	initial water content (average) %	NR. VAR.	SOIURI	water loss in 4 hours (average) %	NR. VAR.	SOIURI	water loss in 20 hours (average) %	NR. VAR.	SOIURI	water loss in 24 hours (average) %
30	GRUIA	278,8	37	LITERA	82,0	15	DOR	91,8	19	ENESCO	175,1
31	GK HATTYU	278,8	29	GK GOBE	81,7	50	TRIVALE	90,6	34	KARLYGASH	173,3
42	ORATORIO	277,7	15	DOR	81,0	33	IZVOR	90,0	15	DOR	172,8
15	DOR	277,2	25	GIAVA	78,9	35	KRISTINA	89,7	23	FLAMURA 85	170,5
47	ROMULUS	277,0	19	ENESCO	78,9	43	ORQUAL	89,7	17	DUNAI	170,3
2	ALEX	276,4	36	LADA	78,3	2	ALEX	89,7	22	FAUR	169,1
13	DELABRAD	275,8	7	SIMNIC 50	78,0	6	BOEMA	88,6	27	GK ELET	168,8
28	GLOSA	275,6	48	SHOHAM	77,3	34	KARLYGASH	88,5	13	DELABRAD	167,6
3	AZTEC	275,4	22	FAUR	76,6	26	GK DAVID	88,4	9	CAROLINA	166,6
17	DUNAI	273,9	4	BEZOSTAIA	76,6	23	FLAMURA 85	87,5	16	DROPIA	166,5
5	BITOP	273,8	20	ESQUISIT	76,3	40	MV PALMA	83,7	47	ROMULUS	166,5
9	CAROLINA	271,9	14	DEMETRA	75,5	17	DUNAI	82,5	35	KRISTINA	164,5
25	GIAVA	271,7	45	NATHAN	75,3	47	ROMULUS	81,5	5	BITOP	163,7
35	KRISTINA	270,3	35	KRISTINA	74,8	25	GIAVA	81,4	12	DARIEL	163,0
49	SIMNIC 30	270,2	27	GK ELET	73,2	16	DROPIA	80,7	26	GK DAVID	161,3
40	MV PALMA	269,1	26	GK DAVID	72,9	13	DELABRAD	80,4	3	AZTEC	161,1
43	ORQUAL	266,8	9	CAROLINA	71,7	4	BEZOSTAIA	78,7	49	SIMNIC 30	161,0
12	DARIEL	266,7	31	GK HATTYU	69,6	7	SIMNIC 50	76,6	25	GIAVA	160,3
26	GK DAVID	266,5	11	CUBUS	69,4	1	AGRON	76,3	1	AGRON	160,2
7	SIMNIC 50	266,1	5	BITOP	68,4	28	GLOSA	74,9	46	ROMANSA	160,1
1	AGRON	264,2	43	ORQUAL	67,0	46	ROMANSA	72,5	28	GLOSA	157,9
16	DROPIA	260,3	12	DARIEL	66,8	30	GRUIA	72,4	43	ORQUAL	156,7
46	ROMANSA	260,0	3	AZTEC	65,7	49	SIMNIC 30	72,1	4	BEZOSTAIA	155,2
4	BEZOSTAIA	258,5	44	JULIUS	62,6	41	NIKIFOR	71,8	7	SIMNIC 50	154,6
	DL 5%	21,4		DL 5%	24,0		DL 5%	20,8			
	F calc/teor	25,77/1,51		F calc/teor	1,4/1,51		F calc/teor	2,55/1,51			

The highest initial water content was recorded by Capo, GK Gobe, Nathan, Demetra and Crina varieties. Of these, only Capo, Gobe and Nathan showed significantly higher values than the average.

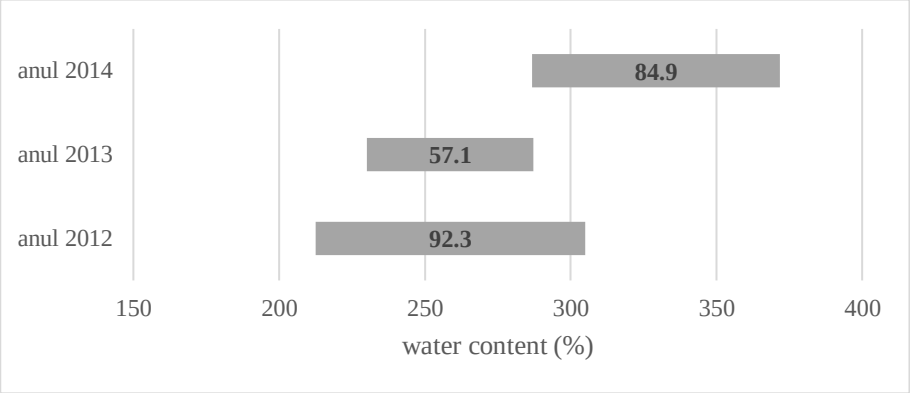

Fig. 1: The amplitude of the initial water content over the three years of testing.

The highest initial water content was recorded by Capo, GK Gobe, Nathan, Demetra and Crina varieties. Of these, only Capo, Gobe and Nathan showed significantly higher values than the average.

Varieties of limited assortment are distributed everywhere in the multitude of the results obtained by the 50 varieties. This suggests that the chosen varieties are representative of the experiment on the leaf water content and the rate of water loss.

Since the critical value for F2,294 = 3,00 is less than F calculated (2112,73) there are significant differences between years in terms of initial water content.

Also, F calculated for varieties is higher than the critical value, the conclusion being that there are significant differences between varieties (Table 2)**.** Interaction variety x years is significant and indicates that the reaction of the varieties differed according to the conditions of the years in which the determinations were made.

The F test to the interaction shows that there may be differences between years but not between varieties when repeating the experience.

Table 2: ANOVA for initial water content.

SOURCE OF VARINCE	**SUM OF SQUARED**	**DEGREES OF FREEDOM**	**MEAN SQUARE**	**F TEST REPORTED OF ERROR**	**F TEST REPORTED OF INTERACTION**
REPETITIONS	99	6	16,5	0,09 (2,09)	0,02 (2,19)
YEARS	746091	2	373045,50	2112,73 (3,00)	548,76 (3,09)
VARIETIES	53070	49	1083,07	6,13 (1,35)	1,59 (3,18)
VARIETY X YEARS	66564	98	679,22	3,85 (1,24)	
ERROR	51911	294	176,57		
TOTAL	**917735**	**449**			

In the first 4 hours, on average, the biggest losses of water were suffered by the Gruia, Capo, Nikifor and Izvor varieties, but the values are not statistically assured. The results are surprising because they do not coincide with the information we have about the tested varieties (Table1).

So Izvor variety known as drought-resistant has a water content after 4 hours significantly higher in 2013, a year with precipitation below the multiannual average. Identical is Simnic 30. In contrast, drought-sensitive varieties (Capo, Bitop and Julius) have significantly lower losses than the average in the same year. On average, none of the varieties are highlighted in any sense, although there are certainly differences between varieties tested under natural conditions regarding to drought toleranceIt is the first studied element in which the varieties of the limited assortment do not capture the entire variability, the vast majority having values above the average of all tested varieties. From this point of view, the results extrapolation can be exaggerated.

The critical value for F2,294 = 3,00 is less than calculated F (146,96) for the water loss in the first 4 hours, so there are differences between years.There are also significant differences between varieties, the F critical being higher than F calculated (Table 3). The interaction variety x years is significant and indicated that the reaction of the varieties was influenced by the year of experimentation. As with the initial water content, the F test to the interaction shows that there may be differences between years but not between varieties when repeating the experience.

Table 3: ANOVA for water loss in 4 hours.

SOURCE OF VARINCE	SUM OF SQUARED	DEGREES OF FREEDOM	MEAN SQUARE	F TEST REPORTED OF ERROR	F TEST REPORTED OF INTERACTION
REPETITIONS	503	6	83,8	0,39 (2,09)	0,15 (2,19)
YEARS	63027	2	31513,47	146,96 (3,00)	48,18 (3,09)
VARIETIES	62427	49	1274,01	5,94 (1,35)	1,95 (3,18)
VARIETY X YEARS	52819	98	538,97	3,05 (1,24)	
ERROR	63044	294	214,44		
TOTAL	**241820**	**449**			

With regard to water loss after 20 hours, on average, they were significantly detached Nathan and Cubus varieties (Table 1). Here too, the vast majority having values below the average of all tested varieties, the extrapolation of the results can be exaggerated. Varieties from the limited assortment no capture all the variability.The statistical calculation shows that there are significant differences in water lost after 20 hours, between years and between varieties. The variety x years interaction is significant and indicates that the reaction of the varieties was influenced by the year of experimentation (Table 4).

Table 4: ANOVA for water loss in 20 hours.

SOURCE OF VARINCE	SUM OF SQUARED	DEGREES OF FREEDOM	MEAN SQUARE	F TEST REPORTED OF ERROR	F TEST REPORTED OF INTERACTION
REPETITIONS	1098	6	183	1,10 (2,09)	0,36 (2,19)
YEARS	537417	2	268708,42	1608,29 (3,00)	530,78 (3,09)
VARIETIES	62637	49	1278,32	7,65 (1,35)	2,53 (3,18)
VARIETY X YEARS	49694	98	507,08	3,03 (1,24)	
ERROR	49122	294	167,08		
TOTAL	**699968**	**449**			

The varieties have lost water differently depending on time, which means a higher or lower speed.

Generally, varieties lost water in the first 4 hours almost equal to the amount lost in the next 20 hours. For example, the Gabriela variety recorded a loss of 96.2% in the first 4 hours and 95.6% in the next 20 hours. Identical, Miranda, Alex, Lovrin 34, Bezostaia, Șimnic 30 varieties (table 1).

From the point of view of water loss within 24 hours, the variability of this element is surprised by limited assortement tested. On the whole, the fact that the water loss in the first four hours the tested varieties belonging to the limited assortment are at the top of the table (above average) and the water loss in the next 20 hours is at the bottom (below average) suggests that varieties that lose water quickly in the early hours have a slower loss over the next few hours. For this reason, the variability of this character is well represented by the limited assortment when considering the entire 24-hour period. The mentioned aspect is evidenced by the relationship between the water loss values in the first 4 hours and the water loss in the next 20 hours, where the determination coefficient is 75.6% (Figure 2).

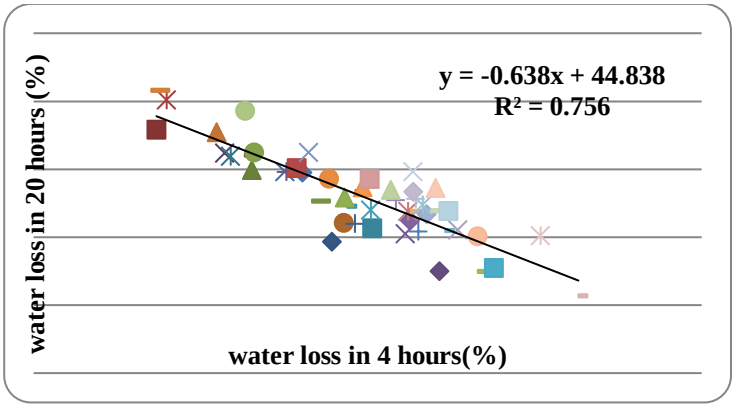

Fig 2: The relationship water loss in first 4 hours - water loss in the next 20 hours.

From the graph below (Figure 3), there is no correlation between the initial water content and the water loss in the first 4 hours, the correlation coefficient being very small (r = -0.091), so we can not say that a variety whose initial water content is high will have water loss in the first 4 hours, more pronounced.

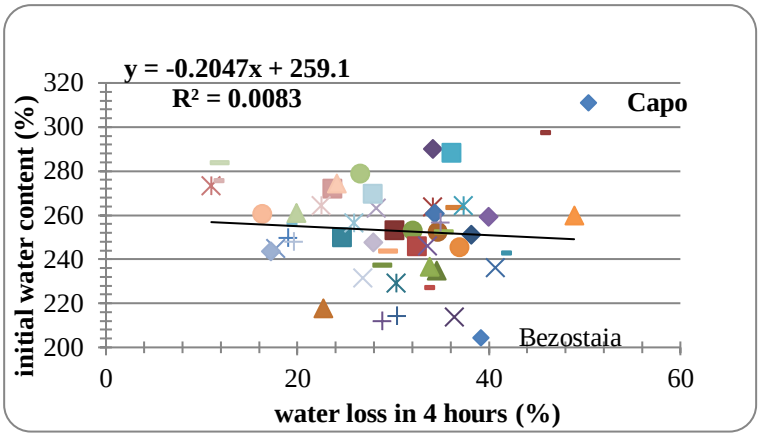

Fig 3: The relationship initial water content - water loss in first 4 hours.

Correlations between the YI (yield index) and the initial water content, between the YI and the water loss after 4 hours were poor. There were no correlations between YI and water loss after 20 hours and YI and total water loss in 24 hours (Table 5). The study was carried out on the limited assortment (13 wheat varieties tested for 14 years under the conditions of Simnic).

Table 5: Correlations between the YI yield index and the water loss speed.

Varieties	YI	Initial water content (%)	Water loss after 4 hours (%)	Water loss after 20 hours (%)	Water loss after 24 hours (%)
GLOSA	1,252	275,6	82,9	74,9	157,9
GRUIA	1,220	278,8	103,6	72,4	176,0
IZVOR	1,219	295,8	101,1	90,0	191,1
ALEX	0,955	276,4	86,4	89,7	176,0
FAUR	1,153	279,5	76,6	92,5	169,1
DELABRAD	1,116	275,8	87,2	80,4	167,6
CRINA	0,996	299,7	88,6	111,0	199,6
DROPIA	0,941	260,3	85,9	80,7	166,5
SIMNIC 30	0,895	270,2	88,9	72,1	161,0
BEZOSTAIA	0,854	258,5	76,6	78,7	155,2
BOEMA	0,847	282,3	91,4	88,6	180,0
ROMULUS	0,800	277,0	85,0	81,5	166,5
LOVRIN 34	0,753	284,6	89,8	92,7	182,5
Correlation with YI		**0,25**	**0,28**	**-0,14**	**0,05**

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Identification of wheat varieties tolerant to water stress based on ratio between the stem growth measured in seedlings after 20% PEG treatment and the stem growth measured after water treatment 15 days after sowing

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Summary

Recent studies thorough previously performed the same team showed that the ratio between the steam measured on 20% PEG treatment and that measured at the control (water treatment) at 15 days after sowing and on average on three determinations (15, 24 and 35 days from sowing) was significantly correlated drought tolerance index YI calculated on the basis of the behavior of 13 varieties tested in field entire 14 years period. Izvor variety showed high values of the ratio between the strain measured on 20% PEG treatment and that measured at the control (water treatment) 15 days after sowing.

In relation to this variety and the Boema variety which proved to be intolerant (subunit ratio) were tested in the laboratory 75 varieties of wheat to different origins.Six seedling in three repetitions of each wheat genotype of 20% PEG treatment and six of the water treatment (control) in plastic pots was introduced into the Sanyo Growth Chamber, previously adjusted to the temperature, light and atmospheric humidity parameters for the proper growth of wheat plants.

Of the varieties tested: Acrobat, Andalou, Attilio, Avantaj, Bhash, Gasparom, Simnic 60, Unitar, Norin, Solveig, Magistral and Marsallshowed high values of the ratio.Izvor variety was distinctly significant exceeded only by: Simnic 60 and Solveig.

The lowest ratio has been registered by Lupidur, durum wheat variety (0,558). The highest ratio has been registered by Solveig (1,176).

Introduction

Half of the area cultivated with wheat in developed countries and over 70% of the area in developing countries suffer from long drought periods. Drought can occur throughout the growing season of culture in areas with low precipitation.Plants that are exposed to water and heat stress reduce their yield all over the world.The combined effect of the two on yield is stronger than the effect of each stress (Dreesen și colab., 2012; Rollins și colab., 2013).

Although the climate of Romania is generally characterized as "moderately continental", in recent years there have been extremely large variations in both the total amount of precipitation from one year to another year and its distribution over the year, which determines water deficits (often associated with heat) frequent during crop vegetation in almost all areas of the country (Păunescu, 2017).

The plant's response to drought is also influenced by the intensity, duration and frequency of stress, as well as the plant-soil-atmosphere interaction.Many morphological and physiological strategies have been identified in response to water stress, ranging from avoiding dehydration

to tolerance to dehydration (Carolina și colab., 2012).

Selection for early tolerance drought tolerance is most commonly practiced using PEG 6000 (Rauf și colab., 2006; Ahmad și colab., 2013). The PEG can be used to alter the osmotic potential of the nutritive solution of the culture and can induce a plant deficit under relatively controlled conditions close to natural experimentation.

The 15% polyethylene glycol (PEG) concentration and the two day duration were considered optimal for the induction of adaptive processes (Petcu și colab., 2007).

Experiences with PEG also performed Frorgóné (2009), which proposed to compare the changes in physiological parameters in the plant stage under osmotic stress and during the filling of the grain during water shortage and to identify the correlations existing on the basis of climate change and aberrant changes in the stage of emergence that can characterize drought tolerance and finally the yield of grains. Two cultivation tolerant to drought - Plaisman and MV Emese and two sensitive - GK Élet and Cappelle Desprez, were studied.Osmotic stress was induced 7 days after germination by adding PEG 6000 to the culture medium on day 7 with 100 on day 9 with 200 and on day 11 with the final value of 400 mOsm.

The ratio between the stem measured after 20% PEG treatment and that measured in the control (water treatment) 15 days after sowing, as well as on average on three determinations (15, 24 and 35 days from sowing) was identified as the best indicator for drought tolerance selection (Păunescu, 2018).

Materials and methods

At Simnic, the experiment was performed in the laboratory using the PEG 6000 solution at 20%. We determined stem length at 15 days from sowing date.

Seventy five wheat varieties of various origins were tested in the laboratory to detect differences for the ratio of stem growth under stress and growth in control in terms of their length. Sowing was done in pots containing the same amount of soil. Eighteen seedlings of each wheat genotype of control (water) were transferred to plastic pots each with 6 plants and introduced into the Sanyo Growth Chamber, previously adjusted to the temperature, light and atmospheric humidity parametersfor the proper growth of wheat plants.

The following experimental variants were established:

- control: the plants were maintained in optimal conditions allduring the experiment;
- treatment 1: plants were treated during 15 days with polyethylene glycol (PEG) at a concentration of 20%.

Varieties were grouped into three experiments (25 variants x 3 repetitions for each of the graduations: control and treatment 1). In each of them, the Izvor and Boema varieties were introduced, the tolerant (first) and susceptible variety (the second).

Results and discussions

In the first experience, Simnic 60 (variety created at ARDS Şimnic) and Solveig (a variety with good yield results in the area, indifferently of the environmental conditions) detached with distinct significant values superior to the Izvor variety validated as drought-tolerant variety by

correlationratio between the stem growth measured in seedlings after 20% PEG treatment and the stem growth measured after water treatment 15 days after sowing and YI (yield index) (Table 1).

The same two varieties have detached more (very significant) from the sensitive Boema variety.

Acrobat, Andalou, Attilio, Avantaj, Gasparom, Unitar, Norin wheat varieties showed hight values ofratio between the stem growth measured in seedlings after 20% PEG treatment and the stem growth measured after water treatment 15 days after sowing but they were not significant. In these varieties, lenghtof stem stressed by the addition of PEG withdose of 20% was greater than stem length when wetted only with water.

The study also highlighted the varieties whose ratio PEG 20% / control for stem lenght were inferior, with statistical assurance, to the Izvor variety:

- Adagio și Adelina – significantly lower;
- Hogoz, Moisson și Palatos – distinct significantly lower;
- Dana – very significantly lower.

The results suggest that these last varieties do not tolerate drought and the water absence places them under the yield performance of the Izvor variety.

Table 1: Identification of wheat varieties tolerant to water stress based on ratio between the stem growth measured in seedlings after 20% PEG treatment and the stem growth measured after water treatment 15 days after sowing – first experience.

CULTIVARS	RATIO PEG20%/CONTROL FOR STEM LENGHT	DIFFERENCE FROM THE TOLERANT VARIETY	SIGNIFI-CATION	DIFFERENCE FROM THE SENSITIVE VARIETY	SIGNIFI-CATION
ACROBAT	1,010	-0,030		0,014	
ADAGIO	0,945	-0,095	o	-0,051	
ADELINA	0,948	-0,092	o	-0,048	
ANDALOU	1,049	0,010		0,053	
ATTILIO	1,026	-0,014		0,030	
AVANTAJ	1,002	-0,037		0,006	
DANA	0,867	-0,172	ooo	-0,129	oo
DESAMO	0,973	-0,067		-0,023	
ENSTEIN	0,955	-0,084		-0,041	
GASPAROM	1,004	-0,036		0,008	
HOGOZ	0,924	-0,116	oo	-0,072	
MOISSON	0,913	-0,127	oo	-0,083	o
MOLDOVA 83	0,996	-0,044		0,000	
PALATOS	0,902	-0,138	oo	-0,094	o
PITAR	0,999	-0,041		0,003	
RETEZAT	1,000	-0,040		0,004	

CULTIVARS	RATIO PEG20%/CONTROL FOR STEM LENGHT	DIFFERENCE FROM THE TOLERANT VARIETY	SIGNIFI-CATION	DIFFERENCE FROM THE SENSITIVE VARIETY	SIGNIFI-CATION
RODITOR	0,972	-0,068		-0,024	
SIMNIC 60	1,158	0,118	**	0,162	***
SOLVEIG	1,176	0,137	**	0,180	***
SORRIAL	0,970	-0,070		-0,026	
UNITAR	1,069	0,029		0,073	
VICTORIA	0,968	-0,072		-0,028	
WW NORIN	1,008	-0,032		0,012	
IZVOR (drought-tolerant variety)	1,040	0,000		0,044	
BOEMA (drought-sensitive variety)	0,996	-0,044		0,000	
DL 5%		0,077			
DL 1%		0,105			
DL 0,1%		0,140			

In the second experience, none of the varieties were superior to the Izvor variety. The Israeli variety Bhash was noted at which the ratio was higher than one but was not significant. This variety entered in the wheat collection at Simnic as a tolerant drought and this experiment confirmed this feature (Table 2).

Most varieties were inferior to Izvor, as follows:

- Fridoline – significantly lower;
- Ades, Akteur, Combin, Cordiale, Feria, Gabrio și Boema – distinct significantly lower;
- Apache, Arezzo, Autan, Bercy, Columna, Dallara, Euclide, Giacometti și Giovani – very significantly lower.

Table 2: Identification of wheat varieties tolerant to water stress based on ratio between the stem growth measured in seedlings after 20% PEG treatment and the stem growth measured after water treatment 15 days after sowing – second experience.

CULTIVARS	RATIO PEG20%/CONTROL FOR STEM LENGHT	DIFFERENCE FROM THE TOLERANT VARIETY	SIGNIFI-CATION	DIFFERENCE FROM THE SENSITIVE VARIETY	SIGNIFI-CATION
ADES	0,871	-0,136	oo	-0,030	
AKTEUR	0,872	-0,134	oo	-0,029	
AMICUS	0,921	-0,085		0,020	
APACHE	0,822	-0,185	ooo	-0,079	
AREZZO	0,804	-0,202	ooo	-0,097	o
AUTAN	0,782	-0,224	ooo	-0,119	o
BERCY	0,786	-0,220	ooo	-0,115	o
BHASH	1,022	0,016		0,121	o
CEZANNE	0,932	-0,074		0,031	
COLUMNA	0,832	-0,174	ooo	-0,069	
COMBIN	0,847	-0,159	oo	-0,054	
CORDIALE	0,855	-0,151	oo	-0,046	
DALLARA	0,761	-0,245	ooo	-0,140	oo
DISCUS	0,977	-0,029		0,076	
EUCLIDE	0,810	-0,196	ooo	-0,091	
FERIA	0,872	-0,134	oo	-0,029	
FORZOR	0,941	-0,065		0,040	
FRIDOLINE	0,891	-0,115	o	-0,010	
FRINI	0,951	-0,055		0,050	
GABRIO	0,856	-0,150	oo	-0,045	
GIACOMETTI	0,811	-0,195	ooo	-0,090	
GIOVANI	0,797	-0,209	ooo	-0,104	o
GK MURA	0,937	-0,069		0,036	
IZVOR (drought-tolerant variety)	1,006	0,000		0,105	*
BOEMA (drought-sensitive variety l)	0,852	-0,154	oo	-0,049	
DL 5%		0,095			
DL 1%		0,123			
DL 0,1%		0,165			

In this and the following experiments, the statistical calculation confirms the previous researches in which it was discovered that the Boema variety is more sensitive than the Izvor variety under drought conditions.

In last experience, it did not exist a superior variety to the Izvor (Table 3). Magistral and Marsall showed high values of the ratio but without significance. The Koska, Messino, Petur, Tolbiac and Boema varieties had values of ratio PEG 20% / control for stem lenght significantly lower than Izvor. The Hazera, Josef, Mariska, Syllon, Thalts and WW Agil varieties were distinctly inferior and Lupidur and Pobeda - very significantly inferior. The lowest value of the ratio was recorded by the Lupidur variety, durum wheat variety (0,558).

Table 3: Identification of wheat varieties tolerant to water stress based on ratio between the stem growth measured in seedlings after 20% PEG treatment and the stem growth measured after water treatment 15 days after sowing – third experience.

CULTIVARS	RATIO PEG20%/CONTROL FOR STEM LENGHT	DIFFERENCE FROM THE TOLERANT VARIETY	SIGNIFI-CATION	DIFFERENCE FROM THE SENSITIVE VARIETY	SIGNIFI-CATION
GORDIAN	0,872	-0,129		0,033	
HAZERA	0,759	-0,242	oo	-0,080	
JOSEF	0,771	-0,230	oo	-0,068	
KOSKA	0,794	-0,207	o	-0,044	
LIVADA	0,910	-0,091		0,071	
LJILJIANA	0,891	-0,110		0,052	
LUPIDUR	0,588	-0,413	ooo	-0,251	oo
MAGISTRAL	1,004	0,002		0,165	*
MARISKA	0,740	-0,261	oo	-0,099	
MARSALL	1,029	0,028		0,190	*
MESSINO	0,808	-0,193	o	-0,031	
MIRONOVSKA	0,982	-0,019		0,144	
MISKA	0,911	-0,090		0,072	
MV LUCILLA	0,874	-0,127		0,035	
PETUR	0,842	-0,159	o	0,003	
POBEDA	0,715	-0,286	ooo	-0,124	
ROLAND	0,811	-0,190		-0,028	
SOBBEL	0,888	-0,113		0,049	
SYLLON	0,750	-0,251	oo	-0,089	
THALTS	0,783	-0,218	oo	-0,056	
TOLBIAC	0,839	-0,162	o	0,000	
VULCANUS	0,918	-0,083		0,079	
WW AGIL	0,735	-0,266	oo	-0,104	
IZVOR (drought-tolerant variety)	1,001	0,000		0,162	*
BOEMA (drought-sensitive variety)	0,839	-0,162	o	0,000	
DL 5%		0,150			
DL 1%		0,210			
DL 0,1%		0,280			

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SSR marker TSM592 for the detection and for distinguishing rye translocations 1AL.1RS and 1BL.1RS in a wheat background.

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Summary

Searching for new sources of genes for agronomic and resistance improvement has been the main goal of wheat breeders worldwide. Wheat improvement using alien substitutions or translocations of chromosomes between wheat and its relative species played an important role in wheat breading programs. Rye (*Secale cereale L.*) is one the most valuable relative species for the improvement of wheat productivity, adaptive possibilities, disease and insect resistance, therefore, the rye 1R chromosome was used worldwide in wheat breeding programs.

Early detection and selection of rye translocation in wheat backgrounds is an important task in wheat breeding programs. Identification of molecular markers that facilitates the detection of rye translocation in wheat represents one of the first steps in molecular marker assisted selection from any modern wheat breeding programs.

In our study, conducted on 11 cultivars, SSR marker TSM592 clearly distinguished the wheat without rye translocation from the wheat that carries the 1AL.1RS and/or 1BL.1RS rye translocation. Therefore, TSM592 marker could be used in MAS (Marker-Assisted Selection) for 1R rye translocation detection.

Introduction

Rye (*Secale cereale L.*) belongs to the Triticeae tribe of the grasses and contributes to increase crop species diversity particularly in European agroecosystems. Wheat rye translocations have been used by breeders worldwide since the 1930s, rye being a valuable genetic resource for wheat breeding programs. Genes located on the translocated fragment of rye chromosome determine a number of useful characteristics such as high yield, wide adaptation, and disease and insect resistance.

Historically, mainly four sources of translocations and substitutions have been used to incorporate rye chromatin in wheat (two developed in Germany by Salzmünder and Weihenstephan in 1920-30, one developed in Japan in the 1960s, and one developed in the USA in the 1970s). These sources were the progenitors of hundreds of commercial wheat cultivars in different countries on five continents of the world. The source of the 1BL/1RS translocation used by Riebesel was Petkus rye (2x), while other scientists – Kattermann, Tsunewaki and Sebesta – used triticale (8x) (Rabinovich, 1998; Efremova et al., 2014).

Today, there are many wheat cultivars that carry rye translocation, mainly 1BL:1RS and 1AL:1RS that have provided resistance genes to rusts (Li et al., 2016; Crespo-Herrera et al., 2017), powdery mildew (Lu et al., 2014), greenbug (Porter et al., 1991), common bunt (Ciuca, 2011), barley yellow dwarf virus (BYDV) (Crespo Herrera et al., 2013) etc. These translocations have also brought positive impact on wheat yield, adaptability and drought (Howell et al., 2014). Saulescu et al. (2011) identified variation for first and second leaf width

among lines derived from triticale × wheat crosses.

Detection of rye chromatin in wheat using molecular markers is a valuable tool and any information regarding the identification of useful markers could help the breeding programs. Kofler (2008) developed SSR (Simple Sequence Repeat) marker primer pairs, named TSM (Tulln Secale Microsatellites) specific for the short arm of rye chromosome 1.

This study aimed to search for molecular markers that highlight rye chromatin and can be used to distinguish different wheat-rye chromosomal translocations.

Materials and methods

Plant material was obtained from NARDI Fundulea, Romania and consisted of 11 cultivars, 5 cultivars without rye translocation, 3 cultivars with 1B:1RS wheat-rye translocation and 3 cultivars with 1A.1RS wheat-rye translocation (table 1).

DNA extraction was performed on dry seeds using SDS3 method by Cristina et al., 2017.

DNA amplification was performed with "MyTaq Red DNA Polymerase" PCR kit from Bioline in an ABI ProFlex™ 3 x 32-well PCR System. PCR parameters for the amplification were as follows: 15 µL final reaction volume containing 1X reaction buffer, 0.5 mM primers, 1U DNA polymerase and 60-80 ng DNA sample.

PCR programme: initial denaturation at 95 °C for 90 s, followed by 35 cycles: 95 °C – 15 s, 54 °C – 15 s, 72 °C – 20 s, and a final extension at 72 °C for 5 min.

Gel electrophoresis for the separation of digested PCR products was carried out with 2% routine use agarose gels, stained with ethidium bromide and visualized on UV light.

Results and discussions

Characterization of rye translocation in wheat has important practical value for wheat breeding programs, and identification of convenient tools for detection/selection of favorable rye alleles can help obtaining fast and reliable results.

Amplification with SSR marker TSM592 resulted in easy to distinguish electrophoretic profiles, viz. no PCR product for genotypes without rye translocation, a ~260bp specific PCR product for genotypes that carries 1AL.1RS translocation and ~210bp for genotypes with 1BL.1RS translocation. Also, a ~60bp PCR product was observed in all cultivars, except Fundulea 29 cultivar (Figure 1). Molecular results, rye source and seed source for the analyzed cultivars are presented in table 1.

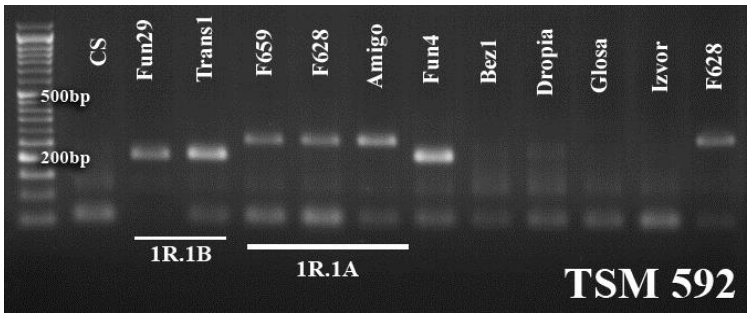

Fig.1: Electrophoretic profiles obtained with TSM592.

Table 1: Biological material and molecular results.

Cultivar	Rye source	Seed source	TSM592
Amigo	Insave FA	IPK Gatersleben, Germany	~260bp
F00628G34-1 (F628)	Unknown (Triticale)	NARDI Fundulea	~260bp
Fundulea-29	Avrora (Petkus)		~210bp
Transilvania-1			~210bp
Fundulea-4			~210bp
F659	Unknown (Triticale)		~260bp
Izvor	No rye		-
Bezostaia 1			-
Glosa			-
CS			-
Dropia			-

Our study showed that SSR marker TSM592 is a valuable tool for rye translocation detection and selection, clearly distinguishing the 1AL.1RS translocation from 1BL.1RS and also from wheat without rye translocation.

Acknowledgements

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Genotypic variations in preharvest sprouting resistance in some Romanian winter naked barley lines

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Summary

Preharvest sprouting is an important agronomic trait related to seed quality and can cause significant reductions in barley grain yield and grain end-use quality. The aim of this study has been the assessment for the first time of preharvest sprouting resistance for some Romanian winter naked barley lines (two row barley) obtained by pedigree and *bulbosum* method. In order to establish the resistance level, the naked genotypes were compared with hulled two row barley.

The analysis of results revealed a highly significant ($p<0.001$) variation for preharvest sprouting, influenced by genotype and a wide variations in sprouting index ranging between 1 (no visible sprouting) and 9 (100% sprouted). As percent, the studied genotypes, registered a mean value between 0% and 80.7%. of sprouted seeds from total seeds number in spike and a positive correlation between those parameters was identified.

The obtained results indicate there is genetic variability, among the barley breeding advances line developed at NARDI Fundulea, in preharvest sprouting resistance. Also, the observed differences indicate the genetic specificity of the studied genotypes. Furthermore, in the next period, it is expected an improvement in preharvest sprouting using the new sources for this trait and also applied the MAS.

Introduction

Pre-harvest sprouting (PHS) is one of complex biological processes of major importance for agricultural production (Börner et al., 2018) and a phenomenon of cereal crops when germination of grains occurs in the spikes before harvest. In the barley production can reduces crop yield to the point of a total damage of the harvest (Lohwasser et al., 2013). Also, pre-harvest sprouting (PHS) significantly reduces grain yield and quality of naked (hulless) barley (NB) (Legdzina et al., 2010) but the sprouting susceptibility is determined mainly by the genotype (Gualano and Benech-Arnold, 2009).

Seed PHS are complex traits controlled by several quantitative trait loci (QTL), which a large variation in dormancy expression patterns among barley genotypes has created (Gu et al., 2005).

Preharvest sprouting in barley cultivars is enhanced if maturing seeds are exposed to rain and warm temperatures, which leads to the loss of seed viability. Majority of hulless barley genotypes have a short dormancy period and an ability to absorb water very fast. Artificial sprouting of barley for seven days demonstrated that extensive damage to starch granules can take place due to an increase in α-amylase activity (Lorenz and Kulp, 1981).

Huang et al., in 1979 reported that the protein matrix was missing or compromised in sprouted wheat samples. The same study demonstrated that proteolytic enzymes broke down the protein matrix, thereby producing a loose structure around the starch granules, which made them more accessible to α-amylase. The relationship between α-amylase activity and PHS resistance may be due to activity of α-amylase that would increase quickly once absorbed enough water and then promoted the seed sprouting (Wang et al., 2008).

Due to climatic changes longer lasting precipitation periods can be expected and the need for naked barley resistance to preharvest sprouting becomes actual. According to the resistance to pre-harvest sprouting naked barley genotypes can be divided in three groups: (1) resistant, (2) sprouting in small extent and (3) susceptible (Legzdiņa et al., 2010). The existence of such groups approves genetic diversity and selection possibilities for pre-harvest sprouting resistance.

Materials and methods

A total of 56 winter barley naked and hulled lines (two row barley genotypes obtained by pedigree and *bulbosum* method) were used for the PHS assessment. Harvesting was done after physiological maturity (after loss of green pigmentation in the spikes and peduncles). Twelve spikes were harvested by cutting with a scissors approximately 10 cm below the base of the spike. Spikes were dried at room temperature for 1 week and after that were subjected to a wetting treatment (50 mm of water as overhead spray was applied over 2 h) then samples were maintained at 20 °C and 100% humidity for the duration of the experiment.

After 10 days, heads were removed and assigned a sprouting score (a visual estimation of the germination rate of intact spikes ranging from 0 to 9%, with 0 = no seed sprouted and, 9 = all seeds sprouted in a spike). A mean score was calculated for each sample as sprouting index (SI) (Liu et al. 2008).

Sprouted and nonsprouted kernels in each spike were counted, and the percentage of visible sprouted kernels (PVSK) in a spike was calculated by dividing the number of sprouted kernels with the total number of kernels in the spike to measure PHS resistance (Liu et al. 2011). In this study, we used PVSK in a spike to reflect PHS resistance for field-grown barley genotypes.

ANOVA, simple linear regressions and correlations were calculated in Microsoft Excel.

Results and discussions

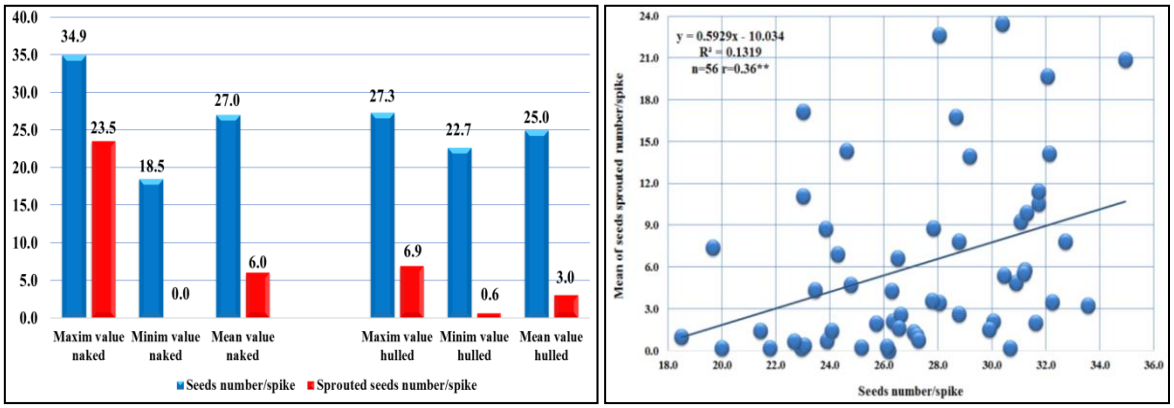
Analysis of variance revealed a genotype significant influence (Table 1) on the number of seeds/spike and sprouted seeds number/spike.

Table 1: F factor for winter naked barley lines (seed number/spike and sprouted seed number/spike).

Source of variation	df	F	P-value	F	P-value
		seed number/spike		sprouted seed number/spike	
Between Groups	55	13.33***	5.07E-30	28.26***	1.03E-45
Within Groups	112				
Total	167				

***Significant for 0.001

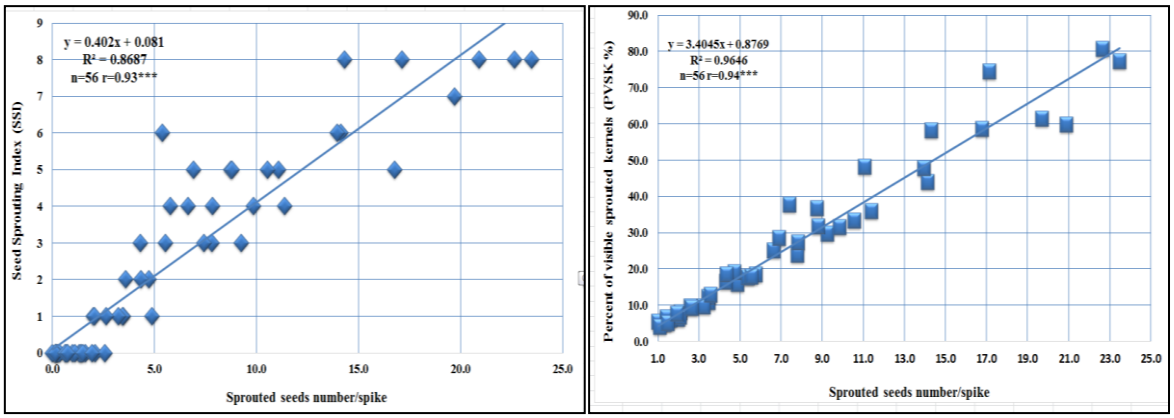
Depending on genotypes, the number of sprouted seeds (Figure 1) ranged as mean from minim 0.0 to maxim 23.5 in the case of naked barley and the value determined for hulled genotypes varied from minim 0.6 to maxim 6.9 sprouted seeds/spike (A).



A B

Fig. 1: The maxim, minim and mean value number of sprouted seeds/spike related by seeds number/spike (A) and the relationship between mean of sprouted seeds number/spike and number of seeds/spike (B).

The correlation coefficients between mean of sprouted seeds number/spike and number of seeds/spike (B) and also between Seed Sprouting Index (SSI) and sprouted seeds number/spike (fig.2) were significant. Regarding the percent of visible sprouted kernels (PVSK%), this was significantly higher for naked barley (from 0.8% to 80.7%) comparing with hulled barley, where the value ranged between 2.8% and 28.5%. The analysis of obtained results (fig. 4) in the case of naked barley indicate that there are some genotypes with good resistance to sprouting (22 genotypes presented a PVSK under 10%, 10 genotypes between 10-20%, 4 genotypes between 20-30%, 6 genotypes between 30-40%, 3 genotypes between 40-50% and between 50-80% were 5 genotype). Also, many of naked genotypes registered good value of this indicator comparing hulled barley (from 2.7% to 28.5%).



A B

Fig. 2: The relationship between Seed Sprouting Index (SSI) and sprouted seeds number/spike (A) and the relationship between percent of visible sprouting kernels (PVSK %) and number of sprouted seeds/spike (B).

Conclusions

There were notable differences of preharvest sprouting resistance among the tested barley genotypes, this variation being explained by a gradual loss of dormancy.

Very good resistance to sprouting was found for 6 naked breeding lines (PVSK% under 1%) and those genotypes can be recommended for use in barley breeding for improving the preharvest sprouting resistance.

A moderate level of preharvest sprouting resistance is desirable for commercial production (seeds) and end use of barley (food products).

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Development of a substitution line of bread wheat with high gluten content in grain and its study for agronomic characteristics

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Introduction

High gluten content in grain and flour correlates with a high bread-making quality. Gluten content strongly correlates with grain protein content and is a classifying trait in grain trading. Earlier, we showed that tetraploid species *Triticum timopheevii* may be the donor of high gluten content in grain as well as the line 821 (L821) obtained on the genetic background of cultivar Saratovskaya 29 (S29) (Budashkina et al. 2001). It carries introgressions in 2A, 2B and 5A chromosomes (Leonova et al. 2001). These chromosomes were supposed to be responsible for high gluten content in grain in the line. To confirm this, as well as to genetically separate the general effect of the introgressions, the new single chromosome substitution line was obtained for cv. S29.

Aim of the work is to introduce 2A chromosome from L821 into cv. Saratovskya 29; to develop a corresponding single chromosome substitution line with subsequent analyses for gluten content in grain, physical properties of flour and dough and yield components.

Materials and methods

The classical backcrossing scheme (Figure 1) with the use of the corresponding monosomic line of S29 was applied for obtaining the substitution line S29 (821 2A). The correctness of chromosome substitution was controlled with the help of the cytological analysis of chromosome set in metaphase I of meiosis in F_1 hybrids of every backcross. On the first stages, the plants were also selected for such morphological traits as awnedness, normal (not speltoid) spike and leaf hairiness typical for S29. The microsatellite markers *(Xgwm636, Xgwm830, Xgwm1539, Xgwm4849, Xgwm4601, Xgwm4276*) were used for the check of substitution correctness on the final stage. The interim control of gluten content in grain at the backcross stages was also used which showed that its high level is retained in generations.

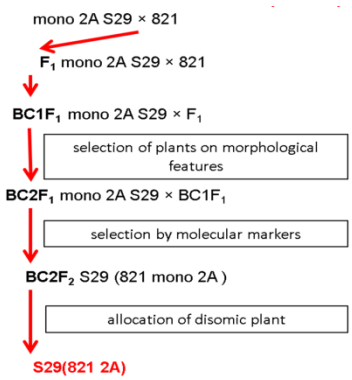

Fig.1: The scheme of development of substitution line S29(821 2A).

The obtained substitution line S29(821 2A) and the initial cv. S29 were grown in experimental fields of the Institute of Cytology and Genetics SB RAS, Novosibirsk (Nsk) and of the Omsk State Agrarian University (Omsk) during spring sowing. This material was also grown in green-house of the Institute of Cytology and Genetics of the SB RAS under normal irrigation and under drought.

Gluten content in grain (%) was hand-washed from 1g of whole meal. Physical properties of flour and dough were studied using Chopin alveograph with 50g mixer (Anonymous. 1988). Analysis of yield components was done in Novosibirsk and Omsk field environments.

Results and discussions

The obtained substitution line S29 (821 2A) consistently showed a higher gluten content in grain comparing to the recipient under diverse conditions (Figure 2). The comparable values of the trait in both genotypes were detected under drought conditions. At the same time, the line retained high physical properties of flour and dough characteristic of S29 (Figure 3). It has a high dough strength and tenacity, although a lower extensibility. P/L ratio, being a lower than in the recipient, at the same time was in the limits of high quality wheats. As well as the recipient, the line can be assigned to a group of 'strong-flour' wheats.

Contradictory results were obtained under one-year field testing of yield components under different environment (Figure 4). In Omsk the line showed the lower values of all components. Overall, the climate in Omsk is more droughty than in Novosibirsk. In June when the intensive growth of wheat plants occurs no rainfalls were registered. In Novosibirsk field conditions the line was more productive that the recipient S29. In the green-house conditions under normal watering the line was also less productive (Figure 4). Under drought conditions in the green-house the line had more tillers and higher number of grains but the grains were light-weight.

The line needs a more detailed evaluation for yield components in additional replicates under more diverse geographical conditions.

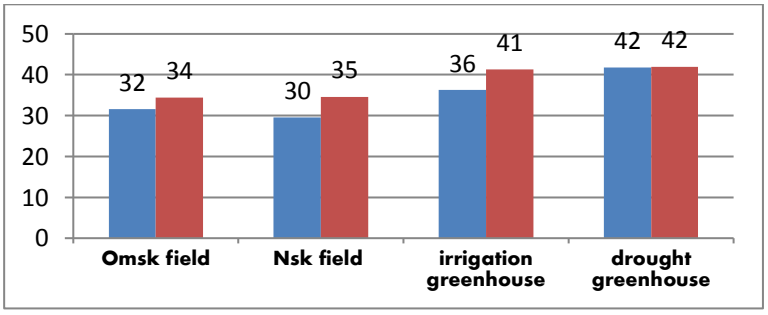

Fig. 2: Wet gluten content in grain, % in the substitution line S29 (821 2A) (red) comparing to the recipient S29 (blue) under different environment.

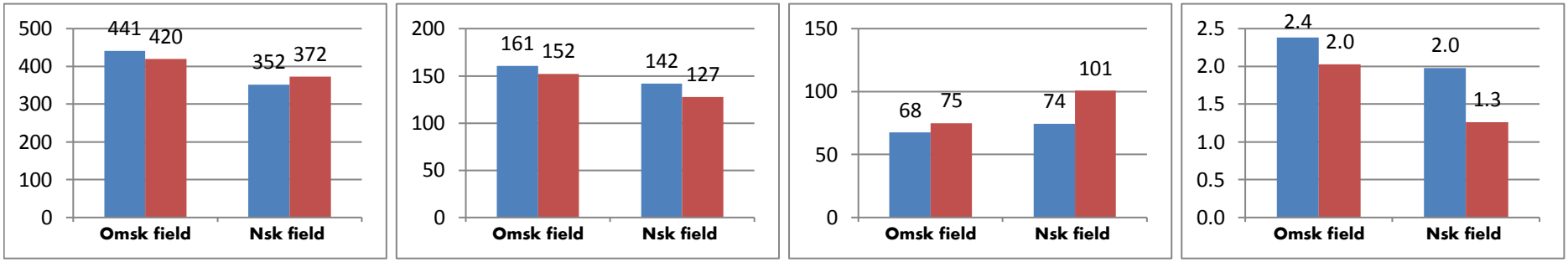

Fig. 3: Physical properties of dough in the substitution line S29 (821 2A) (red) comparing to the recipient S29 (blue) under different environment. From left to right: dough strength ($J \times 10^{-4}$), tenacity (P, mm), extensibility (L, mm) P/L/ ratio.

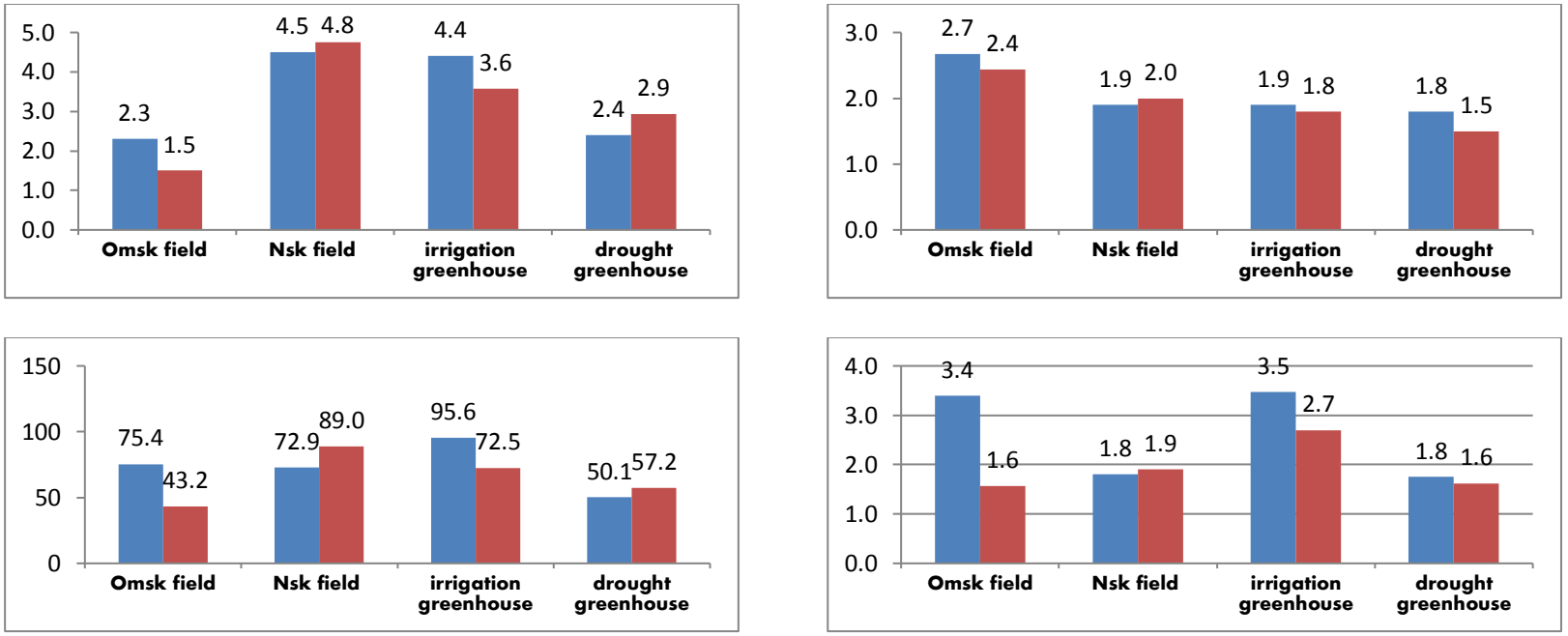

Fig. 4: Yield components in the substitution line S29 (821 2A) (red) comparing to the recipient S29 (blue) under different environment. From left to right: number of tillers, number of grain in the spikelet, number of grains per plant, grain weight per plant (g).

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Agro-morphological evaluation of a barley germplasm collection predominantly from the North African region

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Summary

New varieties improved for the modern agriculture have led farmers to abandon the local landraces and old varieties. These local landraces and old varieties are conserved ex-situ in gene banks could be a source of interesting alleles for future breeding programs. This study aims at assessing the agronomic value of a set of 110 accessions of barley which includes varieties and local landraces from 26 countries. The evaluation was conducted under field conditions, during the 2016-2017 growing season in Algiers, Algeria, in a randomized block design, with three replications and agro-morphological traits such as heading date, maturity date, plant height, growth habit, spike characteristics and resistance to diseases were evaluated. An analysis of variance was carried out for all traits, to evaluate the variability among the 110 genotypes and also between groups of genotypes, grouped according their origin. The results showed that higher variability ($p<0.0001$) among genotypes and also between the groups of origin for all traits studied. Significant correlations among some of the traits were observed. These preliminary results show abundant variability in the barley germplasm collection evaluated, which would be useful for their deployment in barley breeding program of Algeria.

Introduction

Barley (*Hordeum vulgare* L.) is one of the most important crops cultivated worldwide, especially in poor regions, where it is used as a staple food for both animals and humans (Ullrich, 2011). For decades, such as others a cereal, the improvement of barley has been oriented towards uniformity and creation of modern varieties with high yield potential to ensure food security. This situation led farmers to give up the local landraces and old varieties. However, this trend towards uniformity and the loss of agrobiodiversity threatens food security, which would be most affected by climate change. The challenge to ensure food security is to develop new varieties more adapted to changing environmental conditions. New sources of genetic diversity should be used in breeding programs, such as landraces and wild relatives of domestic crops, which have been underused in breeding programs.

The main objective of this work is to evaluate the agronomic potential and the morphology of a barley germplasm collection, predominately from the North African region, in order to exploit this valuable plant genetic resource.

Materials and methods

A set of 110 barley accessions, which includes varieties and local landraces, from 26 countries were evaluated in rainfed conditions, during the 2016-2017 growing season in Algiers (36°45.15′ N and 3°2.5182′ E), Algeria. The experimental design was a randomized block, with

three replications. A plot size was two rows 1m long with 0.25m spacing between rows. Data were collected on the following characters:

In the field

Days to heading (HED): number of days from sowing to the day when the awns appeared in 50% of plants in the plot.

Days to maturity (MAT): number of days from sowing to hard grain stage of 90% of the spikes in the plot.

Grain filling period (GFP): calculated in days, as the difference between physiological maturity and heading time.

Yield (YLD): after harvest, grain yield was estimated in q/ha.

Plant Height (HGT): measured at maturity, from the ground level to the top level of spike excluding awns.

Lodging (LDG) recorded on 1-9 scale as described elsewhere (IPGRI. 1994), where: 1) very low, 2) very low to low, 3) low, 4) low to intermediate, 5) intermediate, 6) intermediate to high, 7) high, 8) high to very high and 9) very high is the expression of the character.

Susceptibility to diseases: net blotch (NBT), powdery mildew (PMD) and leaf rust (LRT) were evaluated using a scale on 1-9 as above.

In the laboratory

At harvesting, 5 representative spikes, in each plot, were randomly sampled. In total 15 spikes from each accession were taken to laboratory for morphological characterization. Spike length (SLG), number of spikelets per spike (NSS), and number of kernel per spike (NKS) were measured.

Data analysis

An analysis of variance was carried out for all traits, to evaluate the variability among the 110 genotypes and also between groups of genotypes, grouped according their origin (Table 1).

Table 1: Distribution of 110 genotypes studied according to region of origin and countries within the region.

Origin	**Number of countries included**	**Number of genotypes**
North Africa (**NAF**)	4	**35**
East Africa (**EAF**)	2	3
Middle East (**MES**)	2	7
Asia (**ASI**)	2	9
Europe (**EUR**)	8	24
Latin America (**LAM**)	6	19
North America **(NAM**)	1	7
Oceania (**OCN**)	1	6
TOTAL	**26**	**110**

A pairwise correlation matrix between all traits was also calculated. These analyses were realized using GenStat discovery 4 software (VSN International, GenStat.co.uk).

Results and discussions

For all the traits studied, the ANOVA showed significant difference among the genotypes, which thus indicate the presence of large variability. Student-Newman-Keuls test used for means comparisons, showed a large number of means groups for most of the traits (data not shown). The huge difference existing among genotypes is appreciated by the standard deviation and the range for the quantitative traits (Table 2). This difference is especially noticeable for days to heading, days to maturity, grain yield and number of kernel per spike.

Table 2: Descriptive statistics for the quantitative traits scored in the germplasm evaluated.

Parameters	Mean	SD	MIN	MAX	Range
Traits					
HED	100.4	7.1	83.0	116.0	33.0
MAT	144.5	5.3	124.0	154.0	30.0
GFP	44.2	3.6	33.0	54.0	21.0
YLD	44.3	26.9	2.0	115.6	113;6
HGT	66.6	10.6	33.5	90.9	57.4
SLG	7.7	1.1	4.7	10.6	5.9
NSS	17.3	2.6	10.0	25.0	15.0
NKS	47.0	9.9	19.0	70.0	51.0

For the discrete quantitative characters, the variability is perceived by the wide distribution of the genotypes through the classes, defined according to the scale used for scoring these traits (Table 3). The wide distribution of the genotypes is particularly observed for the diseases reactions, especially for powdery mildew and net blotch while rust disease was not widespread in the trial.

Table 3: Frequency distribution of the scored values for the discrete quantitative traits, according to scale used, for the 110 accessions evaluated.

Traits	Relatives frequencies								
Classes	1	2	3	4	5	6	7	8	9
LDG	78	26	2	4	0	0	0	0	0
PMD	0	7	14	21	24	23	12	8	1
RST	92	8	6	2	2	0	0	0	0
NBT	0	6	15	33	23	21	9	3	0

The genotypic differences were broken down into between groups and within groups. Mean squares resulted from the analysis of variance comparing between groups were larger than within groups. These results are clearly observed for the quantitative traits such as days to heading, days to maturity, yield, plant height and number of kernel per spike (Figure 1). However for grain filling period, there was no difference between groups. For the discrete quantitative characters, also the difference between groups was slightly larger than within groups. These results suggest that the existing variability is especially present between the groups than within groups.

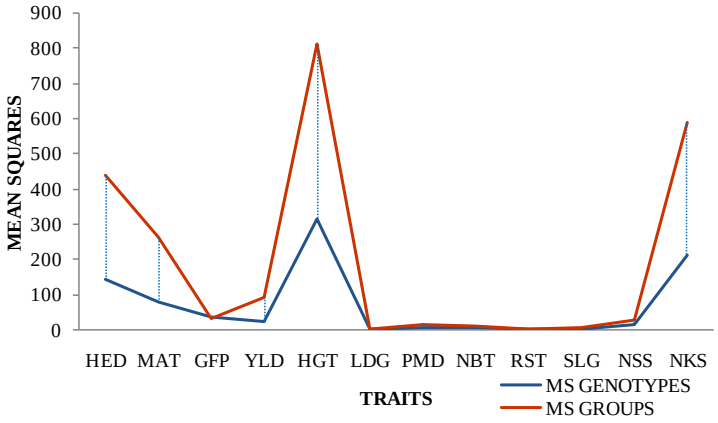

Fig. 1: Means squares among genotypes and among groups, resulted from the analysis of variance, for the all traits scored.

The Asian group of genotypes was the earliest for days to heading and days to maturity, followed by the North African group then the Middle East group , whereas the European and Latin America groups were the latter ones (Figure 2). However, the North African group showed the best yield followed by the European group, then the Middle East group, while the Asian group had the lowest yield.

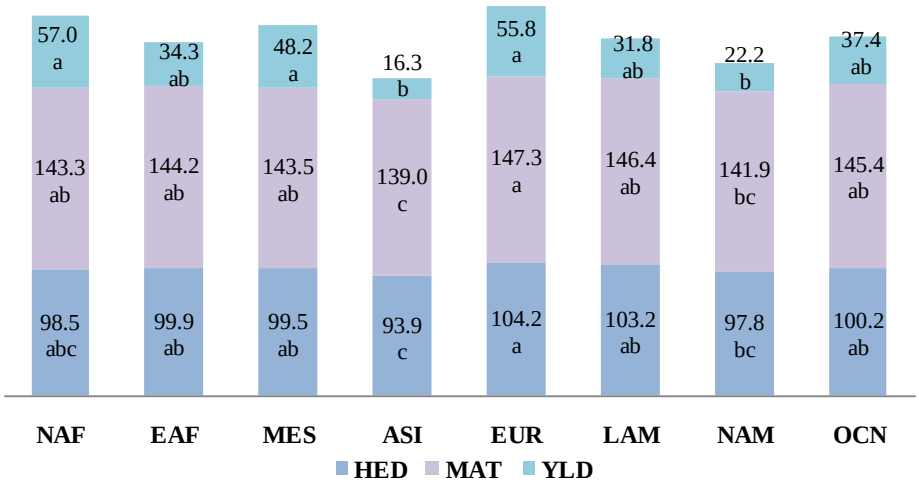

Fig. 2: Results of Student-Newman-Keuls test fo means comparisons among the groups, for days to heading, , days to maturity and grain yield (q/ha).

These results were unexpected. Generally, in our environmental conditions, early genotypes for heading present better yield, because the genotypes escape the drought that can occur during grain filling period. On this issue Garcia del moral et al (2003), mentioned that earlier genotypes generally perform better than the latter one, in the low yielding environments, because of higher water availability at the end of the crop season. This hypothesis is confirmed by the negative correlation observed between days to heading and grain yield and also between days to heading and grain filling period (Table 4). However the performance of the European group, suggest that drought was not the only limiting factor that affected yield in the experiment.

Effectively, as we can see in the Table 4, yield in negatively correlated to powdery mildew and net blotch diseases. The correlation coefficients related to these two parameters suggest that grain yield was more affected by the diseases than the late flowering. This hypothesis may explain the performance of the European group compared to the others, except the North African group. Indeed, the means comparisons of the groups for these traits clearly demonstrate that the North African and the European groups were less affected by the diseases (Figure 3); however the earliest Asian group was classified with the most susceptible groups to diseases. Scott & Griffiths (1980) commented that powdery mildew attack have reduced tiller number, grain size and also grain per tiller. Likewise, Deadman & Cooke (1987) revealed that net blotch have affected adversely spikelet development, leading to a reduction of grain number per spike. In this study, we noticed a reduction in number of spikelet per spike and number of kernel per spike in the Asian group of genotypes (Figure 4). Yahiaoui et al. (2014) observed that heading time, lodging and powdery mildew attacks were the main factors that adversely affect grain yield in barley. In this trial, lodging is correlated positively with grain yield. Even though the most yielded genotypes were the most affected by mechanical lodging, the losses were insignificant, since the harvest was done manually.

Table 4: Pairwise correlation matrix comparing all genotypes, for all traits scored.

TRAITS	HED		MAT		YLD		HGT		LDG		PMD		RST		NBT		GFP		SLG		NSS	
MAT	**0,87**	***																				
YLD	**-0,20**	*	-0,05	NS																		
HGT	0,09	NS	**0,20**	*	**0,44**	***																
LDG	**-0,25**	*	**-0,19**	*	**0,32**	***	**0,23**	*														
PMD	-0,05	NS	-0,04	NS	**-0,35**	***	-0,18	NS	0,05	NS												
RST	0,08	NS	0,09	NS	-0,01	NS	0,02	NS	-0,05	NS	**0,19**	*										
NBT	0,09	NS	0,11	NS	**-0,40**	***	**-0,31**	*	-0,16	NS	**0,45**	***	-0,11	NS								
GFP	**-0,70**	***	**-0,25**	*	**0,32**	***	0,11	NS	**0,20**	*	0,04	NS	-0,01	NS	-0,03	NS						
SLG	0,03	NS	0,04	NS	-0,03	NS	**0,25**	*	-0,04	NS	0,05	NS	0,18	NS	0,00	NS	-0,01	NS				
NSS	0,01	NS	0,08	NS	**0,41**	***	**0,27**	*	0,03	NS	0,08	NS	0,02	NS	-0,01	NS	0,09	NS	**0,20**	*		
NKS	-0,05	NS	0,04	NS	**0,51**	***	**0,31**	**	0,12	NS	0,07	NS	-0,07	NS	-0,02	NS	0,15	NS	-0,02	NS	**0,92**	***

NS : no significant; *P<0.05; ** P<0.01; ***p<0.0001

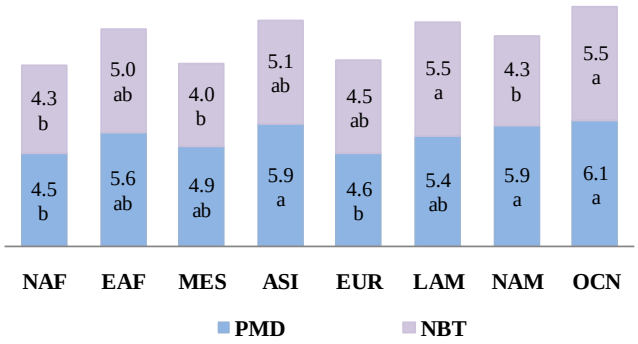

Fig. 3: Results of Student-Newman-Keuls test for means comparisons among the groups, for powdery mildew (PMD) and net blotch (NBT) susceptibility.

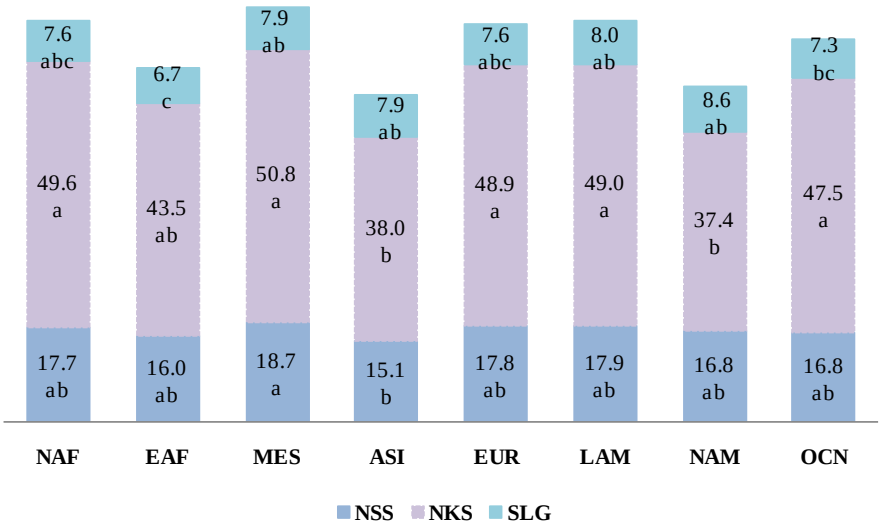

Fig. 4: Results of Student-Newman-Keuls test, for means comparisons among the groups, for number of spikelets per spike (NSS), number of kernel per spike (NKS) and spike length (SLG).

Conclusions

This study allowed to identify the presence of large variability among the genotypes for all the agro-morphological traits observed. This variability was larger among the region groups of genotypes then within groups.

The relative outperformance of the North African group suggests the existence of adaptability.

This variability could be a source of interesting alleles that could be useful for other breeding programs in Algeria.

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The 70th Anniversary of the "AUGUST SESSION of VASKhNIL"

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At August 2018 there was 70th anniversary of August session of the All-Union Academy of Agricultural Sciences of the Soviet Union (Lenin All-Union Academy of Agricultural Sciences) which took place in Moscow (July 31 - August 7, 1948) and was organized under control of the Communist party of the former USSR.

At July 31, 1948, Academician T.D. Lysenko in his opening address "About the Situation in Biological Science" presented his opponents as political opponents of socialist agricultural science and the collective farms system in the Soviet Union and Soviet biological science, in general. This political action has been prepared earlier. At the letter to J.V. Stalin from 27.10.1947, T.D. Lysenko wrote "… Mendelian-Morgan theories, which, as I deeply believe, are false and harmful, are still taught to students in almost all of our biological and agricultural universities. … Mendelism-Morganism continues to influence the scientific theory and practice of plant breeding and seed production and breeding in animal husbandry. This is … the reason for the lagging of the results of selection and seed-growing work in crop production and breeding work in animal husbandry …" (Vavilov Yu.N., 2008) and three days later he has got answer from J.V. Stalin: "… As for theoretical conceptions in biology, I believe that Michurin's theory is the only one scientific theory. Weismanists and their followers, those who negate the inheritance of acquired properties do not deserve to talk about them. The future belongs to Michurin". Those words of J.V. Stalin led to a formal ban on teaching "Mendelist-Weismannist-Morganist" genetics. And there was a strong support of T.D. Lysenko at the session VASKhNIL organized by his colleagues M.A. Olshansky, A.A. Avakian, D.A. Dolgushin. The speech of T.D. Lysenko predetermined further persecution of genetics as a science and geneticists in former USSR until the early 1960s.

Thus the Central Committee of the Communist party, with the direct participation of J.V. Stalin, prepared a new, larger-scale political campaign against genetics. According to this campaign the plan of the main research works of biological institutions of the former USSR Academy of Sciences was organized, the 'anti-scientific Weismann's' projects were removed from the plan of investigations and they were replaced with actual projects that supported the tasks of socialism construction (Esakov, 1994).

The reform affected scientific structures and personnel policy. For example:

- the most important biological institutions were "strengthened" with qualified Michurin's scientists;
- the plan for the training of graduate students were revised and guided in the interests of the development of Michurin's biological science;
- the strengthening of the editorial boards of periodicals of Biological Sciences were done by leading Michurinists.

In the reality the Michurinists were presented by Lysenkoists which convey the politics of Lysenko.

The defeat of genetic science in August 1948 at the session of the Academy of Agricultural Sciences had directly or indirectly results in: distroying scientific plans of hundreds of scientists and deforming theoretical convictions. Scientists were forced to go abroad. According to analysis of State archive documents professor D.P. Ursu from The Department of Modern and Contemporary History of Odessa National I.I. Mechnikov University searched out: "Only during two months after the August session of the Academy of Agricultural Sciences, 115 people were dismissed from their jobs, including 10 deans, 65 department chairs, 9 professors, 23 associate professors and 8 assistants. Among the dismissed persons were deans of biological faculties of Lviv and Uzhgorod Universities, heads of departments of Kiev and Kharkov Universities, two institutes of Kharkov - medical and agricultural, Lviv Medical Institute, Uzhgorod University. In the Crimea the dean of the Faculty of Natural Science and the head of the Department of the local pedagogical institute, Professor V.M. Borovsky, a graduate of the University of Heidelberg, who completed an internship in the United States in 1929 were fired. Academicians of the Academy of Sciences of the Ukrainian SSR I. Schmalgausen, N. Grishko, D. Tretyakov, N. Kholodny lost their positions" (Ursu, 2010).

EWAC conference as a tribute and respect to the memory of geneticists affected by Lysenkoism recalls these events.

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New materials and methods in common winter wheat breeding

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During last 15 years my work was concentrated on the next three groups of breeding materials:

Classical wheat breeding

The modern wheat cultivars must be with high yield capacity – more than 10 t/ha and moderate quality. This mean increasing of the spike productivity, stem stability and tillers ability, or SST complex (spike-stem-tillers). For improving the spike architecture CIMMYT materials were used. Several crosses were made to improve cold tolerance and quality. The new lines possessed ability to form more than 85 grains and 3 g/spike with good grain shape. Such spike need strong stem and some Italian cultivars were used. Some French varieties were used to improve tillers ability, bearing ears. The new lines form 1.5 tillers/grain/plant, stem height 75-80 cm and desirable spike. Spike is like dream, follow it. The main agronomical characteristics are in standard level. A new series of crossing with well adapted cultivars is made to create variety(s), effective for wild practical use.

Black grain wheat

This wheat is created by using the translocation 4DL/4AgL from *Agropyron elongatum* (2n=70) to have new source for bread making. The material is divided in two groups – with high and low content of gluten and protein. The lines are genetically stable, 2n=42, and possessed good agronomical characteristics, resistant to mildew, septoria and leaf rust, probably due of translocation. This new wheat lines can be used for ordinary and dietary bread with antioxidant nature and also for industrial starch production.

Hybrid wheat

The new (m) system for male sterility is used. The (m) cytoplasm is transferred from *Aegilops mutica*, 2n=14, Mt. This cytoplasm is very strong male sterilizer and all cultivars and lines introduced into (m) are male sterile and with good female fertility. Side effect is not detected, the growth is normal. The problem still now is fertility restoration. A complicate crosses are made to transfer the original Rf mutica gene(s) to wheat. Stable results are still expected.

Red listing as a tool for wheat genetic pools conservation for Romania

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During the humankind history, wheat became one of the seven pillars of world nutrition. In Europe, as well as in Romania, archaeological evidences have shown that wheat cultivation has a history of over 7,000 years. After the Second World War in Romania it was recorded the establishment of a growing network of research institutes and stations devoted to crops breeding up to 1990, when it continuously collapsed up to 2004. Their gene banks were either transferred to other institutes in our country or abroad, either lost. During the past 70 years, at least 5 stages of genetic erosion were distinguished, grounding the red listing methodology for crop varieties in Romania that was discussed in 2012 under the International Union on Nature Conservation. To ensure food security, we should economically assess the balance sustainability between *in situ* and *ex situ* conservation strategies, as an integral part of the national biodiversity strategy and action plan (NBSAP). The evaluation of 134 wheat varieties that were officially registered in the National Catalogue of Plant Varieties and Hybrids, between 1956 and 2017, has shown that at least 78 are relevant to be red listed as extinct or threatened wheat cultivars with erosion. Moreover, considering the history of these breeding varieties, the list may grow further up to 150 cultivars that may be threatened with extinction or erosion. This study includes cross-evaluations regarding scientific knowledge that is relevant for each wheat cultivar breeding as well as recommendations for further developing the national strategy for wheat breeding.

Spike morphology genes for wheat taxonomy and breeding

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Searching for ways of biodiversity increasing and preservation is the key point in biology of the 21st century, whereas preservation of cultivated wheat species biodiversity is a strategic task of food security. Wheat species are not good model objects for genetic investigations and taxonomy of cultivated plants. Studying them has always been conditioned by the demands of breeding as wheat is one of the basic food crops. Expanding not only wheat biodiversity, but also using traits that have not been widely used in recent breeding are goal of investigations. Comparative-genetic analysis carried out in cultivated wheats parallel with their related species will allow not only to determine the origin of certain genes in the first ones, schedule the strategy of introgressive hybridisation but provide a clear picture of their origin and differentiation into species. The presentation examines the state of knowledge about the genes which control the architectonics of wheat plant (spike morphology). It is shown that molecular genetic studies, which are started at the present time, allow to find both the ortholog genes from relative species of wheat (barley, rye, etc.) and genes, which were not previously used for the breeding process in wheat. Using these genes for further breeding allows to produce a modern wheat commercial cultivars with novel genetic biodiversity.

Acknowledgements

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Exploring the genomic diversity of the AE Watkins bread wheat landrace collection

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Understanding the genomic complexity of hexaploid bread wheat (*Triticum aestivum* L.) will be useful for future improvement of the crop, particularly in the light of changing environments.

Exploiting the genetic diversity present in the AE Watkins bread wheat landrace collection to explore this genomic diversity is part of the current pre-breeding programme Designing Future Wheat and previous programmes. A nested association mapping (NAM) panel of segregating bi-parental populations were developed from the 119 accession strong core set, selected to cover the majority of genetic diversity (Wingen et al 2014). The modern spring elite variety, `Paragon', was used as common reference parent of the NAM panel. Genetic maps were constructed following identical rules. In total, more than 1,600 linkage groups were identified in 60 maps, based on recombination from estimated 126,300 crossover events. A consensus map constructed from these maps contained nearly 2,500 genetic loci. These genetics tools were used to investigate the rules underlying genome fluidity, e.g. by looking at the conservation of marker distances and marker orders. In general, marker order provides support for strong synteny between bread wheat accessions, however, cases of incongruent linkage groups were also present. Evidence for translocations, falling into different classes, were found in at least 36 of the maps. Moreover, loci involved in recombination rate were identified. Many of the populations have also been trialled under field conditions. Future research to pool results in order to identify useful genetic diversity for breeding is under way.

All developed genetic resources are freely available from http://wisplandracepillar.jic.ac.uk/.

The study of the Siberian collection of spring barley

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Barley (*Hordeum vulgare* L.) is one of the world's earliest domesticated and most important crop plants. The improvement of yield, disease resistance and tolerance to environmental stress as well quality improvement are the main tasks of barley breeding programs. DNA-markers for accelerated and more precise selection have been developed in many studies. However, Russian barley germplasm has not been widely assessed using molecular genetics approaches. Current study was aimed on association mapping of the Siberian germplasm from ICG collection "GenAgro", based on SNP-genotyping and phenotyping for a wide range of traits related with yield, biotic and abiotic stress tolerance. Genomic regions associated with a number of traits assessed in 2 experimental ICG fields, as well as resistance to spot blotch, net blotch and root rot as well as drought and salinity resistance assessed in the laboratory screenings are reported.
This study was supported by RSF 16-14-00086.

Light spectrum dependent regulation of freezing tolerance and yield quality in cereals

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The recent break-through in LED lighting technology make it useful as lighting source in indoor plant production and the flexibility of this system allowed the development of new, adjustable light spectrum lightening devices.

We have used LED illumination (1) to investigate the light-quality and temperature dependent regulation of freezing tolerance in cereals. (2) Moreover the effects of light intensity and spectral composition on plant metabolism and nutritional quality were also evaluated.

1) The reduced red/far-red ratio at 15 °C induced the CBF gene expression and it also increased the freezing tolerance of winter wheat and winter barley. From the CBF cluster only the *HvCBF4* subgroup genes expressed in late afternoon or early night and these genes showed circadian rhythms, as well in barley. It was also revealed that. The CBF gene transcript accumulation had appeared four hours earlier and more intensely under supplemental far-red illumination. Most likely the photoreceptors phyA and phyB plays important regulatory role in this acclimatization process.

2) Comparing different spectral compositions and light intensities revealed significant differences in growth and development, leaf photosynthesis, thiol and amino acid metabolism as well as in yield quantity and flour quality of wheat. Benefits of LED light over fluorescent lighting were manifested in both yield quantity and quality. Our results demonstrated that the LED lighting technology can provide high fluency and customized wavelength for plant cultivation, and through modification of light quality LEDs make it possible to manipulate the metabolism to obtain desired traits and products.

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Molecular background of 5A chromosome induced changes in phytohormone homeostasis in wheat

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Short- and long-term cold treatment on the abscisic acid (ABA) and cytokinin (CK) metabolism, and their main biosynthesis- and signaling-related genes were examined in freezing-sensitive and freezing-tolerant wheat genotypes. Freezing-tolerant Cheyenne and Chinese Spring substituted with the 5A Cheyenne chromosome were compared with the freezing-sensitive Chinese Spring variety.

Using oligonucleotide-based microarray, altogether 636 differentially expressed genes responding to cold-treatment were identified.

Apart from the already known effect on major cold inducible genes, including CBF14, Cor14b, and WCS120, we found that chromosome 5A is associated also with regulation of hormone-related genes and metabolites underlying the freezing tolerance. Dynamics of the expression of freezing tolerance marker genes was correlated with the changes in phytohormone contents, as well as with transcription pattern of the hormone related genes. Overrepresentation analysis of the differentially expressed genes supported the ABA-signaling and revealed some pronounced biological GO categories associated with the cold-shock response of the genotypes. Protein network analysis indicated differences between the genotypes, suggesting different biochemical and cellular strategies in their reaction to cold.

Based on our results we conclude that wheat chromosome 5A regulates, both directly and indirectly, the establishment of freezing tolerance. The most relevant differences between the two tolerant genotypes and CS may become useful as molecular markers of freezing tolerance.

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Analysis of the expression of selected genes encoding antioxidant and proline biosynthesis pathway enzymes under drought stress conditions in common wheat (*Triticum aestivum* L.) substitution lines

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Water deficit is one of the major factors that negatively affects quantity and quality of the field crops yield worldwide. For common wheat (*Triticum aestivum* L.) the exposure of plants to drought stress can cause over 50% reduction in the yield.

At the physiological level drought stress induces the reactive oxygen species (ROS) production. In plant cells ROS are rapidly detoxified by various mechanisms based on antioxidant enzymes. Another defensive physiological mechanism of ROS quenching in plant cells is based on free proline.

The aim of the presented study was analysis of the drought stress influence on expression of the genes encoding selected antioxidant enzymes (ascorbate and guaiacol peroxidases) and proline biosynthesis enzymes (delta-1-pyrroline-5-carboxylate synthetase and reductase) in wheat seedlings. For examinations 18 substitution lines of 'Saratovskaya 29' (drought tolerant) × 'Janetzkis Probat' (drought sensitive) were used. Analyzed seven-day-old plants were grown in the hydroponic culture. Stress conditions were chemically induced by the addition of 10% polyethylene glycol (PEG 6000) to the medium. The tissue for RNA extraction was harvested after 1, 3 and 6 hours of stress.

Obtained results allowed for characterization of the alteration of analyzed genes at the functional level and revealed that both systems (antioxidant enzymes and proline dependent) were activated at the transcriptome level during drought stress, however obtained patterns of response were differentiated and genotype dependent. Furthermore, using of the single chromosome substitution lines allowed for determination of the wheat chromosomes putatively linked to improved tolerance to water deficit.

Validation of published gene-based markers for enhanced thousand-kernel weight and identification of novel loci in large elite germplasm panels

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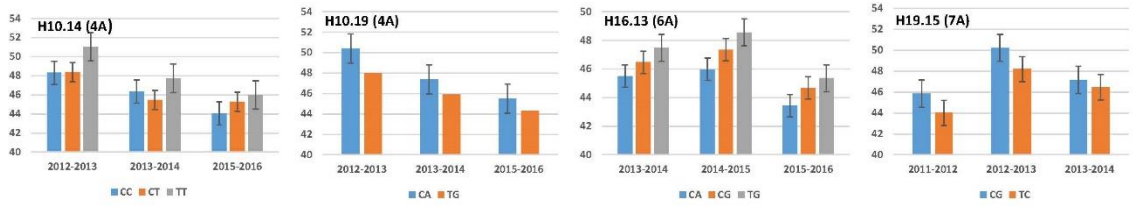
Of all yield components that affect grain yield (GY), grain weight is relatively stable and highly heritable, hence an important selection target for the genetic improvement of GY. In wheat, a number of gene-based markers related to enhanced thousand kernel weight (TKW) are available that are suggested to be used for marker-assisted selection. However, effects of most of these TKW genes have been validated only in limited genetic backgrounds. We therefore tested the published TKW related markers in two diverse panels of CIMMYT's advanced lines to validate the effect of the observed allelic variation on TKW via candidate gene-based genome wide association study. Of the 10 genes investigated, only two genes (*TaGs3-D1* and *TaTGW6_A1*) showed association with TKW in the two diverse panels, respectively. Furthermore, the reported favorable alleles of these two genes, *TaGs3-D1a* and *TaTGW6_A1a*, were found to be unfavorable in CIMMYT germplasm. This example suggests that the published markers cannot be used directly to improve GY via indirect selection.

To further investigate the genetic determinants of TKW in CIMMYT germplasm, a haplotype-based GWAS was conducted in five large elite panels, which identified 4 haplotypes on chromosomes 4A, 6A and 7A with significant effects on TKW (on average by 1.21 to 2.73 g, Fig. 1). KASP assays have been designed for these haplotypes to validate the underlying TKW loci. Going towards cloning of these novel genes will expand the opportunities for developing new functional markers that will be useful in breeding.



Transcriptional regulators of flavonoid biosynthesis: MYB, bHLH and WD40 gene families in Triticeae

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Flavonoids are plant secondary metabolites, having multiple biological functions in plants as well as health benefit for plant food consumers. These compounds are synthesized by most of the higher plants including cereals. Activation of flavonoids biosynthesis occurs via regulatory MBW complex forming by transcription factors MYB, bHLH/MYC and WD40. The goal of the current study was the characterization and comparison of duplicated copies of the *MYB*, *bHLH*/*MYC* and *WD40* genes in Triticeae. Hightly homologous *MYC* genes in barley and wheat were identified in homoeologous groups 2 and 4 chromosomes. In homoeologous groups 4 and 7 chromosomes *MYB* gene copies were found. The *PAC1* orthologues (the maize gene encoding WD40 factor regulating flavonoid biosynthesis) were described for the first time for Triticeae. Copies are located on chromosomes 6A, 6B and 6D in wheat and 6H in barley. Using qRT-PCR on cultivars differing by anthocyanin coloration we compared activity of certain MBW genes. We showed that the main regulator of the blue pigmentation of the aleurone layer is the bHLH-encoding *HvMyc2* gene (4HL). Information on allelic differences in this gene was used for development of convinent CAPS-marker, useful for both mapping this gene in segregation population and marker-assisted selection. Besides we identified the candidate gene for MYC-encofing factor necessary for synthesis of anthocyanins in wheat coleoptile. In general, the results obtained demonstrate that the duplicated flavonoid biosynthesis genes are often maintained in Triticeae species due to their functional specialization. The studies were partially supported by RFBR (16-04-00912) and by RSF (№ 16-14-00086).

Evaluation of Algerian collection of bread wheat (*Triticum aestivum* L.) varieties by agronomic and trait-linked molecular approaches

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For successful breeding program, analysis of genetic diversity among genotypes is fundamental, providing relevant information for the selection of the parental lines. Usually, varieties exhibiting great genetic distances are less related to each other and their original genetic materials may not have common pedigree. Genetic distances could be estimated by the use of morphological and agronomic traits or various molecular markers. The purpose of this study was to estimate the genetic distance among 40 bread wheat (*Triticum aestivum* L) varieties. Genetic distances were estimated based on data from molecular analyses using molecular markers linked to important loci controlling photoperiod response (*Ppd*), plant height (*Rht*), leaf rust (*Lr*), yellow rust (*Yr*), and stem rust (*Sr*); and agronomic characteristics such as plant height, thousand kernel weight, tillage and diseases resistance. The preliminary results show higher variability ($P<0.0001$) between the genotypes for most of the agronomical traits analysed. A significant genetic diversity among the genotypes was also found for key rust diseases resistance genes (*Lr34*, *Lr68*, *Lr37* and *Sr24)* and dwarfing gene (*Rht1*). Theses primary results are useful for targeting crosses in marker-assisted wheat breeding in Algeria.

Analysis of the relationship between the genetic similarity and yielding for Polish *Triticale* breeding materials

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Triticale (×*Triticosecale* Wittmack) combines in one genus the resistance of rye and high quality and yield of wheat. In contrast to other cereals, triticale did not pass the natural process of evolution, what cause that its gene pool is quite narrow.

The aim of the presented study was analysis of the yielding capacity of Polish triticale breeding materials and determination of the relationship between this trait and level of the genetic similarity. In analysis 470 hexaploid winter triticale breeding inbred lines were included. Kernels of analyzed triticale forms were sown in the conditions of field experiment in six spaced locations in Poland. Obtained results were combined with genetic similarity level estimated by means of the DArTseq technique. In order to obtain more precision, the level of genetic similarity was analyzed based on markers from each chromosome separately.

Our results revealed that difference in yield between distant genotypes was dependent on chromosome localization of DArTseq markers. The lowest difference in yield between genetically distant genotypes was shown for the markers located on chromosome 1A (0.01 kg per 1 m^2), while the largest difference was noticed for markers located on chromosome 6R (0.44 kg per 1 m^2).

Obtained results indicate that determination of the level of genetic similarity based on high throughput analysis techniques, especially for the purpose of heterosis prediction, should be based on specific region of the genome rather than whole pool of markers.

Acknowledgement

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Genetic analysis of developmental traits in old Russian spring wheat cultivars

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Older varieties of wheat can be used as sources of a new variability for obtaining the modern wheat varieties adapted to adverse climate changes. The spring wheat cultivar Saratovskaya 29 (S29) was developed almost 60 years ago in the Volga region and is characterized by a high ecological plasticity. Even earlier obtained spring cultivars Caesium 111 and Sibirka 1818 are adapted to the conditions of Western and Eastern Siberia. These two varieties were crossed with S29 for genetic analysis of hybrid dwarfism, productive tillering and developmental rate. It has been shown that the two Siberian varieties differ from S29 by a non-allelic gene of hybrid dwarfness in 2D chromosome, as well as by the genes that determine the higher plant height, high productive tillering, later flowering and maturation.

Acknowledgements

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Chromosome specific DArTseq markers analysis as an alternative approach for genetic similarity determination in polyploid cereals

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High throughput molecular marker systems are currently one of the most important tool for genetic diversity analysis. In polyploid plant species distribution of the markers is not equal for all regions of the genome. The objective of the study was determination of the influence of DArTseq markers chromosomal localization on the results of genetic diversity analysis for Polish triticale genotypes.

The research material consisted of 470 winter triticale breeding lines. Genotyping of these lines was performed by means of DArTseq technique (Diversity Array Technology, Canberra, Australia).

As a result of DArTseq analysis 87 493 markers were obtained for each of the tested genotypes. After preliminary analysis 24 353 markers were selected for subsequent analyses. The analysis of tested triticale genotypes genetic similarity was performed separately for markers localized on certain single chromosome. Cluster analyses were performed by means of the XLStat software. The UPGMA with Jaccard's similarity index was used.

Obtained results showed that maximal genetic distances between analyzed triticale genotypes were different and dependent on chromosome localization of the markers. For maximal genetic distance, the highest value was noticed for 3R chromosome markers (0.9838) and the lowest for 5A chromosome (0.8382).

Our results suggest that determination of the genetic diversity based on selected chromosome(s) could be better approach than use of whole marker set. This information could be especially useful for heterosis effect prediction in breeding programs.

Acknowledgements

The results of the study were obtained within the framework of the project funded by Polish Ministry of Agriculture and Rural Development entitled 'Identification of the genome regions and DNA markers linked to heterosis in hexaploid winter triticale'.

Cultivar Canyon – effective source against oat powdery mildew

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Powdery mildew caused by *Blumeria graminis* f.sp. *avenae* is one of the most damaging foliar disease of oats. It appears annually and causes large yield losses in many parts of the world. The most known economical and environmentally friendly method to reduce the occurrence of powdery mildew is introducing cultivars with effective resistance genes. Numerous host-pathogen tests have shown that the cultivar Canyon is characterized by a high level of resistance to powdery mildew. Moreover, this cultivar possesses different pattern of infection than lines and cultivars with known resistance genes, thus indicating that resistance in the cultivar Canyon is conditioned by a new gene or is the result of interaction of few effective resistance genes.

The aim of this work was to characterize the inheritance of the source of resistance identified in the cultivar Canyon.

The subject of the study were individuals of the F_2 populations: 'Canyon' × 'Fuchs' and 'Canyon' × 'Sam' obtained as a result of crossing the cultivar Canyon with susceptible cultivars Fuchs and Sam. The assessment of resistance segregation was performed using the host-pathogen assays based on 3 powdery mildew isolates avirulent to the cultivar Canyon. The segregation ratio of the F_2 populations were analysed using Chi-square tests for goodness of fit. In both analysed F_2 populations segregation for resistant and susceptible plants was obtained. We found that this segregation fit to the ratio 3:1. The obtained results confirmed the monogenic way of inheriting the powdery mildew resistance in the cultivar Canyon.

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Preliminary screening of *A. sterilis* L. for resistance to crown rust

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Cultivated oat is affected by a number of fungal diseases, among which crown rust caused by *Puccinia coronata* f. sp. *avenae* is the most widespread. The use of race-specific genes is the primary mean of control. *Growing* resistant varieties is not only economical, but also ecological approach. Crown rust resistance in oat has usually monogenic inheritance. So far, a number of potential Pc genes have been identified in oats, both in cultivated *A. sativa*, *A. byzantina* and wild *A. sterilis, A. strigosa, A occidentalis, A. barbata* species. The best source of resistance genes to *P. coronata*, with about 45 described, and about 10 *Pc* introduced to common oat is *A. sterilis* L. Unfortunately, current sources of resistance to crown rust rapidly lose their effectiveness due to fast evolution of pathogen virulence; therefore, recent efforts have focused on identifying new *Pc* genes.

The aims of presented studies were analysis of the level of resistance to crown rust of 65 genotypes belonging to wild oat species *A. sterilis* and identification potential sources of resistance to this pathogen. Analyses were conducted based on host-pathogen tests. Two highly virulent crown rust isolates were used to estimate the level of resistance in analyzed genotypes. Tested *A. sterilis* accessions were characterized by very low level of resistance and most of genotypes were susceptible or showed intermediate response Only 4 (6,1%), among 65 tested, were resistant to both isolates used in the study and, after verification inheritance pattern, could be valuable sources of resistance to crown rust.

The current status of wheat breeding for heat tolerance at NARDI Fundulea

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Fundulea is situated in a region with temperate continental climate, with summers generally warm to hot, the average maxima being around 29 °C, and frequent temperatures over 35 °C. Temperatures after wheat anthesis are often much higher than the optimum for photosynthesis and grain filling. Continuos selection during several decades for improved grain filling allowed the optimization of vegetation period and on the other hand a heat tolerance level of the cultivars released by the Fundulea breeding program, relatively higher than many imported cultivars.

However, higher temperatures have been recorded in the recent years and even higher temperatures are forecasted in the future. This prospect prompted increased efforts in breeding for heat tolerance. These have included:

- testing membrane thermostability in seedlings;
- testing the response of grain weight to increased temperatures in the field under plastic cover;
- increasing genetic diversity in the breeding program by including cultivars from regions cronically affected by heat waves;
- high-throughput phenotyping for traits associated with high temperatures tolerance;
- using molecular markers associated with tolerance to high temperatures.

Evaluation of eyespot resistance in breeding collection on hexaploid wheat (*Triticum aestivum* L.)

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Eyespot is one of the most dangerous stem base diseases of cereals caused by two fungal species: *Oculimacula yallundae* and *Oculimacula acuformis*. Use of cultivars resistant to eyespot is the most economic and environmental friendly way to minimize economic loss of wheat. Therefore, an incorporation of genes for resistance to eyespot is an important aim of varietal improvement. So far only two eyespot resistance genes: *Pch1* and *Pch2* have been characterized and markers made available to plant breeders. *Pch1* is reported as the most effective source of resistance to eyespot, however the chromatin segment with this gene introduce also undesirable traits determining yield reductions. The *Pch2* gene, can constitute an alternative for maintenance the resistance without negative influence on the agriculturally important traits.

The main aim of this study was to determine resistance to eyespot in breeding collection (150 genotypes) of hexaploid wheat. We evaluated the presence of enzymatic and molecular markers referred to the *Pch1* and *Pch2* resistance genes and performed inoculation tests during the seedling and stem elongation stages. Analysis were extend to observations of natural infection by *Oculimacula* species. What is more, the yield components were estimated and compared with molecular results to determine the influence of eyespot resistance genes on phenotype values of breeding lines.

We have identified 3 genotypes with pyramidization of both *Pch1* and *Pch2* genes. The lowest infection of seedlings was observed in genotypes carrying both eyespot resistance genes (0,84 in 0-4 scale) and highest infection of seedlings in genotypes without this genes (1,70 in 0-4 scale). What is more, the lowest infection of stems in field inoculation test was determined for genotypes with *Pch1* gene (mean 1,3%), whereas genotypes with *Pch2* (mean 24,8%) or without eyespot resistance genes (mean 29,2%) were the most infected by *O. yallundae* and *O. acuformis*.

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Studying the flowering and maturity gene complex in spring wheat

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Alberta is one of the three Prairie provinces of western Canada where most of the country's 20-30 million t of spring wheat are grown annually. The University of Alberta's spring wheat breeding program is the most northerly in Canada, in a region where early maturity is of great importance due the very short growing season. We have been actively studying the flowering and maturity gene complex in spring wheat for over 15 years. The results of a number of genetic studies involving *Vrn*, *Ppd* and earliness per se genes/QTL will be discussed. We will also discuss the breeding and registration of early maturing, high yielding and high grain protein spring wheat cultivars from the University of Alberta over the last decade.

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