European Cereals Genetics Co-operative Newsletter 2016

Proceedings of the 16th International EWAC Conference
24 - 29 May 2015
Lublin, Poland

www.ewac.eu

Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany
and
Institute of Plant Genetics, Breeding and Biotechnology,
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Edited by

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MUZEUM
EWAC EUCARPIA Cereals Section International Conference
24-29 May 2015, Lublin, Poland

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Preface

A. Börner

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The 16th International EWAC Conference was organized by Krzysztof Kowalczyk and his co-workers in Lublin, Poland from May 24 – 29. As already in 2011, the conference was jointly organized by EWAC and the Cereals Section of EUCARPIA, the 'European Association for Research on Plant Breeding'. Forty-seven participants from 14 countries came to Lublin in order to discuss their recent results on cereals genetics. During the opening session outstanding EWAC members were awarded on occasion of the approaching 50th anniversary of the co-operative. The awards f long standing cooperation in cereal genetics research and joint scientific projects implementation were passed to Andreas Börner, Sabina Chebotar, Matilda Ciuca, Gábor Galiba, Aurel Giura, Elena Khlestkina, Borislav Kobiljski, Viktor Korzun, Krzysztof Kowalczyk, Svetlana Landjeva, Colin Law, Danuta Miazga, Márta Molnár-Láng, Katerina Pankova, Thomas Payne, Tatyana Pshenichnikova and John Snape (alphabethical order).

During the conference 20 oral and 24 poster presentations were provided during four sessions:

- EWAC – The Story of Successful Cooperation
- Genetic Diversity *vs.* Plant Breeding
- Trait Evaluation and Genetic Mapping
- Wide Crosses, Physiology and Adaptation

In addition a workshop was organized by the Global Crop Diversity Trust entitled: 'The Expert Working Group on Wheat Genetic Resources'.

At the business meeting of the conference the participants discussed the possibility that EWAC may get the status of an EUCARPIA Working Group within the Cereals Section of the Association. It was agreed to use the EUCARPIA platform to increase the visibility of EWAC. Head and Deputy Head of the new Working Group will be Andreas Börner and Tatyana Pshenichnikova, respectively. Sylwia Okoń volunteered to become the Secretary.

For the next EWAC meeting three offers were made: Belarus, Bulgaria and Romania. After voting of the participants a clear decision was made for Romania. Matilda Ciuca agreed to organize the next EWAC Conference in Fundulea.

The local organisation of the conference was excellent. Many thanks to Krzysztof Kowalczyk and his team for preparing and running this successful conference in a very kind and friendly atmosphere. Everybody did enjoy the days in Lublin very much.

Now we are looking forward to the 17th EWAC Conference.

The history of EWAC and whatever happened to wheat aneuploids?

C. N. Law

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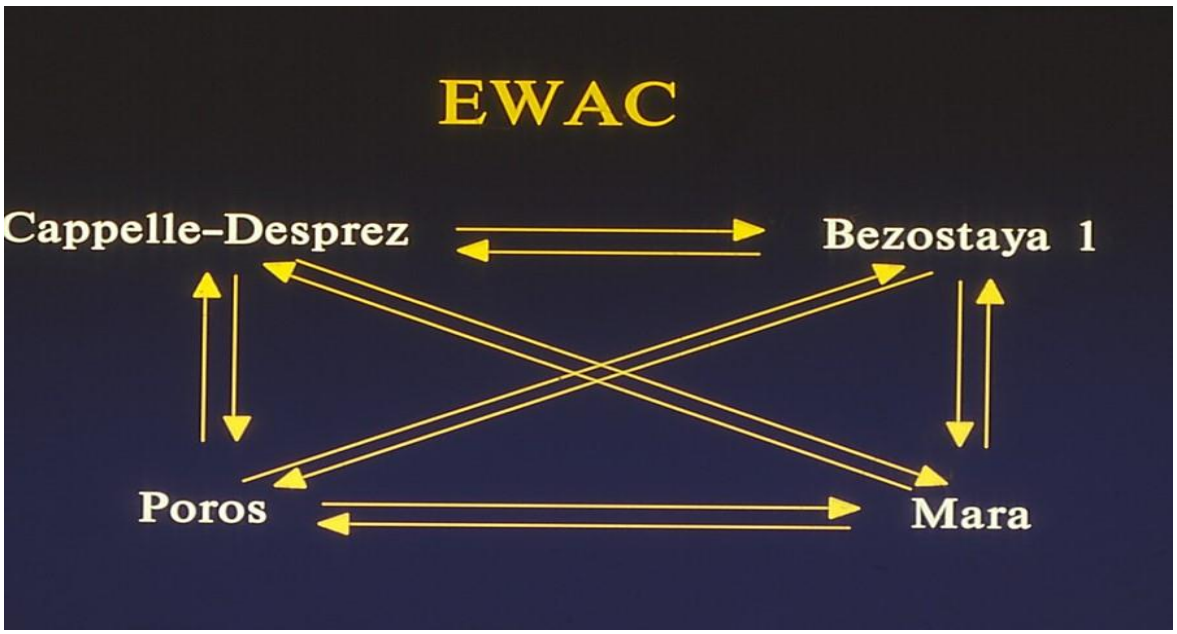
The whole idea of setting up a cooperative venture using wheat aneuploids was founded on the ground breaking work of Ernie Sears in the USA. He had assembled the full range of aneuploids in the variety Chinese Spring and had described their phenotypes in a publication "The Aneuploids of Common Wheat". This was published in 1954 and in many ways was regarded as our Wheat Cytogenetics Bible. Sears also showed how aneuploids could be used in genetic analysis to locate genes and to substitute chromosomes from another variety into Chinese Spring and in this way to carry out chromosome assays. This was seized upon by Kuspira and Unrau who used the substitution lines developed by Sears to do the first field trials on this kind of material, the results of which were published in 1957. It was the first attempt to correlate quantitative characters with chromosomes in a crop plant. It soon became clear - particularly in relation to wheat breeding objectives - that Chinese Spring was not a suitable background in which to conduct chromosome assays. This started the rush to develop monosomic series in more agronomically acceptable varieties using the existing Chinese Spring monosomics as a starting point in a lengthy backcross programme. It was at this point that I was employed at the Plant Breeding Institute in Cambridge to develop a monosomic series in the variety Cappelle-Desprez with the ultimate aim of substituting chromosomes from other varieties into it. This would take several years before I could get any results so I started substituting Cappelle-Desprez chromosomes into Chinese Spring and to using Sears' already existing substitution lines of Hope into Chinese Spring to explore what could be done with such material in investigating the genetics of agronomic characters. I suppose it was around this time that Ralph Riley and I became aware that there were monosomic series being developed in other countries within Europe. In many cases this seemed to be nothing more than insurance because there was no clear objective in mind. It was with these considerations in mind that the idea of setting up a cooperative programme within Europe began to emerge. I was sent on a European trip to gauge interest and whip up enthusiasm before launching a meeting in Cambridge in the summer of 1967. This was attended by 50 scientists from around Europe and included Ernie Sears (USA), J W Morrison (Canada) and I Watson (Australia) as observers.

To develop the complete set of monosomics in a currently leading variety and then to use these to create inter-varietal chromosome substitution lines was of course a formidable task involving the crossing and cytological screening of many lines over several generations. The choice of variety and donor variety was very important. Also, to be meaningful as a tool for wheat breeding then more than one variety would need to be chosen. This was the reason for the cooperative – to share the load across different laboratories in different countries.

Four what were called key varieties were chosen. These were Cappelle-Desprez, a very successful French bred variety grown widely in Western Europe at the time, Mara, an Italian wheat involving varieties bred by N. Strampelli in its parentage, Bezostaya 1, the very successful Russian variety bred by Lukyanenko and Poros, a variety developed in what was then East Germany.



The idea was to make single chromosome substitution lines between these varieties having first completed monosomic series in each of them. Moreover, because I was obsessed with identifying between chromosome interactions, the substitution lines would be made reciprocally. Each participating laboratory would thus be producing 63 substitution lines so that altogether 252 lines would be developed and be made available for screening. Quite an ambitious target!! For those developing monosomic series in other varieties, they were encouraged to use one of the key varieties as a donor in developing any substitution lines. This would have enabled the chromosome sampling to be more widely undertaken.

In the event, the aims of this programme proved to be far too ambitious - not surprising really considering the resources required and the need to maintain this effort over several years. The latter was particularly telling since the completion of the task would in nearly all cases be 10 years or more down the line. A time span which was much larger than the career expectations of the scientists involved so that in most cases they could be expected to move to other jobs well before completion was even in sight. The work at Cambridge was the exception, partly because the monosomic development of Cappelle-Desprez was almost complete at the outset. Here all three substitution sets were sufficiently advanced to commence screening for chromosome effects on field trials.

The development of the Cappelle-Desprez substitution lines did give rise to one important discovery and this was the identification of the genes originating from the Japanese variety Akagomughi. In the 1920s the Italian breeder Strampelli in seeking new sources of dwarfism and earliness, introduced this variety into his breeding programme and was successful in achieving both these objectives in the varieties he produced. Mara was one of the varieties emerging from hybrids involving Strampelli wheats. The study of the Cappelle-Desprez (Mara) substitution set revealed that two chromosomes 2D and a translocated chromosome 5BS-7BS gave reduced heights. Moreover, subsequent analysis showed that the 2D effect was due to two linked genes, one directly affecting height, *Rht8*, and the other indirectly affecting height due to its influence on flowering time. This was the gene, *Ppd1* for daylength insensitivity. Strampelli was thus doubly fortunate that his aim of reduced height and early maturity could be achieved by two closely linked genes on chromosome 2D.

Strampelli's wheats have had a major influence on breeding programmes throughout the world. They were introduced into South America where *Ppd1* has had a major influence. This is almost

certainly the origin of the *Ppd1* gene found in CIMMYT varieties – a crucial contribution to the success of the Green Revolution wheats. Ardito - also a Strampelli wheat - was introduced into Russian breeding programmes and was one of the parents of Bezostaya 1, another EWAC key variety and one of the most successful varieties bred in the last century. The presence of *Rht8* and *Ppd1* in this variety was verified from the study of the Cappelle-Desprez (Besostaya1) substitution lines. EWAC was thus the means by which this story was unravelled - a major success from just a small part of the cooperative programme set up in 1967.

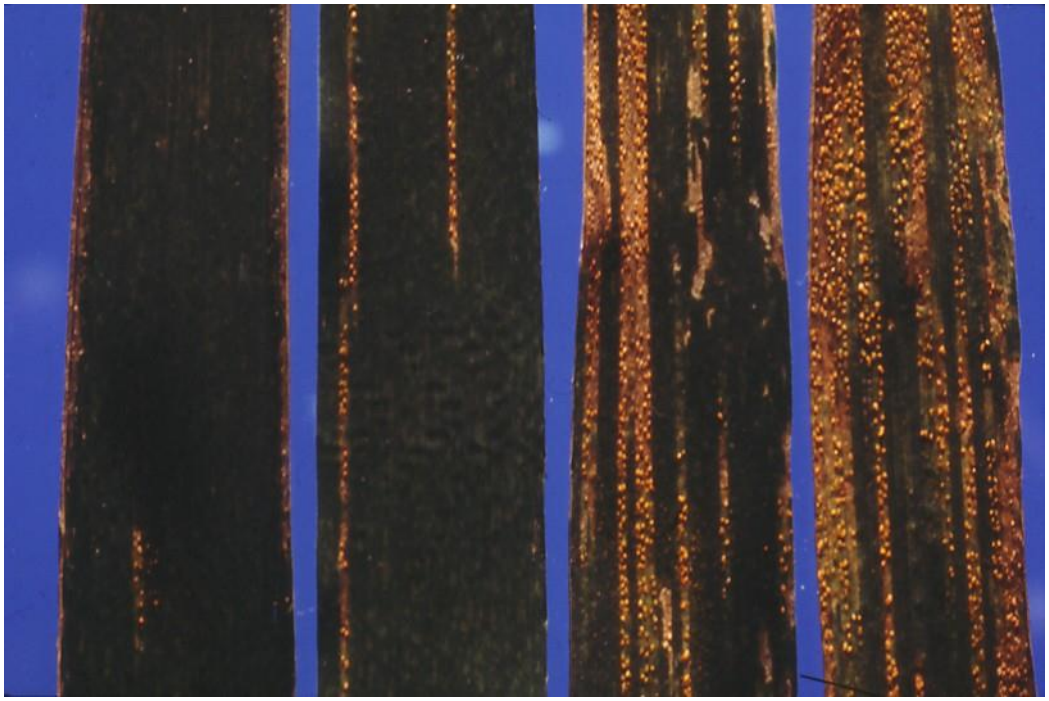
However, it is important to realise that the formation of EWAC created a network of laboratories, all of which were involved in wheat cytogenetics. This provided the basis of much collaborative work particularly in the exchange of material and information. This was a major benefit resulting from the formation of EWAC and, although techniques and the emphasis may have changed, is still evident today.

The paucity of developed substitution lines actually completed is of course a disappointment. This included the development of lines outside the four key varieties. There were some notable exceptions. Olga Maystrenko and her team in Novosibirsk completed monosomic and substitution lines in a number of varieties, for example in the important spring wheat, Saratovskaya 29. It must be acknowledged that there was much concern about the authenticity of some of the substitution lines. Several lines were shown to have undergone chromosome "shift" or "switch" and were incorrect. To answer these concerns three established sets of substitution lines were selected in 1998 for close scrutiny using molecular markers. Although the majority of the lines were as expected, several of the lines were shown to be wrong and needed to be re-established. Undoubtedly these errors have encouraged researchers to move away from using substitution lines even though many of the lines are known to be correct.

Many monosomic series were developed during the time that EWAC has been in being. At one count in 1988 at the 7th International Wheat Genetics Symposium, over 80 monosomic series had been developed or were being developed throughout the world. Whatever happened to all these lines? Many of them reside in seed banks in a number of European Countries and are probably no longer viable. This is a pity since they would have provided a useful resource for studying wheat genetics and cytogenetics.

It is an interesting exercise to have another look at the Wheat Cytogenetics Bible – "The aneuploids of common wheat" by Ernie Sears. He cites for example that all the chromosomes of group 2 carry genes for awn promoting. May be these are the same genes that we know on the group 2 chromosomes that affect plant height. The Bible is full of little snippets of information like this.

In developing monosomics and ditelosomics in Koga II, Bersee, Cappelle-Desprez and Hobbit we uncovered many examples where deficiencies of entire chromosomes or chromosome arms produced marked phenotypic effects. For instance, Koga II ditelosomic 7D^{L} turned out to be sterile due to asynapsis but Chinese Spring and Bersee 7D^{L}s were normal. Crossing these with Koga II monosomic 7D to produce hybrids and an F2 lacking 7D^{S} gave a 3: 1segregation indicating a single recessive gene for asynapsis. At least two genes were thus implicated in the control of chromosome pairing, one of which was found on the short arm of 7D.



Yellow rust infection on Cappelle-Desprez Euploid, Cap Ditelo 5BS, Cap Ditelo 7BS and Cap Nullisomic 5BS-7BS.

My favourite aneuploid result, partly because it has yet to be fully resolved and partly because of its potential importance in controlling disease in wheat, concerns the group 5 chromosomes Both Tony Worland and I along with contributions from Roy Johnson and David Pink spent a lot of time with this problem. We never completed it so I would dearly like someone to take it over and find the answers. May be some of you have already got the answers. It goes back to the early days of EWAC when monosomic series were just being developed. The varieties of Western Europe carry a reciprocal translocation involving chromosomes 5B and 7B to give two chromosomes with the configuration 5BL-7BL and 5BS-7BS. Separately and independently, reports came in that plants nullisomic for the smaller of this translocated chromosome, 5BS-7BS, were showing high levels of susceptibility to yellow rust in the varieties Vilmorin 27 and Caribo. At the same time we were finding similar results in the varieties Bersee and Cappelle-Desprez and later on in Hobbit sib. All these varieties carry the translocation and show varying degrees of adult plant resistance to yellow rust. Some could claim to have durability since the level of resistance to the disease had been maintained over many years. It was therefore thought that the 5BS-7BS effect might have something to do with durability. This was soon dispelled because the highly susceptible variety Hybride du Joncquois was shown to have an identical 5BS-7BS to those in Bersee and Cappelle-Desprez. The study of ditelosomics for 5BS and 7BS showed that the effect was due to an unknown number of genes for resistance on 5BS and not 7BS. The story then moved on to look at the group 5 chromosomes more generally, starting with the tetrasomics of Chinese Spring. We had for a long time noticed that in the glasshouse these tetrasomics were very susceptible to mildew. Even Ernie Sears in his Bible seems to have missed this. If these lines were exposed to yellow rust the same pattern emerged. Further studies demonstrated that this dosage effect was due to genes on the long arms. In other words the long arms carried genes promoting susceptibility whereas, in contrast, the short arms have the reverse effect and have genes promoting resistance. Moreover this pattern was identical for two very different types of pathogen. Could this be the generalised resistance that might be related to durability? Reduced dosage of the long arm as in monosomics confirmed that these were more resistant to both diseases. A very striking illustration of this was found in a chimeric plant of Chinese Spring mono-isosomic for 5AL in which part of the plant was tetrasomic for 5AL (di-isosomic for 5AL)

and was susceptible, another part was euploid (mono-isosomic 5AL) and partly susceptible, and yet another part was monosomic for 5A (nullisomic for 5A) and was resistant.

I have searched through reports on the inheritance of adult plant resistance to yellow rust and have never found any reference to genes on the short arm or indeed on the long arm. We know that there is allelic variation on the short arm because we have shown that substitutions of the 5BS arm from varieties lacking the translocation are susceptible. So I hope that someday someone will resolve this and come up with an answer. In the meantime we have tried to exploit these findings by trying to induce deletions in the long arms of the group 5 chromosomes. We await the results of these endeavours.

Chimeric plant of Chinese Spring, di-isosomic 5AL on the left, mono-isosomic 5AL in the middle and nullisomic 5A on the right.

So much for aneuploids and their potential for providing useful insights into the genetic structure of wheat.

Turning now to the work on alien introgression through wide crossing. Looking through previous EWAC Newsletters it would seem that this area of wheat genetics and cytogenetics is having a new lease of life. The expectation was that this approach would be overtaken by molecular genetic engineering but this has not been the case. An increasing number of projects are seeking to introduce new genes from the close and distant relatives of wheat using a variety of techniques. It is good to see that EWAC is helping these approaches through cooperation. As always, and this goes for molecular genetic engineering as well, the difficulty is to identify the useful genes from the bad in making transfers. There is also the difficult problem of selecting appropriate recombinants although the availability of many, many molecular markers should help this enormously. This should be a fruitful field for adding new genes to help in breeding the new varieties of the future.

There is one area of alien genetic variation that is often neglected and that is the cytoplasm. It is after all the home of the chloroplast and mitochondrion. Alloplasmic lines were developed mainly in Chinese Spring using a range of Aegilops donors. Most of this work was done in Japan or the USA in the 1970s. The motivation behind this effort was to exploit cytoplasmically induced male sterility for use in producing hybrid wheat. What was hardly considered was the profound effect that these alien cytoplasms had on a range of characters other than male sterility. For example, I can recall the major delay in flowering and concomitant increase in biomass in alloplasmic lines involving Ae.ovata cytoplasm. There were many other characters identified ranging from cold induced leaf variegation to striped leaves, all induced by particular

cytoplasms. Future research may find useful properties stemming from cytoplasmic variants which could be profitably exploited in breeding new varieties.

Finally, I must mention individuals who have contributed greatly to keeping EWAC alive and kicking. My former and late colleague, Tony Worland, took over the running of EWAC from me in its early days, ably assisted by Andreas Borner who was, and still is, very much involved in organising cooperative projects and meetings after Tony's untimely death. John Snape, Victor Korzun, and Tatiana Pshenichnikova have also been mainstays in organising and supporting EWAC. Looking through previous EWAC Newsletters the main thing that stands out is not just the stocks that have been created, important though they have been, but the number of cooperative ventures that have been undertaken. The number of isogenic lines carrying *Rht* genes that have been distributed around is extensive, as indeed are the assays of these genes in different backgrounds. It is however the European dimension that stands out. The identification of *Rht8, Ppd1, Rht1* and *2* and their roles in different parts of Europe is something that EWAC can be proud of, so please go on and find some more agronomically important genes. They are out there for sure but in so doing don't forget the aneuploids!

Forty years of cooperative research within EWAC in Poland

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Collaborative research of Institute of Plant Genetics, Breeding and Biotechnology with scientific centres across Europe within EWAC was initiated by professor Danuta Miazga. In early seventies of the 20th century she participated in a year-long internship at Plant Breeding Institute in Cambridge (currently John Innes Centre). Being there she started the research on wheat aneuploids cytogenetics, genes localization on chromosomes and the transfer of monosomics to Polish wheat cultivars 'Grana' and 'Luna' through crosses with aneuploid lines of 'Chinese Spring' and 'Cappelle-Desprez' (Miazga 1978, 1984; Miazga, Lipko 1982).

The collaborative research of Institute of Plant Genetics, Breeding and Biotechnology within EWAC can be divided into several periods. In the first period, the studies were focused on wheat aneuploids cytogenetics, the development of monosomics and chromosomal localization of genes by means of monosomic analysis. This period lasted from 1971 to 1995 (Miazga 1978; Cyran et al. 1996). The research conducted in the second period was a part of the European program concerning climatic adaptability of dwarfing genes and photoperiod insensitivity in wheat. This program was coordinated by Tony Worland up to 2001. This second period of research lasted from 1990 to 2007 (Kowalczyk et al. 1997a; Miazga et al. 1997; Kowalczyk et al. 2008a). During the third stage of collaboration the research was focused on the dwarfing genes in barley and the analysis of introgression lines. The study was carried out in co-operation with Andreas Börner from the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben in years 2005-2011 (Kowalczyk et al. 2008b, 2012). The fourth period includes the study of the effect of plant growth regulators application on transcript levels of genes involved in gibberellin biosynthesis pathway in common wheat and barley lines carrying different dwarfing genes. Moreover, by the inspiration of the research conducted within EWAC, in the Institute of Plant Genetics, Breeding and Biotechnology the study on identification of fungal disease resistance genes in wheat was performed. The study involved the analysis of host-pathogen interactions and the use of DNA markers. What is more, DNA markers for new dwarfing gene in triticale were developed and the study on *Vrn* genes in common wheat, triticale and barley was conducted (Kowalczyk et al. 2008c, 2008d; Nowak et al. 2012, 2014).

First works carried out by Professor Miazga within EWAC were focused on the analysis of monosomics. Genes controlling important quantitative traits were localized using a F_1 and F_2 hybrids of 'Grana' and 'Luna' cultivars with monosomic lines of 'Chinese Spring'. Conducted research revealed similarities, but also some differences, in chromosomal location of genes in those varieties. Genes controlling the protein level in 'Luna' were found to be recessive. Moreover, depending on the line the amino acids level was different. The most favourable changes occurred in 5D line. The development of the set of monosomic lines of 'Grana' cultivar successfully completed the research. The frequency of plants with 41 chromosomes in those lines was determined. It was concluded that lines belonging to the same homeologic group had usually similar frequency of monosomics. 'Grana' 5D monosomic line was used in hybridization in order to develop the substitution line carrying 5D chromosome from 'Atlas 66' variety. Based on the study it was found that the replacement of 5D chromosome in 'Grana' with 5D

chromosome from ‘Atlas 66’ did not significantly increase the protein content in ‘Grana’ variety (Miazga 1978; Miazga, Lipko 1982).

After the development of the set of monosomic lines of cv. ‘Grana’, the research focus on the creation of wheat lines with added rye chromosomes. The study resulted in the development of the complete set of wheat-rye addition lines ‘Grana’/‘Dańkowskie Złote’. It was the first achievement of this kind in Poland. By the use of addition lines, gene *An1* was located on 2R chromosome of ‘Dańkowskie Złote’ rye. This gene is crucial for the anthocyanin biosynthesis. Genes *Reg* and *Rog* controlling the shape and colour of kernels were located on 6RL chromosome, whereas gene *Alt* controlling aluminium tolerance in rye was positioned on 3RS chromosome. Genes responsible for secalin biosynthesis were located on 1R and 2R chromosomes. Moreover, a set of Grana-Dańkowskie Złote addition lines was used to reveal chromosomal location of genes controlling physical properties of kernels, the composition of polysaccharides and also to determine the α-amylase genetic regulation (Miazga, Chrząstek 1987; Chrząstek, Miazga 1988; Miazga et al. 1988).

Within EWAC Miazga and Petrovic (1987) studied the chromosomal structure of several wheat cultivars: ‘Grana’, ‘Cappelle-Desprez’, ‘Sava’, ‘Bezostaya 1’ and ‘Chinese Spring’. The authors put forward a hypothesis that cultivars differ from each other in the number of translocations. The study confirmed the hypothesis that translocations play an important role in the evolution of wheat at the level of differentiation of species into varieties. Further studies in this field performed by professor Miazga (1988) showed that in comparison to 'Chinese Spring', 'Grana' differs by one translocation between 2D and 7B.

Afterwards the research was focused on the analysis of substitution lines. The 'Chinese Spring/Cappelle-Despres' substitution lines obtained from Cambridge had been studied for two years in an international experiment. As a result, the genotype-environment interaction of substitution lines carrying different genes controlling vernalization and photoperiod sensitivity was determined (Law et al. 1974, Sutka et al. 1981, Worland et al. 1984). Moreover, the effect of chromosomes on the level of protein and amino acids in the protein fractions was also identified (Tarkowski, Otłowska-Miazga 1976; Tarkowski, Miazga 1979; Miazga, Chrząstek 1982; Miazga, Tarkowski 1983).

In another experiment the physical properties of stalk associated with lodging were analysed in ‘Cappelle Desprez/Bezostaya’ substitution lines. The genetic analysis of substitution lines and their parental genotypes showed that those traits were controlled by genes located on chromosomes: 1B, 1D, 2B, 3A, 3B, 5A, 6D and 7A. Lines 1D, 2B, 5A and 5BL-7BL and 5BS-7BS had significantly longer peduncle than the variety Cappelle-Desprez. The substitution of chromosomes 3A and 7A by chromosomes from Bezostaya resulted in shortening of peduncle. The substitutions of chromosomes 1B, 3B, and 6D from Bezostaya into ‘Cappelle-Desprez’ caused a significant increase in the outer diameter of the stem. As a result of this collaborative study several research papers were published (e.g. Doliński et al. 1996).

Lodging is an important factor limiting the yield of cereals, therefore the selection of high-yielding forms with short, stiff stems is of great importance. One of the best ways to obtain lodging-resistant cultivars is the introduction of dwarfing genes. However, not all of the dwarfing genes can be used in cereals breeding because some of them are associated with yield reduction. Pleiotropic effects of the GA-insensitive dwarfing genes were investigated using near isogenic lines of Maris Huntsman, Maris Widgeon, Mercia and Bezostaya. Isogenic lines with different dwarfing genes were kindly provided by A. Worland (John Innes Centre) at the beginning of 90’s. The research was carried out within European Program of Climatic Adaptation of Dwarfing Genes at Wheat which was coordinated by Tony Worland. Studies aimed to evaluate the pleiotropic effects of selected *Rht* alleles on plant height as well as yield

and its components, moreover to study climatic adaptation of these genes and possibility to utilize them in Polish conditions. Experiments were carried out over six growing seasons (1991-1996) in Czeslawice near Lublin. Plant height, number of days form 1st May to ear emergence, number of spikelets per spike, number of grains per ear, weight of grains in ear, 1000 kernel weight as well as spikelet fertility and grain yield per plot were analysed.

Pleiotropic effects of GA-insensitive dwarfing genes on yield and its components in common wheat was shown. The experiments revealed advantageous influence of *Rht-B1b, Rht-D1b, Rht-B1d* and *Rht-B1e* genes on yield and its components in common wheat grown in Poland. Reduction of 1000 kernel weight caused by these genes was fully made up by the higher fertility of spikelets and as a result the higher set of the grains in ears (Miazga et al. 1995; Kowalczyk et al. 1997a).

Using gibberellic acid, we tested Polish cultivars and breeding lines of common wheat involved in Polish Research Centre for Cultivar Testing (COBORU) experiments in 1992-1995, as well as isogenic lines 'Maris Widgeon' *Rht-B1b* and 'Maris Widgeon' *Rht-D1b*. After test completion, coleoptile length and seedling height was analyzed for studied forms. Among cultivars and breeding lines of winter wheat, only in four ('Elena', 'Parada', SMH 1693 and STH 594) as well as in five spring cultivars ('Broma', 'Henika', 'Polna', 'Santa' and 'Sigma') the presence of dwarfing genes insensitive to gibberellic acid were found. These forms reaction towards GA_3 was similar as in isogenic lines of 'Maris Widgeon' containing genes *Rht-B1b* or *Rht-D1b* (Kowalczyk et al. 1997b, 1999a; Miazga et al. 1998). In order to determine which dwarfing genes insensitive to exogenous gibberellic acid are present in Polish cultivars 'Elena' and 'Parada', I tested, using GA_3, F_2 hybrids of these cultivars with isogenic lines 'Maris Widgeon' *Rht-B1b* and 'Maris Widgeon' *Rht-D1b* as well as isogenic line 'Maris Widgeon' *rht* and 'Elena' and 'Parada' as controls. On the basis of achieved frequency distributions for coleoptile length and calculated values of χ^2-test for studied F_2 hybrids, it was found that in both studied cultivars *Rht-D1b* gene was present (Kowalczyk 1997b).

Genes *Rht-B1b, Rht-D1b, Rht-B1d* and *Rht-B1e* are worth recommendation for application in Polish common wheat breeding programs, due to their good adaptation and positive influence on yield and its components. Reduction of 1000 kernel weight caused by these genes is fully compensated by higher spikelet fertility, which in consequence cause setting more kernels in spike (Kowalczyk et al. 1999b, 2003a).

The next aim of research was analysis of allelic variation in *Xgwm 261* locus and identification of *Rht8* gene in new Polish common wheat cultivars. The highest allele frequency was observed for a 192 bp fragment. This fragment, linked to *Rht8* dwarfing gene, was observed in 6 modern spring cultivars: 'Bombona', 'Griwa', 'Hewilla', 'Histra', 'Kosma' and 'Napola'. Among winter wheat cultivars only two: 'Legenda' and 'Naridana' had 192 bp allele. The 165 bp DNA fragment, correlated with an increase in plant height, was observed in 7 winter wheat cultivars: 'Finezja', 'Fregata', 'Muza', 'Radunia', 'Rapsodia', 'Rywalka', 'Sława', 'Turnia'. The 174 bp allele neutral with respect to plant height was observed in 5 winter cultivars: 'Batuta', 'Izyda', 'Kobiera', 'Nadobna', 'Satyna'. This allele was present only in one spring cultivar 'Żura'. Moreover, in winter wheat cultivars 180 bp DNA fragment was observed in 'Ostka Strzelecka' and 198 bp in 6 cultivars: 'Bogatka', 'Nutka', 'Parabola', 'Smuga', 'Sukces', 'Tonacja' (Kowalczyk et al. 2008e; Kowalczyk, Okoń 2009). In Polish old cultivars registered before 1975, the highest frequency was observed for a 197 bp fragment in 8 cultivars (Biała Kaszubska, Biały Krzyż, Konstancja Granum, Konstancja Wierzbieńska, Litwinka, Niewylegająca, Wysokolitewska Ołtarzewska and Wysokolitewka Sztywnosłoma). Moreover, 165 bp fragments were observed in 7 cultivars (Antonińska Wczesna, Choryńska, Dańkowska Graniatka, Malwa, Sobieszyńska 44, Stieglera 22 and Strzelecka) and 174 bp fragments 7 cultivars (Balta, Bożena, Dana, Jana, Komorowska, Leszczyńska Wczesna and Olza) DNA fragments of size 203 bp were

present in cultivars: Bogatka, Halina, Jasnocha Włoszanowska, Płocka, Puławska Wczesna. 192 bp fragment size linked to *Rht8* dwarfing gene was observed in three cultivars: Aria, Grana and Luna. Pedigrees of these cultivars contain Etoile de Choisy cultivar from France (Kowalczyk 2006).

Another approach in lodging prevention involves the use of plant growth regulators. Plant growth regulators are widely used in cereal production in many countries over the world. They cause reduction of the length of the stem. In this way plant growth regulators increase resistance to lodging. Numerous studies show that common wheat varieties carrying dwarfing genes insensitive to gibberellic acid (GA_3) have disrupted biosynthesis of gibberellins. Few studies conducted in England showed that the application of CCC caused the reduction in yield in the lines carrying *Rht-B1b* and *Rht-D1b* genes. As wheat varieties carrying these genes are grown in Poland, I initiated the research, which aimed at determining the effect of chlormequat chloride and ethephon on yield components in isogenic 'Bezostaya' lines with different *Rht* genes. The value and changes in the correlation of some quantitative traits in those lines were also analysed. The research showed that the use of plant growth regulators causes the reduction in the plant height. The greatest reduction in the stem length was observed in the lines that primarily were the highest – namely in lines *Rht-D1a* and *Rht-D1b*. The study revealed that in order to reduce lodging in short-stemmed wheat forms it is better to use products containing ethephon, due to its beneficial effect on some yield components such as the number of kernels per spike and weight of kernels per spike (Kowalczyk et al. 2008f, 2009).

Barley is one of the most important crop plants and lodging is a serious problem in cultivation of this cereal. The reduction of stem length in barley is associated with the increase in plant yield. The dwarfing genes are used in barley breeding in order to shorten the plant height. The literature shows that many new cultivars contain *sdw1 (denso)* and *Gpert* genes which are sensitive to gibberellic acid. In the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben (Germany) dwarfing genes insensitive to GA_3 were also identified – that is *Dwf2* and *gai* (*Rht-H1*, *GA-ins*) genes. In cooperation with Andreas Börner a study was undertaken to identify GA_3 insensitive dwarfing genes in Polish cultivars of barley. The screening included 41 barley cultivars from Polish register, among which 8 have winter growth habit and 33 have spring growth habit. After GA test only 8 barley cultivars were found to be GA_3-insensitive. Their coleoptiles length did not differ significant from control form with *gai* gene. Among these cultivars Bażant, Bursztyn, Gil, Horus, Lomerit have winter growth habit and Atol, Rastik, Rodos have spring growth habit (Kowalczyk et al. 2008b).

Another research we prepared in co-operation with John Innes Centre. These research concerned pleiotropic effects on yield and yield components of lines with *Ppd* genes. Day length is one of the most important factors affecting the rate of wheat development. Genetic control of day length insensitivity is determined by many genes present on different chromosomes. Genes *Ppd-D1*, *Ppd-B1* and *Ppd-A1* localized on chromosomes of the second homeologous group (2D, 2B and 2A, respectively) have the major influence on this trait expression. In order to evaluate precisely the effects of genes *Ppd-A1*, *Ppd-B1*, *Ppd-D1*, the series of homozygous recombinant lines were created in John Innes Center, England. There were Avalon, Brigand, Brimstone, Cappelle-Desprez, Mercia, Norman and Randezvous. These lines were tested in several countries. Particularly wide research with *Ppd-D1* lines were made (Kowalczyk et al. 2003b, 2006). During experiments recombinant lines with *Ppd-D1* gene had accelerated ear emergence more than recombinant lines with *Ppd-B1* and *Ppd-A1* genes. The recombinant lines with *Ppd-D1* had accelerated ear emergence about 4 to 5 days during three years of experiments (it was depended on the year). The recombinant lines with *Ppd-A1* had accelerated ear emergence about 2 to 3 days and the recombinant lines with *Ppd-B1* had accelerated ear emergence only about one day in comparison to the control lines (Kowalczyk et al. 2003b, 2006, 2008a). The differences were significant between analyzed recombinant lines and their control. Plants of recombinant lines

with different *Ppd* genes were shorter than their control lines, but significant reduction of plant height as compared to control forms was found in recombinant lines Mercia containing genes *Ppd-D1*. On the basis of six-year study it was found that number of spikelets in spike, number and weight of kernels in spike as well as spikelet fertility in analyzed forms depended mainly on the year. No significant differences for these traits, were recorded between lines with *Ppd-A1*, *Ppd-B1* and *Ppd-D1* genes in comparison to their control forms (Kowalczyk et al. 2003b, 2008a). Another analysis performed with the use of CAPS markers revealed that *Ppd-B1* gene is present in the following Polish cultivars: Asta, Dańkowska Graniatka, Kaja, Konstancja Wierzbieńska, Konstancja Granum and Płocka (Okoń et al. 2012).

Vernalization requirement in barley is mainly controlled by three loci: *Vrn-H1*, *Vrn-H2* and *Vrn-H3*. In barley the *Vrn-H1* locus has been mapped on the long arm of chromosome 5H, the *Vrn-H2* locus in the distal part of chromosome arm 4HL and the *Vrn-H3* locus on chromosome 1H (Laurie et al. 1995). Gene *Vrn-H2* is dominant for winter growth habit, whereas *Vrn-H1* and *Vrn-H3* are dominant for spring growth habit (Dubcovsky et al. 2005). The alleles for spring and winter habit are epistatic thus the only vernalization-responsive genotype is *vrn-H1*, *Vrn-H2*, *vrn-H3*. All other combinations reveal spring growth habit (Karsai et al. 2005, Dubcovsky et al. 2005). Allelic variations at the *Vrn-H3* locus occurs mainly in barleys from high or low latitudes (Yasuda et al. 1993), therefore to determine growth habit for most cultivars a two-locus model is sufficient (Yasuda et al. 1993, Laurie et al. 1995, Karsai et al. 2005). The identification of *Vrn* genes in Polish barley cultivars with the use of STS markers was conducted. The dominant *Vrn-H2* allele was observed in winter cultivars: Bażant, Bursztyn, Horus, Gil and spring cultivars: Boss, Bryl, Edgar, Rabel, Rastik, Refren, Rodos. The recessive *vrn-H2* allele was observed in spring cultivars Atol, Binal, Blask, Granal, Lot, Nadek, Nagrad, Poldek, Rasbet, Rataj, Rodion, Ryton and Start. (Kowalczyk et al. 2008d).

The determination of flowering time in wheat is controlled by three major groups of genes: photoperiod response genes (*Ppd*), vernalization response genes (*Vrn*) and developmental rate genes (*Eps*) (Snape et al. 2001). *Vrn* genes have been mapped on the long arms of chromosomes 5A, 5B and 5D (Tóth et al. 2003) and designated as *Vrn-A1*, *Vrn-B1* and *Vrn-D1*. Dominant alleles of these genes are inhibitors of vernalization requirement. The dominant allele of *Vrn-A1* completely inhibits vernalization requirement, dominant *Vrn-B1* and *Vrn-D1* inhibit it partially (Košner and Pánková 1998). We used molecular markers for identification of *Vrn-B1* alleles in 20 Polish wheat cultivars (10 have spring growth habit and 10 have winter growth habit). Our results confirmed presence of dominant *Vrn-B1* allele in spring cultivars: Hewilla, Histra, Kosma, Monsun, Triso, Zebra and Żura. Dominant *Vrn-B1* allele was found also in winter cultivars Parabola and Radunia. The lack of 709-bp DNA fragment in winter cultivars: Alkazar, Finezja, Fregata, Nutka, Rywalka, Satyna, Smuga and Tonacja confirmed recessive character of *vrn-B1* alleles in this forms. Recessive alleles *vrn-B1* were found also in spring cultivars: Bombona, Bryza and Rubens (Kowalczyk et al. 2008c).

Moreover, in cooperative with Leibniz Institute in Gatersleben lines carrying the segments of chromosomes from D genome were investigated. Experiments were carried out over seasons 2006-2008. During all three seasons the heading time of analyzed introgressive lines were significantly different than noticed for Chinese Spring. The earliest were introgressive lines with 2D and 7D chromosome segments and the latest were 5D. Plant height of introgressive lines depended on the year, but the shortest were 1D, 6D and 7D lines. More differences were found in number of kernels from spike. In 2006 year 1D, 2D, 3D and 6D lines set more kernels in spike in comparison to control Chinese Spring. Value of this trait in 2008 year for some lines was similar to control cultivars. All introgressive lines had similar spikelet fertility and low value of 1000 grains weight (Kowalczyk et al. 2012).

The Bezostaya wheat isogenic lines with different *Rht* genes developed in John Innes Centre and barley lines obtained from Leibniz Institute in Gatersleben were used in the research analysing the transcript levels of selected genes encoding enzymes involved in gibberellin biosynthesis pathway. The real-time PCR (qPCR) method was used to analyse the expression of genes encoding ent-kaurene synthase (KS), ent-kaurenoate oxidase (KAO), GA20-oxidase (GA20ox), GA3-oxidase (GA3ox), GA2-oxidase (GA2ox), ent-copalyl diphosphate synthase (CPS), ent-kaurene oxidase (KO) and the GID1 gene encoding GIBBERELLIN INSENSITIVE DWARF1 receptor (Nowak et al. 2012).

The results obtained from the study indicate clearly the linkage between the presence of dwarfing genes in cereals genome and the expression levels of genes involved in gibberellin biosynthesis. The profiles of expression obtained in both wheat isogenic line carrying *Rht12* gene that is sensitive to GA_3 and wheat isogenic line without dwarfing gene were often similar. When it comes to genes insensitive to gibberellic acid: similar profiles of expression were frequently observed in lines carrying *Rht-B1b* and *Rht-B1e* genes, whereas the profile of expression in *Rht-B1d* line was different. In barley similar reactions were observed in control variety Morex and the Triumph variety carrying *sdw1* gene.

The analysis of the effect of plant growth regulators on expression profiles of selected genes involved in gibberellin biosynthesis was also performed. The study conducted in wheat revealed that in most cases the plant response to the application of chlormequat chloride and ethephon was similar, while the use of trinexapac ethyl resulted in different profile of expression. In barley similar profiles of expression were observed for CCC and trinexapac ethyl, whereas the application of ethephon usually caused different changes in the expression of analysed genes. These results indicate the species-specific character of molecular response to the application of plant growth regulators. The application of plant growth regulators showed to have an influence on molecular mechanisms associated with gibberellin biosynthesis. The short-stemmed phenotype caused by the application of plant growth regulators generally associated with the changes on the biochemical level may also be the result of the changes in the functioning of plant genome.

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EWAC - the past 25 years (1991 – 2015)

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This presentation is a personal review about 25 years of co-operation on cereals research. A period between 1991 when the 8th EWAC Conference was held in Cordoba, the first one I did attend, and 2015 the year of the 16th EWAC Conference in Lublin will be considered. We will give examples for 25 years of successful co-operation within the frame of EWAC. Genetic studies for a range of different traits are presented.

In 1991 already series of genetic stocks including monosomics, chromosome substitution lines, alloplasmic lines, single chromosome recombinant lines, introgression lines, isogenic lines etc. were available. At the Conference in Cordoba we presented co-operative studies on the genetics of plant height including the investigation of pleiotropic effects of near isogenic *Rht* lines grown in the UK and Germany. Four complete sets of lines isogenic for *rht* (tall), *Rht1*, *Rht2*, *Rht3*, *Rht1+2*, and *Rht2+3* in the genetical backgrounds of the cultivars 'April Bearded', 'Berssee', 'Maris Huntsman' and 'Maris Widgeon' were investigated. The effects of *Rht* alleles on plant height obtained in Germany are given in figure 1. The height reducing effects of the *Rht* genes had the same gradation over all backgrounds (*rht* < *Rht1* < *Rht2* < *Rht1+2* < *Rht3* < *Rht2+3*). The most consistent pleiotropic effect of the *Rht* genes was to increase the number of grains per ear (Worland et al. 1992, Börner et al. 1993).

Another early collaborative study between the John Innes Center (UK) and the IPK (Germany) was performed using the tetrasomic lines of 'Chinese Spring' (CS). The lines of the homoeologous groups 2, 5 and 7 of CS together with the euploid standard were screened at the seedling stage for sensitivity to exogenously applied gibberellic acid (GA_3). Whilst the seedling length of lines tetrasomic for group 2 chromosomes were taller and those for chromosomes 5A, 5D and 7D shorter in both treatments (with and without GA_3) compared to the euploid control, tetrasomics for chromosomes 5B, 7A and 7B were shorter than the euploids in the GA variant only (Fig. 2). It was suggested that these chromosomes are carrying genetic factors for GA insensitivity (Börner et al. 1992).

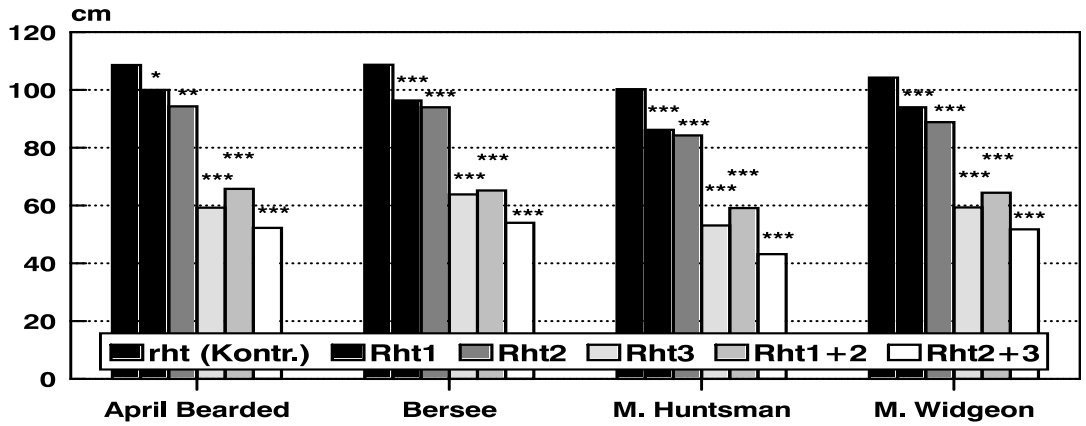

Fig. 1: Height reducing effects of *Rht* alleles in near isogenic lines of 'April Bearded', 'Bersee', 'Maris Huntsman' and 'Maris Widgeon' grown in Germany (Börner et al. 1993).

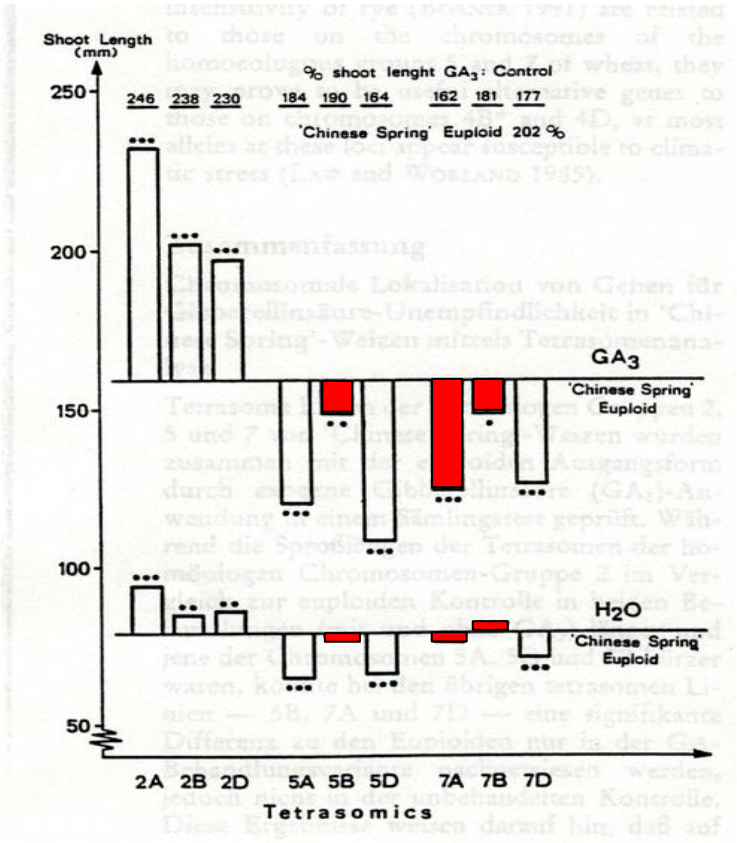

Fig. 2: Differences in seedling lengths of GA_3 treated (above) and untreated (below) tetrasomic lines from 'Chinese Spring' compared to the euploid control. The red bars indicate the lines with a significant difference to the euploids in the GA_3 variant only (Börner et al. 1992).

In the early 1990s molecular marker techniques were developed. In order to assign the markers to certain chromosomes cytogenetic stocks (mainly nulli-tetrasomic lines) became an important tool (Plaschke et al. 1995). On the other hand molecular markers were used to confirm the authenticity of cytogenetic stocks. Inter-varietal chromosome substitution lines were checked using microsatellite markers (Korzun et al. 1997; Pestsova et al. 2000, Salina et al. 2003).

Molecular markers were intensively used for the mapping of major genes but also quantitative trait loci. The knowledge gathered from the stock investigations became very useful and often was the pre-requisite for a precise mapping of the target loci on certain chromosomes or chromosome arms. Molecular markers were also used to develop new precise genetic stocks. Pestsova et al. (2001, 2006) developed introgression lines based on the 'CS/Synthetic 6x' D genome substitution lines. The parental 'Synthetic 6x' had been obtained in the 1940s by McFadden and Sears (1947) from a cross of tetraploid emmer and *Aegilops tauschii*. Thus, in the set of substitutions for the D-genome, individual chromosomes of *Ae. tauschii* replaced the homologous chromosomes of CS. Through repeated backcrossing to the recurrent parent CS and microsatellite markers selection 84 'CS/*Ae. tauschii*' introgression lines were developed (Fig. 3).

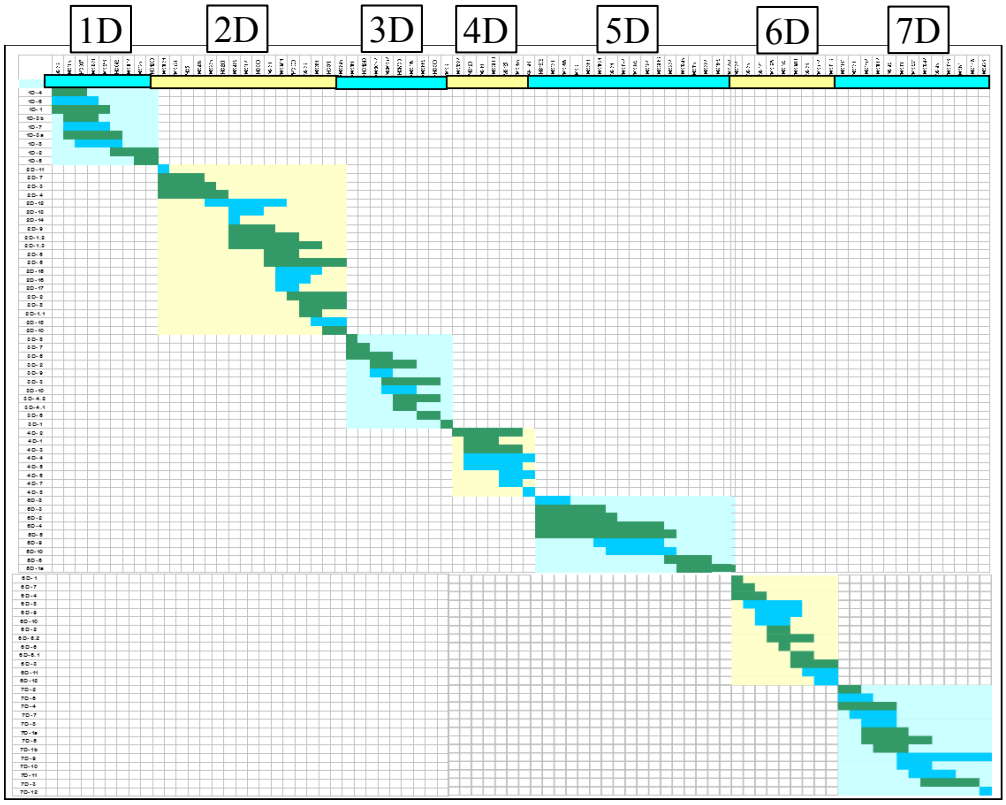

Fig. 3: Set of 84 'CS/*Ae. tauschii*' introgression lines covering the seven D genome chromosomes

A subset of these introgression lines, the thirteen chromosome 7D introgression lines were used to study the resistance to Septoria tritici blotch in a collaborative project between Argentina and Germany (Simon et al. 2007). Chromosome 7D was identified to be resistant against two virulent Argentinean isolates of the disease by analysing the set of 'CS/Synthetic 6x' single chromosome substitution lines (Simon et al. 2001, 2005). Results of the introgression line analysis are given in figure 4. Considering the introgressions being significantly different to 'CS' in at least two (light blue bars) or even four (dark blue bars) independent experiments it is clearly indicated, that the disease resistance locus acting at the seedlings and adult plant stages is present in the centromeric region of the short arm of chromosome 7D. Because the position of the locus detected was highly comparable with that, described by Arraiano et al. (2001) we concluded that the major gene *Stb5* was tagged causing resistance against isolates from Europe (Portugal, the Netherlands) but also from South America (Argentina).

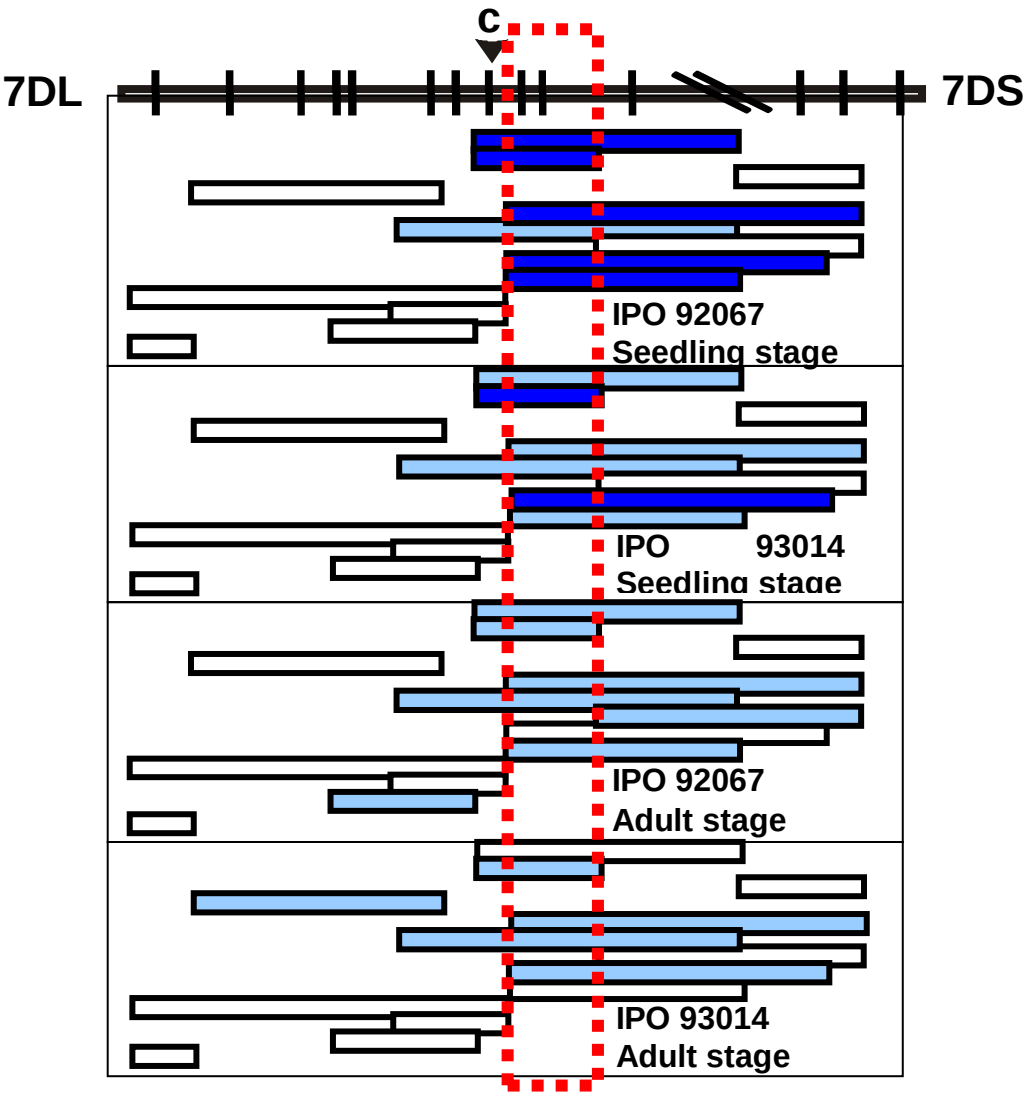

Fig. 4: Wheat/*Ae. tauschii* chromosome 7D introgression lines inoculated with septoria tritici blotch isolates IPO 92067 and IPO 93014 at seedlings and adult plant stages. Lines significant different to 'CS' in at least two or even four independent experiments are given in light blue and dark blue colour, respectively. Box in broken red line indicate the position of the resistance locus. L = long arm, S = short arm, C = centromere position.

The same set of lines has been used in a collaborative study between Bulgaria and Germany investigating the traits germination, seed vigour and longevity, and early seedling growth. Seed germination was characterized by a standard germination test (Landjeva et al. 2010). To evaluate for vigour the seeds were exposed for 72 h at 43°C and high (ca. 100%) humidity. Seed longevity was evaluated from the relative trait values. Seedling growth was assessed both under non-stressed and under osmotic stress conditions. QTL were mapped to chromosomes 1D, 2D, 4D, 5D, and 7D. Most of the QTL for germination clustered on chromosome 1DS in the region *Xgwm1291–Xgwm337* (Fig. 5). Chromosome 7DS harboured loci controlling the development of normal seedlings. Seed vigour-related QTL were present on chromosome 5DL linked to *Xgwm960*. QTL for seed longevity were coincident with those for germination or seed vigour on chromosomes 1D or 5D. Finally QTL for seedling growth were identified on chromosomes 4D and 5D.

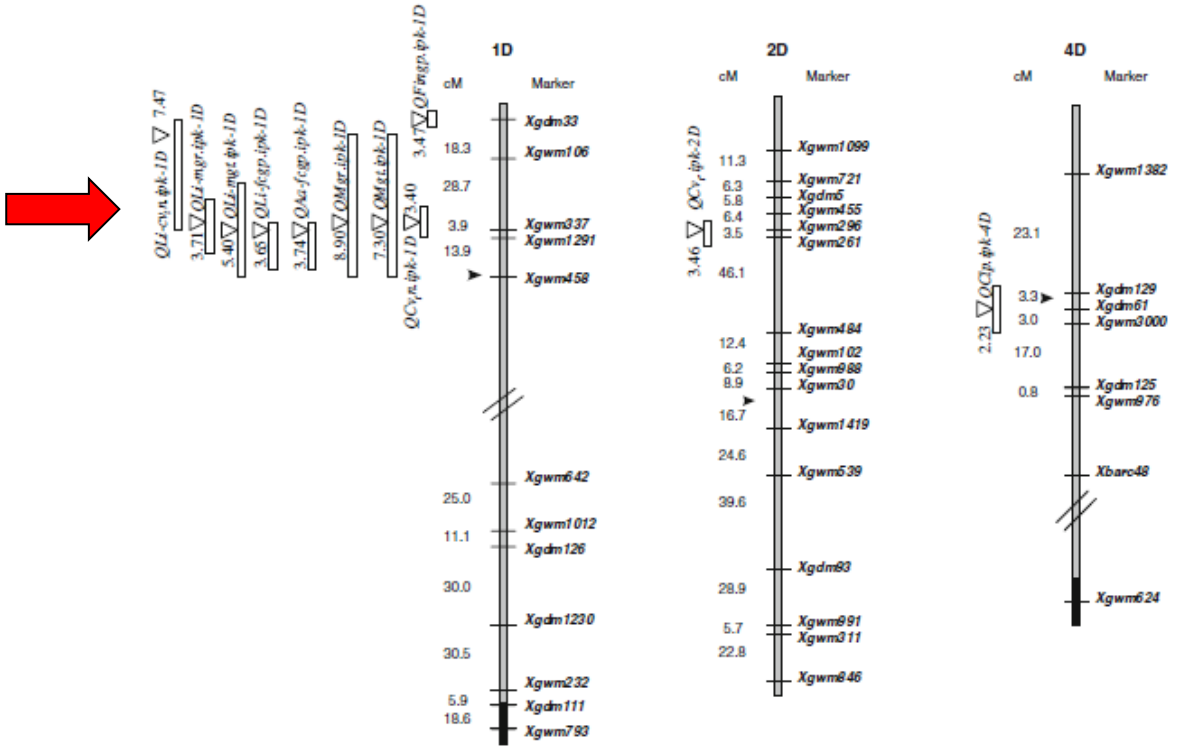

Fig. 5: Genetic mapping of QTL for seed and seedlings traits. Bars indicate major genomic regions defined by the interval mapping analysis. White arrowheads show the position of the maximum LOD. The position of centromeres is marked by black arrowheads (Landjeva et al. 2010).

Further studies exploiting these lines for agronomic traits were performed in the frame of a joint Polish–German research. Between 2006 and 2008 the material was grown in Czeslawice (Poland) and analysed for the agronomic traits ear emergence, plant height, number of kernels per spike, weight of kernels per spike and 1000 grain weight. Details are given by Kowalczyk et al. (2012).

A co-operation between Romania, Russia and Germany was established in order to map a gene for flowering time on chromosome 7B. In an earlier study of 'Favorit/F26-70' substitution lines Giura and Ittu (1986) identified chromosomes 4B and 7B of 'F26-70' to flower three days earlier compared to 'Favorit' (Fig. 6). In consequence a set of wheat single chromosome (7B) recombinant lines was developed from the cross 'Favorit' x 'Favorit/F26-70 7B'. The lines were genotyped and grown with and without vernalisation under short and long daylengths (Khlestkina et al. 2009). It became obvious (Fig. 7) that the flowering time gene on chromosome 7B was a new photoperiod response gene designated *Ppd-B2*. It was mapped on the short arm of chromosome 7B, 8.8 cM distal and 20.7 cM proximal to microsatellite markers *Xgwm0537* and *Xgwm0255*, respectively. In contrast to the *Ppd-1* genes on the group 2 chromosomes, which are expressed under short day conditions, *Ppd-B2* was detectable only when the plants were exposed to long photoperiod. Earliness in flowering time due to *Ppd-B2* was correlated with an increase in grain protein content and a major gene for this character was mapped 4.4cM proximal to *Ppd-B2* (Fig. 7). This gene designated *Gpc-B2* (*Grain protein content – B2*) does not affect grain size and is therefore probably involved in nitrogen uptake and/or translocation.

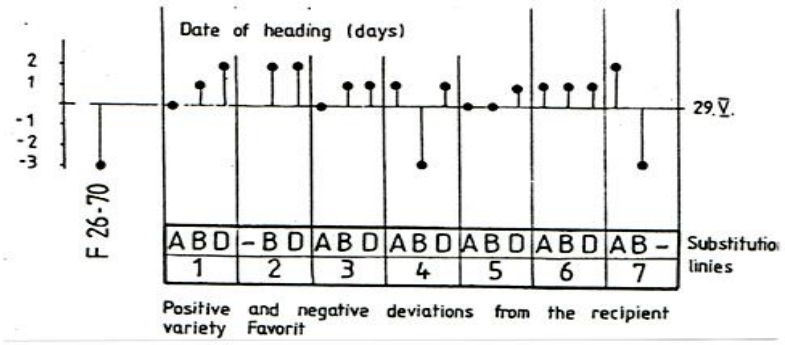

Fig. 6: Analysis of 'Favorit/F26-70' substitution lines for flowering time in comparison to 'Favorit' Giura and Ittu 1986)

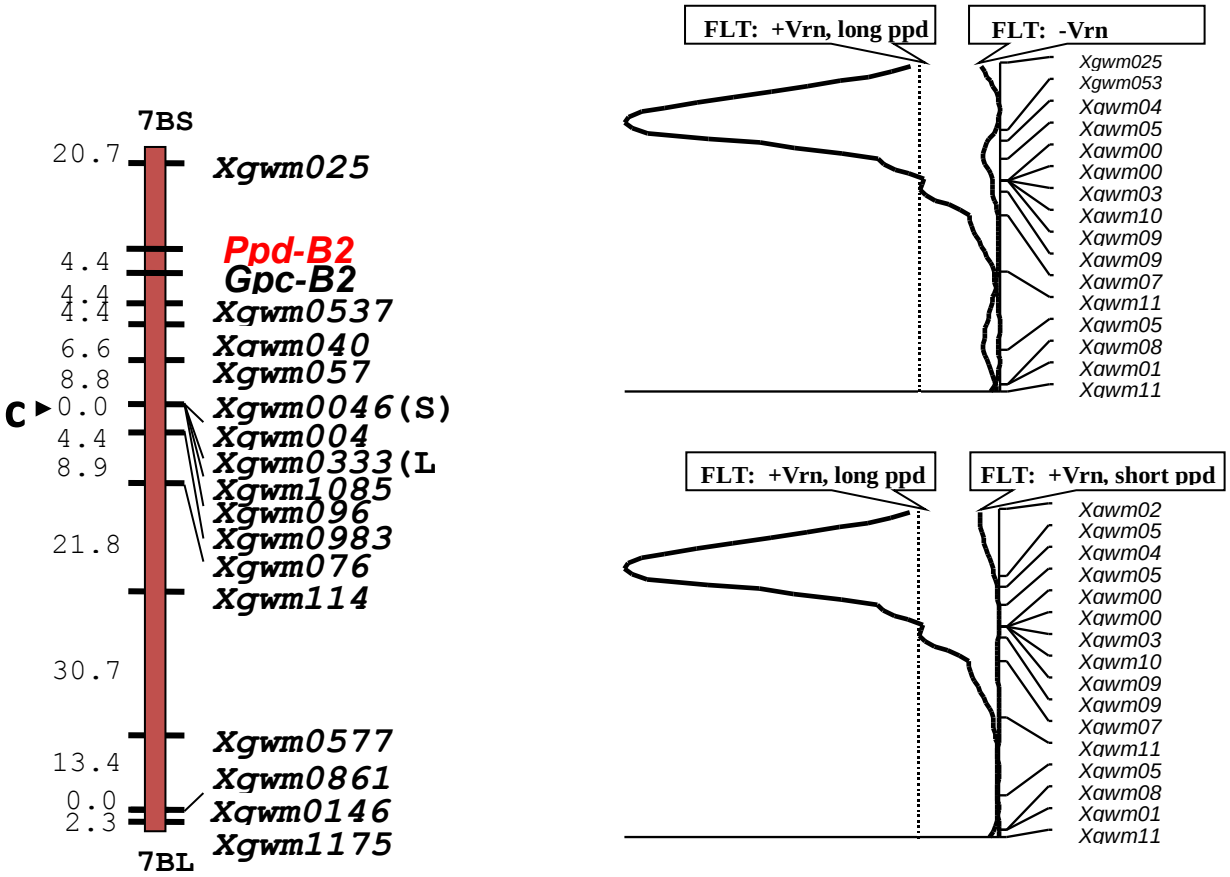

Fig. 7: Genetic map of chromosome 7B showing positions of *Ppd-B2* and *Gpc-B2* (left); QTL interval mapping for flowering time under different environments with respect to vernalisation and photoperiod (right) (Khlestkina et al. 2009).

Resistance to abiotic stress is an important target in wheat breeding. Among the stresses, drought plays an important role. However, drought tolerance is a very complex trait. In order to elucidate the genetics behind the trait the set of 'CS/Synthetic 6x' substitution lines was investigated under drought stress in a Russian-German collaborative project. The reaction to drought stress was quantified, based on grain yield components. Enhanced drought tolerance was associated with the presence of the 'Synthetic 6x' chromosomes 1A, 5A, 1D, 3D, 5D and 6D, and enhanced

susceptibility with chromosomes 3A, 4B and 7D (Osipova et al. 2001a). In addition the 'CS/Synthetic 6x' substitution lines were used to identify the relationship between dehydroascorbate reductase (DHAR) and catalase (CAT) activities in leaves of wheat plants and the stability of yield components under water deficit. The lines carrying chromosomes 1B, 1D, 2D, 3D or 4D all expressed a low constitutive level of DHAR (Fig. 8) and the lines carrying chromosomes 3B, 1D, 2D and 3D a low constitutive level of CAT. All were able to increase this level (by fourfold for DHAR and by 1.5-fold for CAT) in response to stress caused by water deficit. It was concluded that the discovered genetic variability for enzymes activity in leaves of wheat might be a useful selection criterion for drought tolerance (Osipova et al. 2011b).

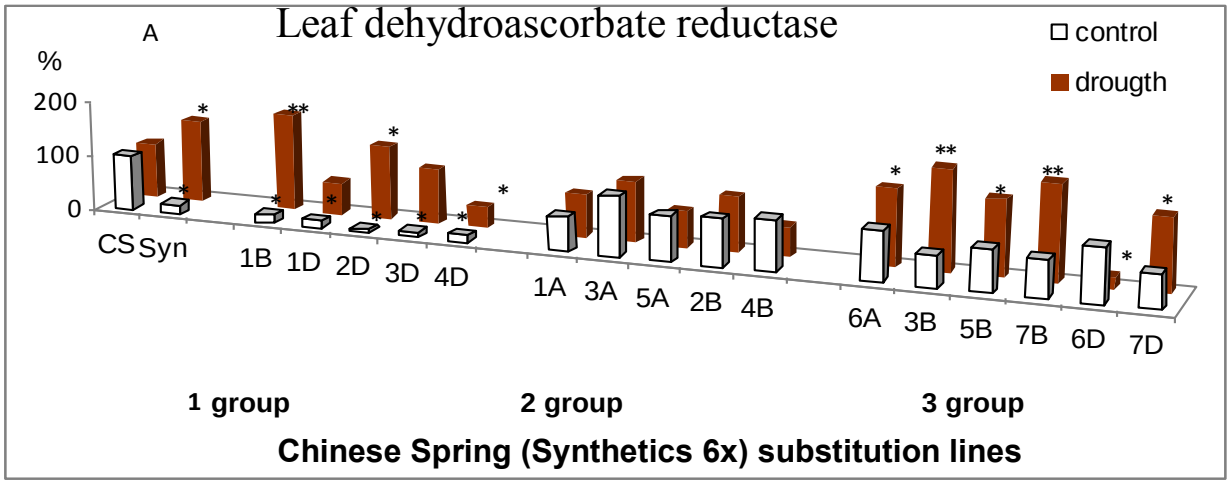

Fig. 8: Relative leaf DHAR activity in the 'CS/Synthetics 6x' lines and their parents at tillering stage (in percentage to the recipient CS.). *P<0.01; **P<0.001, compared to CS

In the present report only a few samples chosen more or less randomly are described in more detail. However, many further joint investigations were performed on different traits with different partners during the last years. A selection is listed in table 1.

Table 1: Selected publications resulting from collaborative activities with the co-authorship of this paper in five years slices.

Authors (Collaborators)	**Title (Topic of collaboration)**	**Journal**
1991 - 1995		
BEN AMER IM, WORLAND AJ, BÖRNER A	*In vitro* culture variation of wheat and rye caused by genes affecting plant growth habit *in vivo*.	Euphytica 61 (1992) 233-240
BEN AMER IM, WORLAND AJ, BÖRNER A	Chromosomal location of genes affecting tissue culture response in wheat.	Plant Breed 114 (1995) 84-85
PLASCHKE J, KORZUN V, KOEBNER RMD, BÖRNER A	Mapping of the GA_3-insensitive dwarfing gene *ct1* on chromosome 7R in rye.	Plant Breed 114 (1995) 113-116
PLASCHKE J, BÖRNER A, XIE DX, KOEBNER RMD, SCHLEGEL R, GALE MD	RFLP-mapping of genes affecting plant height and growth habit in rye.	Theor Appl Genet 85 (1993) 1049-1054
SAGI H, BÖRNER A, BARTOK T, SAGI F	Genetic identification, agronomic performance and technological quality of tissue culture-induced dwarfs of the Mv4 winter wheat.	Cereal Res Commun 21 (1993) 309-315
WORLAND AJ, SAYERS EJ BÖRNER A	The genetics and breeding potential of *Rht12*, a dominant dwarfing gene in wheat.	Plant Breed. 113 (1994) 187-196

1996 - 2000

BEN AMER IM, WORLAND AJ, BÖRNER A	The effects of whole chromosome substitutions differing in alleles for hybrid dwarfing and photoperiodic sensitivity on tissue culture response in wheat.	Euphytica 89 (1996) 81-86
BEN AMER IM, KORZUN V, WORLAND AJ, BÖRNER A	Genetic mapping of QTL controlling tissue culture response on chromosome 2B of wheat (*Triticum aestivum* L.) in relation to major genes and RFLP markers.	Theor Appl Genet 94 (1997) 1047-1052
BÖRNER A, CHEBOTAR S, KORZUN V	Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long term maintenance.	Theor Appl Genet 100 (2000) 494-497
BÖRNER A, KORZUN V, WORLAND AJ	Comparative genetic mapping of mutant loci affecting plant height and development in cereals.	Euphytica 100 (1998) 245-248
BÖRNER A, PLASCHKE J, KORZUN V, WORLAND AJ	The relationships between dwarfing genes of wheat and rye.	Euphytica 89 (1996) 69-75
FLINTHAM JEF, BÖRNER A, WORLAND AJ, GALE MD	Optimising wheat grain yield: effects of *Rht* (gibberellin-insensitive) dwarfing genes.	J Agric Sci 128 (1997) 11-25
KORZUN V, MELZ G, BÖRNER A	RFLP mapping of the dwarfing (*Ddw1*) and hairy peduncle (*Hp*) genes on chromosome 5 of rye (*Secale cereale* L.).	Theor Appl Genet 92 (1996) 1073-1077
KORZUN V, MALYSHEV S, VOYLOKOV A, BÖRNER A	RFLP based mapping of three mutant loci in rye (*Secale cereale* L.) and their relation to homoeologous loci within the Gramineae.	Theor Appl Genet 95 (1997) 468-473
KORZUN V, PLASCHKE J, BÖRNER A, KOEBNER R	Differences in recombination frequency between male and female gametogenesis in rye, *Secale cereale* L.	Plant Breed 115 (1996) 122-124
KORZUN V, RÖDER M, WORLAND AJ, BÖRNER A	Mapping of the dwarfing (*Rht12*) and vernalisation response (*Vrn1*) genes in wheat by using RFLP and microsatellite markers.	Plant Breed 116 (1997) 227-232
KORZUN V, MALYSHEV S, KARTEL N, WESTERMANN T, WEBER WE, BÖRNER A	A genetic linkage map of rye (*Secale cereale* L.).	Theor Appl Genet 96 (1998) 203-208
SALINA E, BÖRNER A, LEONOVA I, KORZUN V, LAIKOVA L, MAYSTRENKO O, RÖDER MS	Microsatellite mapping of the induced sphaerococcoid mutation genes in *Triticum aestivum*.	Theor Appl Genet 100 (2000) 686-689
WORLAND AJ, BÖRNER A, KORZUN V, LI VM, PETROVIC S, SAYERS EJ	The influence of photoperiod genes to the adaptability of European winter wheats.	Euphytica 100 (1998) 385-394

2001 - 2005

ALAMEREW S, CHEBOTAR S, HUANG X, RÖDER MS, BÖRNER A	Genetic diversity in Ethiopian hexaploid and tetraploid wheat germplasm assessed by microsatellite markers.	Genet Res Crop Evol 51 (2004) 559-567
BALINT AF, KOVACS G, BÖRNER A, GALIBA G, SUTKA J	Substitution analysis of seedling stage copper tolerance in wheat.	Acta Agron Hung 51 (2003) 397-404
BÖRNER, A. and A. J. WORLAND:	Does the Chinese dwarf variety 'XN0004' carry *Rht21*?	Cereal Res Commun 30 (2002) 25-29
CASTRO AM, VASICEK A, MANIFESTO M, GIMÉNEZ DO, TACALITI MS, DOBROVOLSKAYA O, RÖDER MS, SNAPE JW, BÖRNER A	Mapping antixenosis genes on chromosome 6A of wheat to greenbug and to a new biotype of Russian wheat aphid.	Plant Breed 124 (2005) 229-233
CHEBOTAR S, RÖDER MS, KORZUN V, BÖRNER A	Genetic integrity of *ex situ* genebank collections.	Cell Mol Biol Lett 7 (2002) 437-444
CHEBOTAR S, RÖDER MS, KORZUN V, SAAL B, WEBER WE, BÖRNER A	Molecular studies on genetic integrity of open pollinating species rye (*Secale cereale* L.) after long term genebank maintenance.	Theor Appl Genet 107 (2003) 1469-1476
KHLESTKINA EK, PESTSOVA EG, RÖDER MS, BÖRNER A	Molecular mapping, phenotypic expression and geographical distribution of genes determining anthocyanin pigmentation of coleoptiles in wheat (*Triticum aestivum* L.).	Theor Appl Genet 104 (2002) 632-637
KHLESTKINA EK, RÖDER MS, EFREMOVA TT, BÖRNER A, SHUMNY VK	The genetic diversity of old and modern Siberian varieties of common spring wheat determined by microsatellite markers.	Plant Breed 123 (2004) 122-127
KHLESTKINA EK, HUANG XQ, QUENUM FJ-B, CHEBOTAR S, RÖDER MS, BÖRNER A	Genetic diversity in cultivated plants – loss or stability?	Theor Appl Genet 108 (2004) 1466-1472
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MALYSHEV S, KORZUN V, EFREMOVA TT, BÖRNER A	Inheritance and molecular mapping of a gene determining vernalisation response in the Siberian spring rye variety 'Onokhoyskaya'.	Cereal Res Commun 29 (2001) 259-265

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QTL analysis of response to water deficit in D-genome introgression lines of bread wheat

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Drought is a most serious abiotic stress affecting crop productivity which is caused by insufficient or irregular precipitations followed by soil water deficit (Fleury et al. 2010, Richards et al. 2010). The future climate change predictions are not favorable. The worldwide drought occurrence has become more and more probable while the earth population is quickly growing up (Lobell et al. 2011). Water will become a limiting factor for sustained production of wheat – one of the most important food crops. Therefore, the need for intensive investigations of genetic basis of drought tolerance is well understood among scientists. At the same time, the multigenic nature of response to drought makes this task very difficult. The physiological dissection of such a complex trait is a first step to understand the genetic control of tolerance (Mir et al. 2012). Many investigations have been fulfilled all over the world using mapping populations and association panels. Several drought related traits, such as canopy temperature depression, carbon isotope discrimination and traits related to water use efficiency and photoprotection have been studied (Panio et al. 2013, Czyczylo-Mysza et al. 2013). Nevertheless, many physiological and biochemical processes, in particular, participating in the adaptation of the photosynthetic apparatus to stress, are still left very poorly characterized in terms of their genetic control.

Although bread wheat carries some measure of drought tolerance new sources of variation for drought tolerance are searched among bread wheat wild relatives as they often demonstrate hardiness to severe droughty environments. The wild relatives of wheat represent a potentially valuable source of genes for stress tolerance. One of these species is the goat grass *Aegilops tauschii*, known to be the donor of the bread wheat D genome, and to harbor allelic variation at a number of genes associated with the stress response (Jia et al. 2013). In the present investigation, the D-genome Chinese Spring (Synthetic 6x) introgression (recombinant lines) (Pestsova et al. 2006) were exploited in order to determine the genetic basis of variation for physiological traits - gas exchange, chlorophyll fluorescence, the maximum electron transport rate, the chlorophyll a and b and carotenoid content, the activity of various Asc-GSH enzymes and CAT, and shoot biomass under both well watered and moisture deficient conditions. Earlier, these lines have been successfully used to localize a number of quantitative trait loci (QTL) for agricultural traits, for germination rate and longevity and for resistance to diseases (Simon et al. 2007; Landjeva et al. 2010).

Materials and methods

The set of 80 CS/Syn introgression lines (ILs), each line harboring a single *Ae. tauschii* segment in the homozygous state (Pestsova et al. 2006), along with their CS and the Syn parents, represented the mapping population. Plants were raised under controlled conditions in climatic chamber CLF PlantMaster (CLF Plant Climatic GMBH, Wertingen, Germany) mounted on the phytotron SIFIBR SB RAS in a 1:1:1 humus:sand:peat mixture, and maintained under a 16 h photoperiod supplied at 600 µmol m^{-2} s^{-1}, a day/night temperature of 23/16°C and 60% relative humidity. One pot was maintained in a well-watered state (60% saturation), while water was withheld from the other pot, starting at the three leaf stage an continuing until the soil moisture content had fallen to 30% of saturation, as determined by Osipova et al. (2011). Shoot biomass, gas exchange and chlorophyll fluorescence of the flag leaf were determined around 55 days after germination. One day after the measurements the flag leaf material was snap-frozen in liquid nitrogen and stored at – 70°C. The response of each trait to moisture stress was assessed in the form of a tolerance index, 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. Stomatal conductance (SC), transpiration rate (TR) and the rate of CO_2 assimilation or net photosynthetic rate (Phr) were determined from the central part of the flag leaf on the leading tiller of eight of the ten plants per line, using a portable gas exchange system (LCi Photosynthesis System, ADC BioScientific Ltd., Hoddesdon, England). Water use efficiency (WUE) was calculated from the ratio net photosynthetic rate/ transpiration rate. Chlorophyll fluorescence was measured on the flag leaf of three plants per line using a PAM-250 chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany). The leaves were enclosed in small light proof chamber for 30 min before the measurement was taken; the parameters recorded were basic chlorophyll fluorescence yield (F_0), electron transport rate at 160 µmol photons m^2 s^{-1} (ETR_{160}), peak electron transport rate (ETR_{max}) and the effective photochemical quantum yield of photosystem (PS) II (Y(II)). Chlorophyll and carotenoid content was determined as a concentration (mg pigment per g fresh weight) represented by the mean of three plants per line, with each assay being carried out in triplicate.

Enzyme extracts were prepared as described by Osipova et al. (2013), except for APX (ascorbate peroxidase) where 1 mM ascorbate was included in the homogenization buffer. Peak activities of CAT, DHAR, APX and GR (glutathione reductase) were determined in batches of three extracts, which were introduced into a flat-bottomed UV-Star microplate (Greiner Bio-One GmbH, Frickenhausen, Germany); reading were taken using an Infinite M200 PRO microplate reader (Tecan Group Ltd., Männedorf, Switzerland). Each measurement represented the mean of three biological replicates, each being run in triplicate. Enzymatic activity was expressed as micromoles of substrate per milligram of protein per minute at 25°C. Total SOD activity was measured spectrophotometrically using an Infinite M200 PRO microplate reader and flat-bottomed Citotest microplate. Protein content was determined according to Bradford (1976), using BSA (Sigma Aldrich, USA) as a standard.

SigmaPlot v12.0 (Systat Software, Inc., San Jose California USA, www.sigmaplot.com) was used to obtain means, standard deviations, perform *t*-tests and to calculate Spearman correlations. Analyses of variance (ANOVA) and the principal component analysis were based on routines implemented in the statistics package PAST (Hammer et al. 2001). Broad-sense heritability (h^2) estimates were obtained from the variance components, according to Dospekhov (1985). Associations between phenotype and genotype were sought using the single marker regression routine implemented in QGENE software (Nelson 1997). The additive effect of a given QTL was given by the expression 100*(BB-AA)/AA, where AA was the phenotypic mean of individuals homozygous for the CS allele at the given locus and BB the phenotypic mean of individuals homozygous for the *Ae. tauschii* allele. A LOD threshold of 3.0 was set for major QTL, and a LOD of between 2.0 and 3.0 for minor ones.

Results

Compared to CS, under well watered conditions, Syn accumulated less shoot biomass and exhibited lower values of both TR and SC (Table 1); its leaf content of each of the measured pigments was significantly lower; and its level of DHAR activity was about nine fold lower. Under water deficient conditions, Syn exhibited a significantly lower Phr and GR activity than CS. The stress negatively affected the performance of both lines, but the effect on both Phr and shoot biomass on Syn was more severe (Table 2). The drought treatment enhanced DHAR activity in the CS leaf but decreased it in the Syn leaf, producing a ~50 fold difference between the two lines. In contrast, moisture stress enhanced APX activity in the Syn leaf by 3.6 fold, while the level in CS was not altered. There was also a genotypic difference with respect to SOD activity (Table 1). None of the chlorophyll fluorescence parameters were affected by the drought stress, and did not vary between CS and Syn.

Table 1: Trait performance of the CS/Syn ILs and their donor lines under the two moisture regimes. *, **, ***: means differed significantly at P < 0.05, 0.01 and 0.001, respectively.

Trait	**Parents**		Introgression lines			h^2 (%)
	CS	Syn 6x	Mean±SD	Range	Max/min	
			Well-watered conditions			
Biomass[a]	4.7	2.2***	4.6±1.0	2.6-8.3	3.2	61.3
TR	1.3	0.70**	1.1±1.0	0.5-1.7	3.4	35.2
SC	0.12	0.05**	0.09±0.03	0.03-0.18	6.0	34.8
Phr	16.0	12.5	16.4±8.0	7.7-48.5	6.3	36.9
WUE	12.8	19.3	15.3±7.0	5.5-35.7	6.5	34.8
F_0	0.10	0.10	0.105±0.024	0.08-0.29	3.6	85.7
Y(II)	0.43	0.57	0.45±0.07	0.15-0.54	3.6	80.0
ETR_{160}	29.1	30.0	30.5±4.1	17.7-36.0	2.0	71.2
ETR max	44.9	46.7	58.7±17.9	21.1-102.6	4.9	58.8
Chl_a	2.12	1.13**	2.24±0.43	1.21-3.25	2.3	65.4
Chl_b	0.88	0.51**	0.92±0.20	0.48-1.56	3.3	53.0
Car	0.50	0.24*	0.48±0.07	0.32-0.63	2.0	35.4
Chl_{a+b}	3.0	1.6*	3.2±0.6	1.8-4.9	2.7	61.9
Chl_{a+b}/car	6.0	7.0	6.6±1.0	5.3-12.8	2.4	26.5
DHAR	186.0	20.0***	190.2±81.6	38.6-336.0	8.7	68.0
GR	37.1	31.9	36.1±8.2	13.0-54.5	4.2	23.6
APX	13.7	7.9	17.0±7.7	7.2-34.7	4.8	10.6
CAT	16.6	15.3	20.4±11.8	5.0-72.1	14.4	25.7
SOD	38.6	56.1	55.7±15.9	15.6-84.9	5.4	64.3
			Soil water deficit			
Biomass	2.8	0.8***	2.5±0.4	1.4-3.2	2.3	41.7
TR	0.60	0.30*	0.46±0.14	0.21-0.81	3.9	88.1
SC	0.04	0.02*	0.030±0.011	0.014-0.054	3.9	47.4
Phr	12.7	6.9*	13.8±5.4	5.5-29.6	5.4	42.2
WUE	24.7	25.9	22.6±14.6	3.3-67.2	20.4	52.5
F_0	0.09	0.09	0.101±0.01	0.07-0.12	1.7	41.2
Y(II)	0.46	0.53	0.46±0.04	0.40-0.54	1.4	50.0
ETR_{160}	30.5	28.9	31.0±2.5	25.0-36.3	1.5	48.9
ETR max	59.7	52.2	67.5±16.6	35.9-105.7	2.9	38.8
Chl_a	2.79	1.65**	2.64±0.39	1.68-3.53	2.1	48.5
Chl_b	1.14	0.81*	1.12±0.26	0.53-1.85	3.5	55.8
Car	0.60	0.32**	0.58±0.09	0.34-0.78	2.3	50.0
Chl_{a+b}	3.9	2.5	3.8±0.6	2.3-4.9	2.1	48.3

Trait	**Parents**		Introgression lines			h^2 (%)
	CS	Syn 6x	Mean±SD	Range	Max/min	
			Well-watered conditions			
Chl_{a+b}/car	6.6	7.8	6.6±1.1	4.2-13.7	3.3	39.5
DHAR	310.0	6.0**	273.8±87.0	104.0-477.1	4.6	58.9
GR	31.4	20.5**	35.6±11.6	10.6-59.9	5.7	43.8
APX	17.0	28.7	19.4±8.9	4.6-47.0	10.2	14.3
CAT	12.8	11.2	19.5±10.8	4.8-38.5	8.0	22.2
SOD	30.7	56.3***	45.4±15.2	10.1-73.5	7.3	63.0

[a] The biomass unit is grams. The transpiration rate (TR) is expressed as $mmol \cdot m^{-2} \cdot s^{-1}$; stomatal conductance of H_2O (SC) is $mol \cdot m^{-2} s^{-1}$; photosynthetic rate (Phr) is $\mu mol \cdot m^{-2} \cdot s^{-1}$; WUE – water use efficiency as net photosynthesis/transpiration; basic chlorophyll fluorescence yield (F_0); effective photochemical quantum yield of PS II (Y(II)); electron transport rate at 160 µmol photons/($m^2 \cdot s$) (ETR_{160}) is $\mu mol \cdot electrons/(m^2 \cdot s)$; maximum electron transport rate (ETR_{max}) is $\mu mol \cdot electrons/(m^2 \cdot s)$; pigment contents is expressed in mg per gram of wet weight. Chl_{a+b}/car – the ratio of total chlorophyll to total carotenoids; CAT, DHAR, GR, APX, SOD - catalase, dehydroascorbate reductase, glutathione reductase, ascorbate peroxidase and superoxide dismutase activity, that is presented as U/mg of protein extract.

Table 2: Tolerance indices of the CS/Syn ILs and their donor lines. *, **: means differed significantly at $P < 0.05$ and 0.01, respectively

Trait	Parents		Introgression lines	
	Chinese Spring	Synthetic 6x	Mean ± SD	Range
Biomass	63	43**	55 ± 15	35 - 112
TR[a]	43	40	47 ± 27	13 - 167
SC	31	40	42 ± 31	8 - 200
Phr	88	55**	77 ± 34	11 - 152
WUE	202	128	193 ± 83.9	51 – 429
F0	93	94	98 ± 13	65 - 138
Y(II)	105	93	104 ± 15	71 - 149
ETR_{160}	105	96	104 ± 15	70 - 165
ETR_{max}	130	112	127 ± 47	48 - 308
Chl_a	132	146	123 ± 30	82 - 211
Chl_b	130	159*	126 ± 39	43 - 228
Chl_{a+b}	131	149	126 ± 38	73 - 249
Car	120	133	122 ± 27	84 - 206
Chl_{a+b}/car	110	111	102 ± 18	66 - 219
CAT	77	73	99 ± 57	23 - 285
DHAR	167	28*	184 ± 136	66 - 663
GR	85	64	103 ± 38	35 - 317
APX	124	363	137 ± 75	34 – 333
SOD	79	100	80 ± 20	24 - 144

There was a transgressed variation among the ILs in both directions for some of the traits both under well watered and drought conditions. The fold difference between the lowest and the highest values ranged from 2.0 (Car and ETR_{160}) to 14.4 (CAT activity) (Table 1) in favorable conditions and from 1.4 (Y(II)) to 20.4 (WUE) in droughty. Some traits showed the reduction of variability under drought, such as chlorophyll fluorescence parameters, stomatal conductance or photosynthetic rate, some retained the variability level (leaf pigments content) and some substantially enlarged the limits of variability. The latter was characteristic of WUE, APX and SOD activity. The h^2 parameter was reduced by the drought stress with respect to shoot biomass

and chlorophyll fluorescence, but was increased with respect to the gas exchange parameters and Car. DHAR and SOD activity level was associated with the highest h^2 values in both the well watered and moisture-stressed plants. Some tolerance indices did not differ between CS and Syn (Table 2) but again CS showed the especially high tolerance of traits biomass, WUE and DHAR under drought. Many differently directed correlations were found under both conditions (data not shown). The pattern of inter-trait correlations was quite different for the normal and droughty conditions. For example, in the non-stressed plants, shoot biomass was significantly correlated with photosynthetic pigment content and the activity level of both DHAR and GR. Under contrasting conditions, shoot biomass positively inter-correlated with SC and TR and CAT activity while the correlation was negative for both GR and SOD activity.

QTL analysis

Forty three QTLs were detected totally. The interval mapping procedure identified 24 major QTL (LOD scores varying from 3.0 to 8.7) (Table 3). Four of these loci were specific for the well watered plants, 9 for the droughted ones and 12 referred to a tolerance index; 19 minor QTL (LOD score ranging from 2.0 to 3.0) were also identified. The most well populated chromosome was 2D chromosome (11 loci) and the least populated was 3D (zero loci) (Table 3). The largest number of positive alleles of the drought-specific QTL was inherited from CS and all the positive alleles for the tolerance indexes QTL were donated by CS. The proportion of the variance explained by each QTL varied from 11 to 21% (well watered specific loci), 11-33% (drought specific loci) and 12-39% (tolerance index QTL). Considerable input in trait variability under water deficit was introduced by QTL for biomass (2 loci), TR (2 loci), SC (2 loci). WUE (2 loci), GR (2 loci) and APX (1 locus) explaining, respectively, 32, 25, 30, 27, 44 and 27 of the phenotypic variance.

Eleven D genome regions harbored QTL associated with traits of relevance to drought tolerance; they were distributed over the four chromosomes 1D, 2D, 5D and 7D. The two regions identified on chromosome arm 1DS. One of them was defined by the microsatellite locus *Xgdm33,* and harbored major QTL underlying the Chl_{a+b}/Car ratio, the tolerance index for this trait and Chl_b content under moisture deficit conditions. The other was defined by *Xgwm337*, and harbored QTL underlying Y(II), ETR_{160}, and ETR_{max} in well watered plants and tolerance index QTL for two of these traits. On chromosome arm 2DS, *Xgdm5* was linked to QTL underlying TR, SC, ETR_{max} and PC1 in moisture-stressed plants. A second QTL region on this chromosome, lying 106 cM away from the first one, was defined by the 24.6 cM long interval flanked by *Xgwm539* and *Xgwm1419*. This region harbored QTL underlying shoot biomass, SC and WUE in moisture-stressed plants, tolerance index QTL for all three traits and a QTL underlying Phr under well watered conditions. The GR activity major QTL denoted *QGr.ipk-2D.1*, which mapped close to *Xgwm484*, was detected in both moisture regimes. An additional locus was detected in droughted plants on chromosome arm 2DL, close to *Xgwm991*. Tolerance index QTL for Y(II) and ETR_{160} were mapped to chromosome arm 5DS, associated with *Xgdm3* and *Xgwm960*, and a major shoot biomass QTL was linked to *Xgwm292* specifically in water deficient conditions. The distal end of 5DL included a small region, defined by *Xgwm272* and *Xgwm1454*, harboring QTL for tolerance index of leaf pigment content. Finally, on chromosome 7D, the large segment lying between *Xgwm1242* and *Xgwm1672* housed QTL underlying Phr under moisture stress and its tolerance index, and for WUE. The region around *Xgwm1672* featured QTL underlying APX activity under both growing conditions. Twenty seven suggestive loci with LODs less than 2,0 were detected (Table 3). Most of them were situated in the chromosome regions as major and minor QTLs mentioned above and their peak LOD score coincided with the positions of molecular markers being already identified.

Table 3: QTL underlying trait performance under the two moisture regimes

	Trait	QTL on chromosome	Marker nearest to LOD max	LOD max	Source of positive allele	r^2, %
		Well-watered condition				
1	Photosynthetic rate	*QPhr.ipk-2D*	*Xgwm1419*	2.3	Syn6x	12
2	Water use efficiency	*QWue.ipk-4D*	*Xgdm61*	3.0	CS	16
3	Y(II)[a]	*QEpq.ipk-1D*	*Xgwm337*	2.1	CS	11
4	ETR_{160}	*QEtr.ipk-1D*	*Xgwm337*	4.1	Syn 6x	21
5	ETR_{max}	*QEtrm.ipk-1D*	*Xgwm337*	2.3	Syn 6x	12
6	Chl_{a+b}/car	*QCcr.ipk-4D*	*Xgwm1382*	3.3	CS	17
7	GR	*QGr.ipk-2D*	*Xgwm484*	3.5	Syn 6x	18
8	APX	*QApx.ipk-7D*	*Xgwm1672*	2.0	CS	11
		Soil water deficit				
9	Biomass	**QBm.ipk-5D**	*Xgwm292*	3.3	Syn 6x	16
10	- " -	**QBm.ipk-2D**	*Xgwm539*	3.0	CS	16
Total[b]						32
11	Transpiration rate	*QTr.ipk-2D.1*	*Xgdm5*	2.4	CS	12
12	- " -	*QTr.ipk-2D.2*	*Xgwm539*	2.6	CS	13
Total						25
13	Stomatal conductance	*QSc.ipk-2D.1*	*Xgdm5*	2.9	CS	15
14	- " -	*QSc.ipk-2D.2*	*Xgwm539*	2.9	CS	15
	Total					30
15	Photosynthetic rate	*QPhr.ipk-7D*	*Xgwm111*	6.3	CS	30
16	Water use efficiency	*QWue.ipk-7D*	*Xgwm111*	2.8	CS	15
17	- " -	*QWue.ipk-2D*	*Xgwm539*	2.2	Syn 6x	12
Total						27
18	Y(II)	*QEpq.ipk-5D*	*Xgwm1629*	2.0	CS	11
19	ETR_{max}	*QEtrm.ipk-2D*	*Xgdm5*	3.5	CS	18
20	Chl_b	*Qchb.ipk-6D*	*Xgdm36*	4.8	Syn 6x	24
21	Chl_{a+b}/car	*QCcr.ipk-1D.1*	*Xgdm33*	3.4	CS	18
22	- " -	*QCcr.ipk-1D.2*	*Xgwm458*	2.8	CS	15
Total						33
23	GR	*QGr.ipk-2D.1*	*Xgwm991*	4.4	Syn6x	23
24	- " -	*QGr.ipk-2D.2*	*Xgwm484*	4.1	Syn6x	21
Total						44
25	APX	*QApx.ipk-7D*	*Xgwm1672*	5.6	CS	27
		Tolerance indices				
26	Biomass	*QBmit.ipk-2D*	*Xgwm539*	5.4	CS	26
27	Transpiration rate	*QTrit.ipk-2D*	*Xgwm539*	5.7	CS	28
28	Stomatal conductance	*QScit.ipk-2D*	*Xgwm539*	5.7	CS	28
29	Photosynthetic rate	*QPhrit.ipk-7D*	*Xgwm111*	3.4	CS	18
30	Water use efficiency	*QWueit.ipk-7D*	*Xgwm428*	2.3	CS	12
31	Y(II)	*QEpqit.ipk-5D*	*Xgwm960*	3.7	CS	19
32	- " -	*QEpqit.ipk-1D*	*Xgwm337*	2.6	CS	13
Total						32

33	ETR_{160}	*QEtrit.ipk-5D*	*Xgwm960*	3.6	CS	19
34	- " -	*QEtrit.ipk-1D*	*Xgwm337*	2.6	CS	14
Total						33
35	Chl_a	*Qchait.ipk-5D*	*Xgwm272*	5.0	CS	25
36	Chl_b	*Qchbit.ipk-1D*	*Xgdm33*	3.7	CS	19
37	Chl_{a+b}	*QTchit.ipk-5D*	*Xgwm272*	2.4	CS	13
38	Car	*QCarit.ipk-5D*	*Xgwm272*	6.0	CS	29
39	Chl_{a+b}/car	*QCcrit.ipk-1D.1*	*Xgdm33*	4.4	CS	22
40	- " -	*QCcrit.ipk-1D.2*	*Xgwm458*	3.0	CS	16
Total						38
41	DHAR	*QDharit.ipk-7D*	*Xgwm1672*	2.7	CS	14
42	GR	*QGrit.ipk-1D*	*Xgwm1012*	8.7	CS	39
43	SOD	*QSodit.ipk-4D*	*Xgdm61*	2.4	CS	13

[a]Y(II) - effective photochemical quantum yield of PS II; ETR_{160} - electron transport rate at 160 µmol photons/(m^2·s); ETR_{max} - maximum electron transport rate. GR, APX, DHAR, SOD - glutathione reductase, ascorbate peroxidase, dehydroascorbate reductase and superoxide dismutase activity; Chl_{a+b}/car – the ratio of total chlorophyll to total carotenoids; [b]total phenotypic variance

Table 4: Suggestive QTLs (LOD<2,0) in the genome D of wheat associated with shoot biomass, gas exchange and chlorophyll fluorescence parameters, chlorophyll and carotenoids contents and enzymes activities under two water regimes and with tolerance indices

QTL	Trait	Chromosome	Marker	LOD	Source of positive allele	r^2, %
		Well-watered condition				
1	Biomass	2D	*Xgwm1419*	1.7	Syn 6x	9
2	- " -	1D	*Xgdm33*	1.6	CS	9
3	- " -	7D	*Xgwm130*	1.6	CS	9
	Total					27
4	Water use efficiency	5D	*Xgwm982*	1.9	CS	10
5	Chlorophyll b	7D	*Xgwm1672*	1.7	CS	9
6	Carotenoids	5D	*Xgwm1454*	1.6	Syn 6x	9
7	DHAR	6D	*Xgwm325*	1.9	CS	10
8	GR	2D	*Xgwm539*	1.9	CS	10
9	SOD	2D	*Xgwm1419*	1.9	Syn 6x	10
10	- " -	6D	*Xgwm325*	1.8	CS	10
	Total					20
		Soil water deficit				
11	Biomass	5D	*Xgwm205*	1.8	Syn 6x	10
12	- " -	2D	*Xgwm991*	1.7	CS	9
	Total					19
13	Stomatal conductance	2D	*Xgwm311*	1.6	CS	8
14	- " -	7D	*Xgwm428*	1.9	CS	10
	Total					18
15	ETR_{160}	5D	*Xgwm1629*	1.9	CS	10
16	Chlorophyll a	5D	*Xgwm205*	1.7	CS	10
17	Chlorophyll b	5D	*Xgwm982*	1.8	Syn 6x	10
18	Total chlorophyll	1D	*Xgwm1012*	1.6	Syn 6x	9
19	- " -	6D	*Xgdm36*	1.6	Syn 6x	9

	Total					18
20	Chlorophyll/ carotenoids	6D	*Xgdm36*	1.9	Syn 6x	10
21	DHAR	6D	*Xgdm132*	1.8	CS	10
22	- " -	6D	*Xgdm98*	1.6	CS`	9
	Total					19
23	SOD	6D	*Xgdm132*	1.6	CS	9
24	- " -	6D	*Xgdm98*	1.6	CS	9
	Total					18
			Tolerance indices (IT, %)			
25	Chlorophyll b	6D	*Xgdm36*	1.8	Syn 6x	10
26	- " -	5D	*Xgwm272*	1.7	CS	9
	Total					19
27	GR	2D	*Xgwm991*	1.8	Syn 6x	10

Abbreviations as in Table 3.

Discussion

The donor and recipient of the CS/Syn ILs differed with respect to a number of parameters of relevance to both the direct (gas exchange, photosynthetic pigment content) and indirect (level of Asc-GSH cycle enzyme activity) effects of drought (Table 1). Syn, an amphiploid derived from the cross *Triticum dicoccoides* x *Ae. tauschii* (McFadden and Sears, 1946), produces narrow leaves and a relatively low gas exchange rate, traits which are likely to evolve in the semi-arid environment co-habited by the two progenitor species. The wheat CS in its drought resistance strategy, in a line with a reduced stomatal conductance uses the powerful protective mechanism manifesting in a high DHAR enzyme activity (Table 1). The set of ILs varied considerably for most of parameters predicted to be associated with drought tolerance, so represented a suitable population for identifying D genome genes with a likely impact on this key performance trait.

Of the seven D genome chromosomes assayable through the use of the set of CS/Syn ILs, four appeared to harbor clusters of relevant QTL. The chromosome arm 1DS segment was shown to house a large number of QTL underlying a mix of the traits. The same genetic interval is known to harbor QTL determining both grain weight (Pestsova et al. 2006) and various germination-related traits (Landjeva et al. 2010), as well as genes/QTL controlling flag leaf temperature in drought-stressed plants (Kumar et al. 2012). A second region associated with gas exchange and shoot biomass was identified on chromosome 2D segment specified by *Xgwm93* and *Xgwm988* (Tables 3, 4). Verma et al. (2004) have mapped a major QTL associated with leaf senescence in response to drought in this region, which also harbors a determinant of grain weight (Pestsova et al. 2006). Working with CS as a parent of mapping population Czyczylo-Mysza et al. (2013) were able to locate a major QTL underlying leaf pigment content in the neighborhood of *Xgwm539*, mapping to this same part of chromosome 2D. The implication is that this region of the second homoeologous group chromosomes harbors the gene(s) with a pleiotropic effect on the stomatal control of photosynthesis and biomass production. A third cluster mapped to the chromosome 7D coincides with identified by Yang et al. (2007) as the site of drought-induced QTL underlying chlorophyll fluorescence parameters. A significant contribution to the drought response determined by this genomic segment may be a major locus underlying APX activity in both well watered and moisture-stressed plants and/or the DHAR activity tolerance index QTL, since both these enzymes act to control cellular levels of reactive oxygen species. A QTL underlying shoot biomass was mapped here to chromosome 5D, in the vicinity of *Xgwm292*. This genomic region houses genetic determinants of a range of agronomic traits (Pestsova et al. 2006) and seedling vigor (Landjeva et al. 2010). The location of the chlorophyll fluorescence parameter tolerance index QTL located on chromosome arm 5DS overlaps with that of a QTL

derived from a germination test (Landjeva et al. 2010). Similarly, the photosynthetic pigment content tolerance index QTL located on 5DL co-localize with a QTL controlling germination following artificial seed ageing (Landjeva et al. 2010). The implication is that this chromosome too carries a number of genes regulating development and the stress response. An attempt has been made here to identify the genetic basis of variation in the activity of antioxidant enzymes in wheat plants exposed to contrasting moisture regimes. For GR activity, major QTL expressed only under water deficient conditions was mapped to chromosome 1D and to chromosome 2D. Major loci active under both non-stressed and/or drought-stressed conditions was identified around *Xgwm484* on chromosome 2D. The same locus for APX was detected around *Xgwm1672* on chromosome 7D.

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Diversity within Bulgarian old bread wheat germplasm

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'Old', or historic varieties are defined as "relatively homogeneous selections made within existing landraces or in the early stages of formal plant breeding programmes that was once on a variety registration list but are no longer registered" (Serpolay et al., 2011). Being a product of natural and purposeful selection carried out by generations of farmers through the years this old germplasm is highly adaptive to particular ecological conditions. Therefore, it represents a rich source of traits and alleles that should be characterized, evaluated and appropriately utilized to widen the diversity within modern crops.

Wheat (*Triticum aestivum* L.) is the most important crop in Bulgaria. The old Bulgarian germplasm grown to the west of the Black Sea was noted for its stable yield, drought tolerance, high protein content, or good bread-making quality (Majdrakov, 1945).

A collection of 60 historic bread wheat varieties released in Bulgaria from the beginning of last century up to the early 1970s was gradually assembled during the period 2005-2012 (Table 1). It comprises both selections within local forms, and varieties having a local material in their pedigree. The majority of accessions (28) has come from the IPK genebank, followed by the entries kindly provided from the Czech genebank at the Crop Research Institute, Prague (25), the Bulgarian genebank at Sadovo (6), and the Dobrudzha Agricultural Institute (1).

Brief characterization of the collection

For 42 out of 60 accessions, the origin - either collection site or release breeding station, is known based on the information listed in the genebanks' databases. Most of the old varieties have come from local breeding stations or nearby farmers' selections from North-Eastern territories and from the central Southern part of the country. Few entries have come from breeding centres near Black Sea coast and Sofia valley.

The botanical composition of the collection was determined on the basis of 5 highly heritable traits: presence of awns, colour of awns, colour of glumes, colour of grain, pubescence. The *erythrospermum* (27) and *ferrugineum* (20) varieties were prevailing, the rest of accsessions were of *lutescens* (7), *graecum* (2), *sardoum* (1), *milturum* (1), *hostianum* (1), and *pseudohostianum* (1) type.

The information about the pedigree of the historic varieties was scarce. Eight of the accessions have varieties Noé and/or Mentana as parents that have been crossed with local varieties (Martynov et al., 2006). Noé itself is a selection from a local wheat from Odessa region (Martynov et al., 2006). The Italian varieties Fortunato and San Pastore are parents in the pedigree of three other varieties, contributing shorter stem and earliness. One variety (Burgas-1) has received the wheat-rye 1B.1R translocation from the German variety Neuzucht.

Since the collection has been established step by step during a period of 7 years, various numbers of accessions have been included in different analyses and different number of growing seasons. The following traits were evaluated: plant growth habit, resistance to lodging and resistance to yellow rust (all accessions - 2014); heading time, final plant height and yield components (30 acc., 4 years-trial), nitrogen use efficiency (NUE) (21 acc., 2013, 2 environments). The material was sown in early to mid October in either 1.2 m^2 drilled plots (2006, 2007, 2008), or in two 1 m-long rows in two replications (2014). Moderate amounts of N fertilizer (N_8) split in two doses were applied during the vegetative season in all filed trials. The experimental design for the NUE study is described below. The genomic diversity was evaluated in 28 accessions based on allelic richness, occurrence of unique alleles, PIC (polymorphism information content), and variety heterogeneity using a set of 24 highly polymorphic wheat microsatellites as described in Landjeva et al. (2015).

Plant growth habit

Plant growth habit was determined at tillering stage in the middle of March. The frequency distribution for this trait was: 10% prostrate, 13% semi-prostrate, 15% semi-erect, 27% intermediate, and 35% erect.

Heading time

Heading time was determined as an average for 30 historic varieties over a 4-year field trial (2006, 2007, 2008 and 2014) conducted at the experimental field of the Institute of Plant Physiology and Genetics, Sofia. Three modern varieties were used for comparison: Sadovo-1 (a long term standard for productivity), Enola (the most widely grown variety in the country), and Katya (distinguished for its good drought tolerance). Heading date presented as number of days after April 1st, displayed large variation spanning from 35 days (Sadovska ranozrejka) to 55 days (variety No165). The old and modern varieties differed significantly ($p<0.05$), the old ones being later by 5 days. Only four of the old genotypes were either comparable or earlier in heading than the modern releases.

Final plant height

Final plant height presented as an average over a 4-year field trial with 30 old genotypes varied within the range from 89 cm (Pldin) to 135 cm (Yubilejna-2). The average plant height of the old varieties (116±21.6 cm) was significantly higher compared to the modern varieties (87±13.6 cm). The variation in plant stature was larger in 2014 which was characterized by extremely high and prolonged spring precipitation that favoured the vegetative growth. The trait varietal mean values ranged from 93 cm (Sadovska ranozrejka-4) to 156 cm (Yubilejna-2) and a mean of 136±19.2 cm which was significantly higher than the mean plant height of the modern control varieties (101±10.9 cm). Eleven old varieties were of short stature comparable with the modern releases, three of them having San Pastore as an ancestor.

Lodging resistance

Year 2014 was characterized by both high precipitation during the vegetative and reproductive stages and numerous heavy storms thereby causing severe lodging for a large part of the field material. Even under such adverse conditions 37% of the historic varieties displayed complete lodging resistance. These included varieties with plant height up to 150 cm, whereas the tallest ones were mostly moderately resistant or susceptible to lodging.

Resistance to yellow rust

Stripe (yellow) rust caused by *Puccinia striiformis* can result in significant loss to wheat yield and grain quality, given appropriate environmental conditions and susceptible varieties. It usually appears as a mass of yellow to orange urediniospores erupting from lesions arranged in stripes on leaves. At severe disease outbreak the glumes and awns could also be affected. During later growing stages and higher temperatures black teliospores appear often on spikes and stems. Usually the disease is not a serious problem in South-Eastern parts of Europe. However, the mild 2013/2014 winter followed by prolonged humid and cool spring ensured favourable environmental conditions for epiphytotic development of stripe rust in 2014. Growing resistant varieties is the most economical way to keep the level of yellow rust low so that other controls are not required. It is considered that the resistance in wheat varieties is based on a very narrow genetic range. The old germplasm is expected to have certain level of resistance that is sufficient to control the limited disease occurrence in non-standardized conditions. This together with the high genetic heterogeneity of the old resources prompted the search for resistance within the collection of old varieties during the "good for yellow rust" 2014. An assessment scale from 1 (highly resistant, HR, no visible symptoms or occasional symptoms of infection without sporulation) to 9 (highly susceptible, HS, abundant sporulation across the whole leaf area with no evidence of stripes) proposed by Wellings and Bariana (2004) was used to describe the genotypic response. The reaction of 20 varieties ranged from 1 (HR) to 4 (moderately resistant with affected area less than 30%) that could be used as donors of resistance genes; the response of the rest varied from intermediate to highly susceptible (Fig. 1).

Nitrogen use efficiency (NUE)

Modern semi-dwarf varieties are highly responsive to N application, but not all of them are efficient in N use. The adoption of responsive and inefficient cultivars in practice results in high expenses with N fertilizers and high risks for ecological contamination. Therefore, in unfavourable nutritional conditions or in organic practices, N efficient and well-adapted genotypes are required. The NUE of 21 old varieties was evaluated in two different environments and two N fertilizer applications in a search of N efficient genotypes. The modern varieties Sadovo-1, Enola and Katya were used for comparison.

The experiment was conducted in Sofia, Western Bulgaria (42°41'N 23°19'E) at the experimental field of the Institute of Soil Science, Agrotechnologies and Plant Protection, and in General Toshevo, North-Eastern Bulgaria (43°42'N 28°2'E) at the experimental field of the Dobrudzha Agricultural Institute at low (N_0) and high (N_{12}) nitrogen fertilization, in a randomized block design with three replications. The two environments differed with respect to the soil type, humus content and pH in the soil, content of P and K, and crop predecessor. The final plant height, grain yield per linear meter, grain yield per spike and TKW were evaluated for each variety x replication x N block combination. Significant effects for the genotype (G), environment (E), N level (N) were detected ($p<0.001$) for all traits, except for the non-significant effect of the N level on plant height. The effects of G x E interaction was significant ($p<0.001$) demonstrating different response of the old varieties at different eco-climatic conditions. At the same time the interactions G x N, E x N and G x E x N did not prove significant for all traits implying a relatively similar performance of all genotypes to the two nutritional regimes and environments. All historic varieties and the 3 modern ones were classified into groups according to N use and diagrams were designed for the two environments. By plotting the values of the yield overall average at the high N onto those at the low N level the diagrams were divided into quadrants showing the response and efficiency of each genotype. The four groups represented N-responsive and efficient, N-non-responsive and efficient, N-responsive inefficient and N-non-responsive inefficient genotypes (Fig. 2). Only 5 varieties appeared to fall into one and the same group in both environments. The rest demonstrated different response and efficiency under conditions differing for soil type, soil composition and preceding crop.

Molecular diversity

With the development and use of molecular markers, the assessment of genetic variation available in the old germplasm at molecular level advanced rapidly. Such information is important for resources conservation to correlate the molecular diversity to the phenotypic one, and for plant breeding to serve as a reference for the choice of parents. The twenty-four microsatellite markers used detected a total of 173 alleles at 25 loci on 14 chromosomes, ranging from 4 to 16, with an average 6.9 alleles per locus and an average PIC of 0.68. Twenty-eight % of the alleles were unique appearing only once in the whole pool of entries. The population was genetically highly heterogeneous (30.9%) with no completely homogeneous accession. The varietal heterogeneity ranged from 16 % (4 heterogeneous loci in variety Okerman-804) to 52% (13 heterogeneous loci in variety Obrascov Ciflik Nr. 16). At locus *Xgwm*261, 10 alleles were detected ranging from 165bp to 219bp. Five of the accessions were heterogeneous for this locus. Interestingly, allele $Xgwm261_{192bp}$ which has long been proposed diagnostic for the semi-dwarfing gene *Rht8* (Korzun et al., 1998) was identified in three varieties (Khebros, Erythrospermum 19-16 and Sadovka) having comparable or slightly longer culms than the modern control varieties. Based on pedigree, the origin of the 192bp allele in Khebros and Erythrospermum 19-16 can be traced back to Akakomugi, the key source of *Rht8*, through the Italian varieties Fortunato and San Pastore, respectively. The genealogy of variety Sadovka involves one local variety from Odessa region and two local Bulgarian materials. This finding is suggestive for the existence of another source of $Xgwm261_{192}$ besides Akakomugi. According to Ellis *et al.* (2007), this allele has been reported also in Daruma, one of the ancestors of Norin 10, and in some Chinese landraces. The suggestion is that haplotypes not associated with *Rht8* would persist in landraces or breeding material with no strong selection pressure for short stem (Ellis et al., 2007).

The dendrogram (Fig. 3) was constructed on the basis of the genetic distance at 21 loci displaying 7 or more alleles by hierarchical clustering of 1-GS using Ward's method (Ward, 1963). All but two genotypes could be differentiated. Some of the varieties formed clusters according to their geographic origin /breeding station, or genealogical relations.

Conclusion

A collection of historic ('old') bread wheat varieties released in Bulgaria from the beginning of last century up to the early 1970s as products of traditional farmers' selection and early breeding activities has been studied with regard to molecular variability, phenotypic characteristics, plant height, earliness, agronomic traits, disease resistance, and nitrogen use efficiency. This old germplasm had evolved from a broader gene pool and therefore is a valuable though yet underutilized resource for breeding purposes. Among this germplasm, potential sources of good productive potential, lodging resistance, N efficiency and yellow rust resistance were identified.

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Table 1: List of Bulgarian old (historic) bread wheat varieties used in the study

Variety name	Botanical type	Contributor	Catalogue No	Xgwm 261 allele (bp)
Beliya	graecum	RICP[a]	K-42783	211
Burgas-1	lutescens	RICP	RICP 01C0102599	
Bulgarische Weizen Nr. 7	sardoum	IPK[b]	TRI 4974	215
Bulgarische Weizen Nr.44	ferrugineum	IPK	TRI 4975	213
Bulgarische Weizen Nr.84	erythrospermum	IPK	TRI 4976	174
Butovo	ferrugineum	IPK	TRI 356	165
Daskot-171	ferrugineum	IPK	TRI 378	205
Dimitrovka 5-11	erythrospermum	RICP	RICP 01C0101820	
Dobrudzhanka-1	lutescens	RICP	RICP 01C0101830	
Dunavka	erythrospermum	IPK	TRI 191	174
Duravko	ferrugineum	IPK	TRI 373	174, 211
Erythrospermum-19-16	erythrospermum	IRGR[c]	BG 1985-TRT-AE-189	192
Ferrugineum-2	ferrugineum	RICP	RICP 01C0101824	
Ferrugineum-113	ferrugineum	RICP	RICP 01C0102194	
Ivanca	ferrugineum	IPK	TRI 377	205
Karnobat-92	erythrospermum	RICP	RICP 01C0101827	
Karnobater frühreifer	erythrospermum	IPK	TRI 3702	174
Khebros	lutescens	RICP	K 49835	192
Kneja	ferrugineum	IPK	TRI 357	213
Koslovez-3	erythrospermum	IPK	TRI 365	165
Mariza	erythrospermum	IPK	TRI 190	211
Nadezhda-2	erythrospermum	IRGR	BG 2001-TRT-AE-154	174
Nedan	erythrospermum	IPK	TRI 368	165, 203
Nova Sadovka	erythrospermum	RICP	RICP 01C0104465	
Obrascov Ciflik Nr.7	ferrugineum	IPK	TRI 185	215
Obrascov Ciflik Nr.14	ferrugineum	IPK	TRI 186	217, 219
Obrascov Ciflik Nr.16	ferrugineum	IPK	TRI 187	213
Obrascov Ciflik Nr.84	erythrospermum	IPK	TRI 188	174
Obrascov Ciflik Nr.159	ferrugineum	IPK	TRI 189	165, 174
Okerman-17	ferrugineum	IRGR	BG 1985-TRT-AE-188	174
Okerman-804	ferrugineum	IPK	TRI 9532	213
Plamak	lutescens	RICP	RICP 01C0105337	
Pldin	lutescens	RICP	RICP 01C0104081	
Poliak	ferrugineum	RICP	RICP 01C0104466	
Popovo	ferrugineum	IPK	TRI 5215	174
Prof. Ackerman	lutescens	IPK	TRI 192	165
Prof. Ackerman	ferrugineum	IPK	TRI 370	174
Sadorka	erythrospermum	IPK	TRI 372	174
Sadovka	ferrugineum	IPK	TRI 194	192
Sadovska ranozrejka-2	milturum	IRGR	IPGR	165
Sadovska ranozrejka-4	ferrugineum	RICP	RICP 01C0201627	174
Slomer	erythrospermum	IPK	TRI 367	205
Sofia-40	erythrospermum	IPK	TRI 5095	215
Sofia-312	graecum	RICP	RICP 01C0201141	
Stalinka	pseudohostianum	RICP	RICP 01C0201003	
Tenscha	erythrospermum	IPK	TRI 369	164
Tolbuhin	erythrospermum	IPK	TRI 7851	213
Vitosa	erythrospermum	RICP	RICP 01C0102207	
Yubilejna-2	erythrospermum	IRGR	BG 2001-TRT-AE-151	165, 174
Zlatiya	erythrospermum	RICP	RICP 01C0102608	
No100-10	erythrospermum	DAI[d]	-	

No127	erythrospermum	RICP	RICP 01C0100626	215
No165	hostianum	RICP	RICP 01C0100962	
No182	erythrospermum	RICP	RICP 01C0100629	174
No264	erythrospermum	RICP	RICP 01C0100630	174
No301	erythrospermum	IRGR	1956-TRT-AE-1	174
No312	ferrugineum	RICP	RICP 01C0100961	
No1153	erythrospermum	RICP	RICP 01C0100633	
No1438	erythrospermum	RICP	RICP 01C0100634	
No2315	lutescens	RICP	RICP 01C0100964	

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[c]IPGR – Institute of Plant Genetic Resources, Sadovo, Bulgaria
[d]DAI – Dobrudzha Agricultural Institute, General Toshevo, Bulgaria

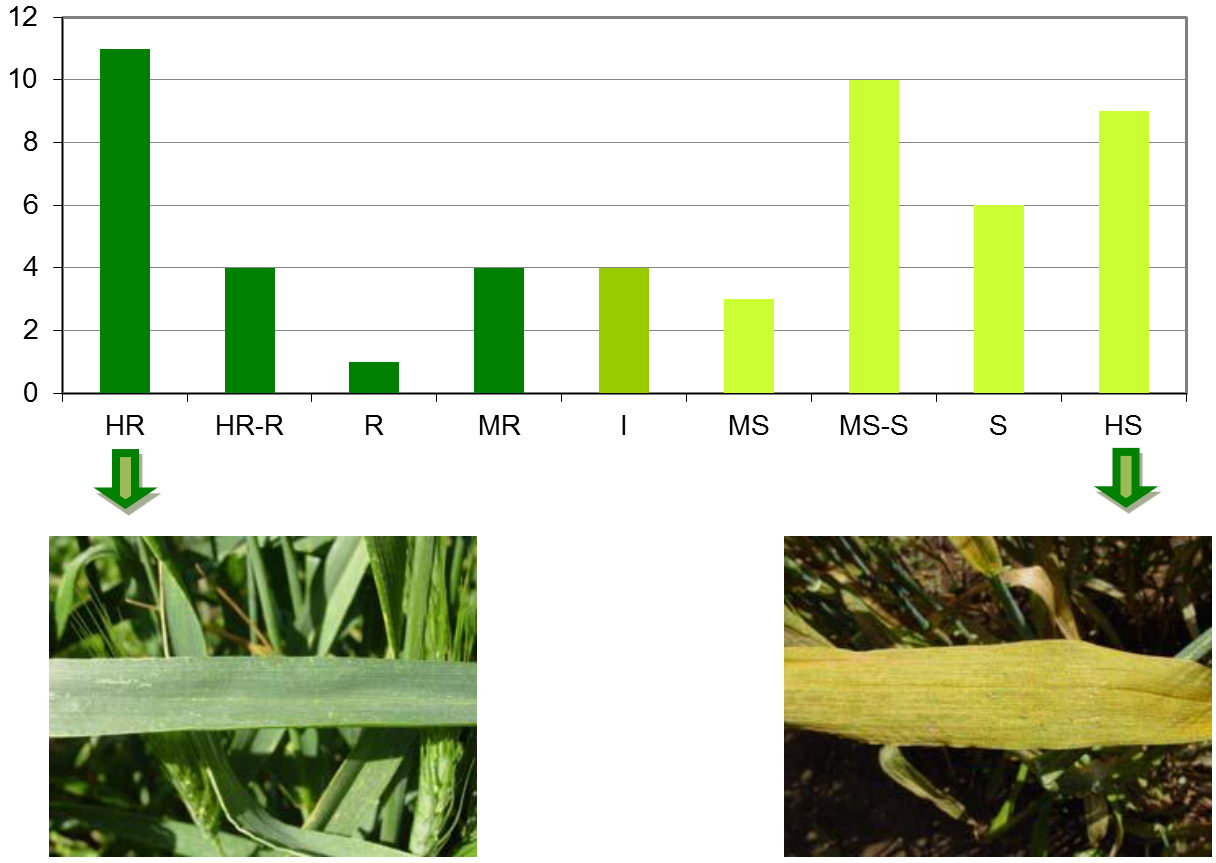

Fig. 1: Frequency distribution of Bulgarian historic (old) bread wheat varieties for resistance to *Puccinia striiformis*, following the assessment scale proposed by Wellings and Bariana (2004)

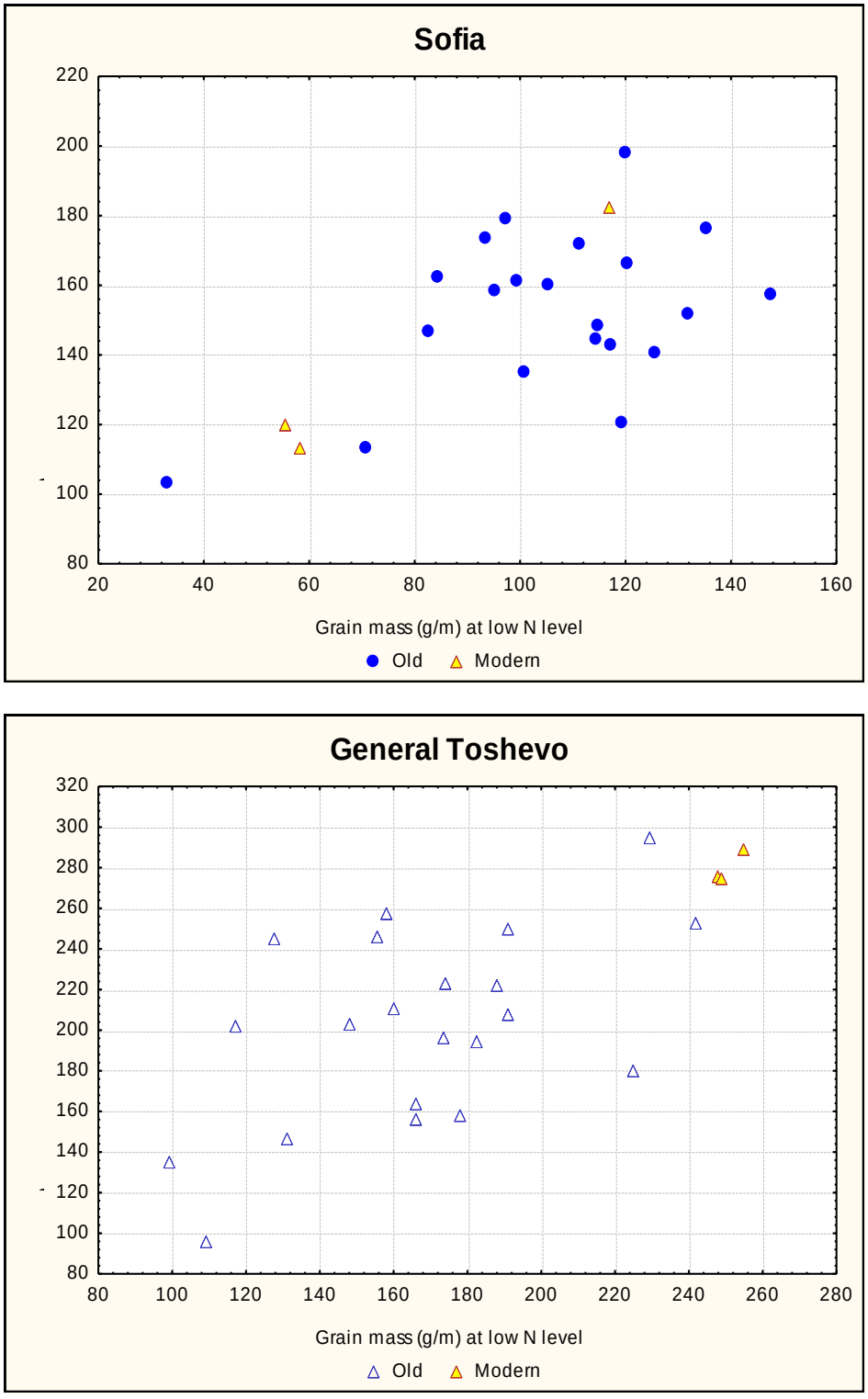

Fig. 2: Bi-plot of grain yield in 21 Bulgarian historic (old) bread wheat varieties and 3 modern ones (controls) at high (N_{12}) and low (no fertilizer N added) N levels in two environments: Sofia and General Toshevo. Broken lines represent means for each N level.

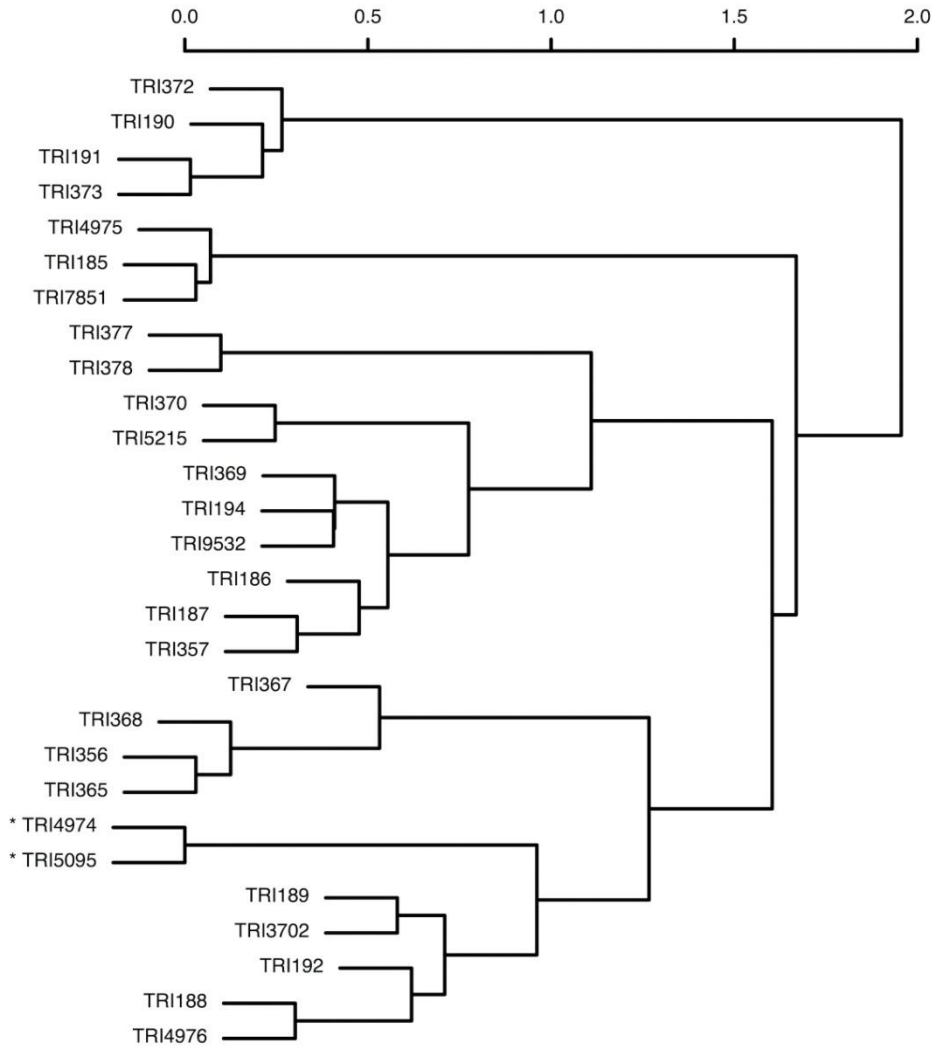

Fig. 3: Hierarchical clustering dendrogram, showing genetic relationships of 28 Bulgarian historic (old) bread wheat varieties. The genetic distance coefficients were calculated as 1-GS from data at 21 microsatellite loci displaying 7 or more alleles. Accessions that are identical at all loci are marked by *.

Genetic diversity of hexaploid wheat in Belarus based on SNP genotyping

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Summary

We used high-throughput array to evaluate diversity within hexaploid wheat growing in Belarus under breeding program through 384 gene-associated SNPs. A total 331 SNPs were genotyped, representing variability of 97.6% loci. We found out some common and contrasting patterns of the haplotype diversity in winter and spring accessions, which suggest different selection forces possibly acting in specific regions of the wheat genome throughout breeding. By neighbor-joing method, 94 wheat accessions were grouped into two major groups with high intra-group diversity in accordance with its type of vegetation and geographic origin. Genetic structure of Belarusian population of wheat appeared similar to Russian and Ukrainian variety and essentially different from west-European varieties.

Observed frequency of minor allele (MAF) was shifted towards alleles with MAF>0.25. Of the 331 markers, only 8 (2.4%) detected MAF≤0.05, and 236 (71.3%) had MAF≥0.2, which showed its high differentiating ability for tested wheat accessions. As a whole, used SNP set has been helpful in differentiating unique germplasm accessions and further allow us to validate allele-trait associations for wheat improvement in Belarus by genomic selection.

Introduction

Wheat is one of the main grain crops of Belarus along with barley, rye and triticale. Mainly Belarus provides its needs for grain, except high-quality elite cultivars. Last decade the structure of grain crops significantly changed in favor of high-yielding cultivars of wheat for 19% (from 20,1 till 32,1%) by reducing the acreage of rye in 3 times. It caused changes of total yield of grain crops (http://mshp.gov.by/agriculture/crop/grain/).

In 2014 year the area under wheat in Belarus was about 500 thousand hectares, two third (336.9 th/he) of which were sowed by winter wheat. Average yield of wheat in last year was 4 tons per hectare and overall reached approximately 2 million and 2 hundred thousand tons.

In the prewar and postwar years, wheat breeding in Belarus had not been in progress and up to middle of 80s there were not local winter cultivars and only some spring wheat with low productive potential (Grib 2009). But annually about 100 thousand ha of area has been sowed by Russian, Ukrainian and Polish cultivars. All of them had not been adapted to the local conditions and so didn't realize its full genetic potential.

In eighties wheat production still had low competitiveness due to export of high-quality grain of strong wheat from different regions of ex USSR. For instance, Ukrainian cultivar Mironovskaya 808 showed of 10 quintals per hectare greater yield in comparison with local Belarusian varieties (Koptik 2010). Evidently, in those years Belarusian wheat breeding extremely needed new sources of valuable genes. Thus, big forces were done for intensive field evaluation of wide varieties from different world breeding centers to collect prospective forms for further wheat improvement in Belarus.

Wheat breeding in Belarus started to grow intensively since 90s of last century, after USSR collapse. Valuable genes were introgressed from foreign variety and fixed in local wheat by applying crosses and selection, and set of high yielding cultivars for different purposes was created during subsequent years.

To date numerous local and foreign wheat accessions are stored in Belarusian GeneBank with poor information about its genetic diversity and possible relationships. In this work we were aimed to characterization of genetic structure in collections (58) winter and (27) spring accession including contemporary local Belarusian and foreign wheat varieties.

Materials and methods

We performed a comparative study 64 accessions (winter wheat: Berezina, Bylina, Zavet, Karavay, Legenda, Melodya, Plejada, Premiera, Prestizh, Sakret, Arina, Garmoniya, Kapylyanka, Epopeya, Avangardnaya, Veda, Suzore, Shtchara, Spectr, Sanata, Kontur, Komfort, Grodnenskaya 23, Grodnenskaya 24, Zarica, Kanveer, Mogilevskaya, Navina, Prinemanskaya, Priozernaya, Epos, Uzdym, Yadvisya, Kalaryt, Slavyanka, Balada; spring: Anyuta, Belorusskaya 80, Viza, Darya, Laska, Lyubava, Rassvet, Rostan, Sofya, Toma, Sudarynya, Lastotchka, Tchayka, Vestotchka, KSI-3_11, KSI-15_11, PSI-74_11, PSI-76_11, PSI-84_11, ESI-3_11, ESI-9_11, MR-1, MR-15, Opal, Festivalnaya, Kontessa, YA-1_5, YA-1_12) of hexaploid wheat growing in Belarus under breeding program with 27 cultivars (winter wheat: Podolyanka, Pereyaslavka, Tripilska, Tchornava, Natalka, Zolotokolosa, Bogdana, Volodarka, Smuglyanka, Sonetchko, Sakva, Solotokolosaya, Plutos, Lgovskaya 4, Nutka, Fineziya, Bogatko, Sukces, Kobra, Bokris, Dromos, Kubus, Avalon, Cadenza, Claire, Apache and spring wheat Opal), represented variety across 5 European countries (Ukraine, Germany, Russia, Poland, England).

To assess wheat genetic diversity we used high-throughput array with 384 gene-associated SNPs from published Cavanagh et al. (2013). Genotyping data were analyzed for basic attributes: minor allele frequency - MAF (Matthew J. et al. 2005), observed heterozygosity - He (Bryc et al. 2013) and polymorphism information content - PIC (Botstein et al. 1980).

All calculations were performed in MS Excel with manually specified equations. The tools available GENEPOP (Rousset 2008) have been used for winter and spring wheat populations differentiation and Fis (Weir 1984) calculation. Hierarchical tree constraction and principal coordinate analyses were performed through DARwin5 software (Perrier et al. 2003).

Result and discussions

Of the 384 used wheat SNPs in our assay, a total 331 SNPs (86%) were genotyped. 295 SNPs distributed on 21 chromosomes and 36 SNPs with not defined localization represented variability 97% loci (Table 1). Base changes at SNP loci include 80.8% transition and 19.2% transversion. The coverage per chromosome ranged from 1 for 4D to 26 for 5B with following distribution through genome: A-genome – 129, the largest B-genome – 138 and smallest D-genome – 28 SNPs. SNP distribution over homeologous groups of chromosomes was mainly uniform, except 4^{th} group.

The most SNPs showed high polymorphisms. But there were some differences in its variability between spring and winter wheat accessions. The highest level of polymorphisms we revealed in winter wheat. But large variation through genome and gomeologous groups was characteristic of spring wheat.

Average polymorphic information content values observed in our study wildly ranged with high proportion of markers with PIC value above 0.35 (61,9%) (Fig. 1). It’s also varied among

chromosomes and genomes. The most informative SNPs were localized on 7D, 6B and 3A chromosomes with average PIC level above 0.4.

Mean PIC values for all markers with chromosome location were also calculated separately for sub-groups of accessions according the type of vegetation. And we found out that observed PIC not only varied among chromosomes, it was dependent on sub-group. Highest level was revealed for winter wheat with SNPs spreading on 7D and 2A chromosomes, whereas for spring wheat the most informative markers were localized on 4A and 7A chromosomes.

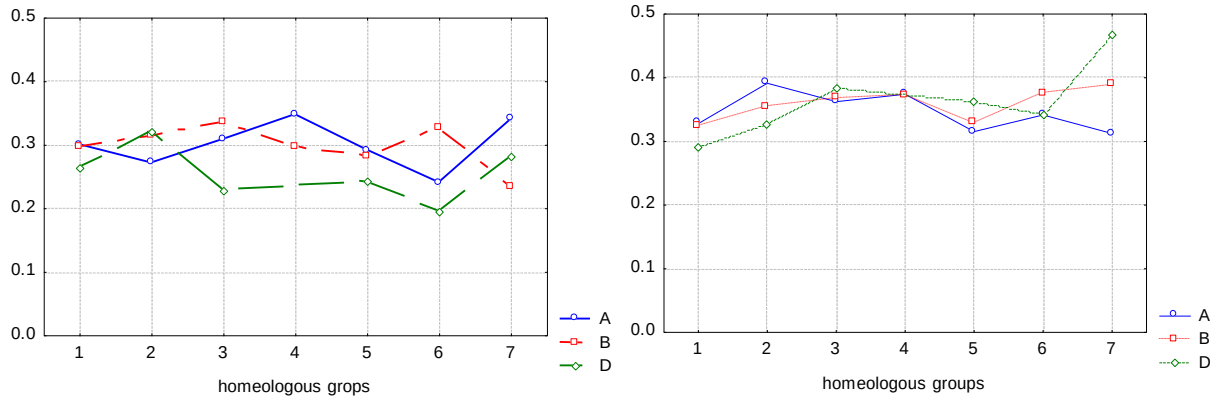
Table 1: SNPs genomic distribution among chromosomes and genomes

Chromosomes		SNP number	% over total number	Spring wheat		Winter wheat		overall
				Poly-morphic SNPs, %	Mono-morphic SNPs, %	Poly-morphic SNPs, %	Mono-morphic SNPs, %	
A-genome	1	20	6.0	90	10	100	0	100
	2	14	4.2	71.4	28.6	100	0	100
	3	19	5.7	100	0	100	0	100
	4	15	4.5	100	0	100	0	100
	5	21	6.3	90.5	9.5	100	0	100
	6	17	5.1	88.2	11.8	100	0	100
	7	23	6.9	82.6	17.4	86.9	13	95.7
In total		**129**	**39.0**	**89.0**	**11.0**	**98.1**	**1.9**	**99.4**
B-genome	1	21	6.3	90.5	9.5	100	0	100
	2	25	7.6	84	16	96	4	96
	3	25	7.6	92	8	96	4	96
	4	11	3.3	100	0	100	0	100
	5	26	7.9	92.3	7.7	92.3	7.7	92.3
	6	17	5.1	94.1	5.9	100	0	100
	7	13	3.9	92.3	7.7	100	100	100
In total		**138**	**41.7**	**92.2**	**7.8**	**97.8**	**16.5**	**97.8**
D-genome	1	7	2.1	85.7	14.3	85.7	14.3	85.7
	2	6	1.8	83.3	16.7	100	0	100
	3	3	0.9	66.7	33.3	100	0	100
	4	1	0.3	100	0	100	0	100
	5	2	0.6	50	50	100	0	100
	6	4	1.2	50	50	75	25	75
	7	5	1.5	80	20	100	100	100
In total		**28**	**8.5**	**73.7**	**26.3**	**94.4**	**19.9**	**94.4**
NA		36	10.9	77.8	22.2	97.2	2.8	97.2
Average		**331**	**100.0**	**83.2**	**16.9**	**96.9**	**10.3**	**97.2**

The single SNPs are bi-allelic, with continuous allele frequency distribution. In our research, observed frequency of minor allele (MAF) was shifted towards alleles with MAF>0.25. Of the 331 markers, only 8 (2.4%) had MAF≤0.05, and 236 (71.3%) - MAF≥0.2, that confirmed its high differentiating ability for tested wheat accessions. The main loci with rare alleles are located on chromosomes *3D, 4B, 5A, 6D.*

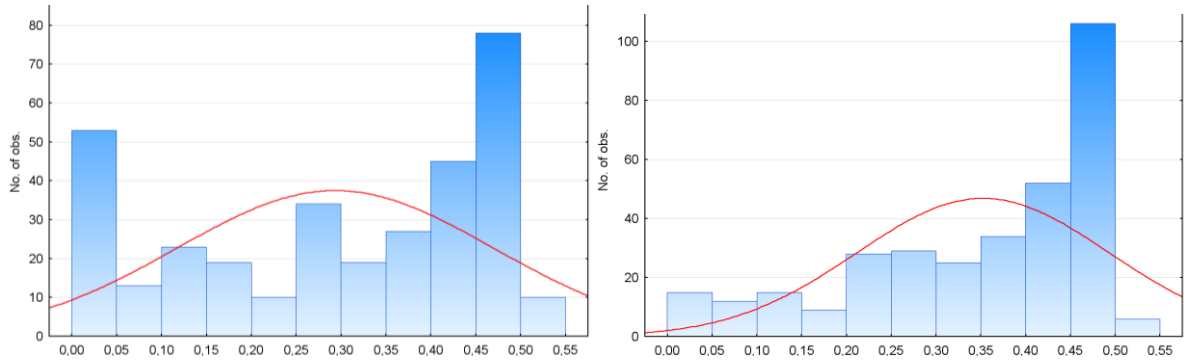
Distribution of MAF in sub-populations revealed differences (Fig. 2): winter wheat with high overall SNP polymorphism had high frequencies of alleles with MAF more 0.25 whereas for spring wheat it is noted a high frequences monomorfic markers and prevalence SNPs with MAF less 0,25. MAF Distribution in sub-groups and the general population significantly correlates (0.53 and 0.85 for spring and winter wheat correspondingly). The low link (r=0.29) is noted among winter and spring accessions, that indicates differential accumulation of SNPs through artificial selection in wheat. We found coincidence of alleles in winter and spring accessions in 72,5% of SNPs whereas 27,5% of loci showed switching of its frequencies.

Thus, our results revealed some common and contrasting patterns of the haplotype diversity in winter and spring accessions, which suggest different selection forces, which had been possibly acting in specific regions of the wheat genome throughout breeding.



A B

Fig. 1: Average polymorphic information content (PIC) values over A-, B-, D- genomes and seven homeological groups of chromosomes in spring (A) and winter (B) wheat accessions



A B

Fig. 2: Minor alleles frequences (MAF) distribution in spring (A) and winter (B) wheat

Gene pools of winter and spring wheat significantly differ by frequencies of 248 variants of SNPs. Genetic diversity of spring wheat is 1.2 times lower both overall (π=0,37) and winter wheat (π=0,36) values. Higher proportion of low-frequency alleles and heterozygosity of a spring accessions versus winter accessions is confirmed by fixation index Fis (0.4 vs. 0.48 respectively). By neighbor-joing method, 94 wheat accessions under research were clustered into two major groups with high intra-group diversity in accordance with its type of vegetation and geographical origin (Fig 3).

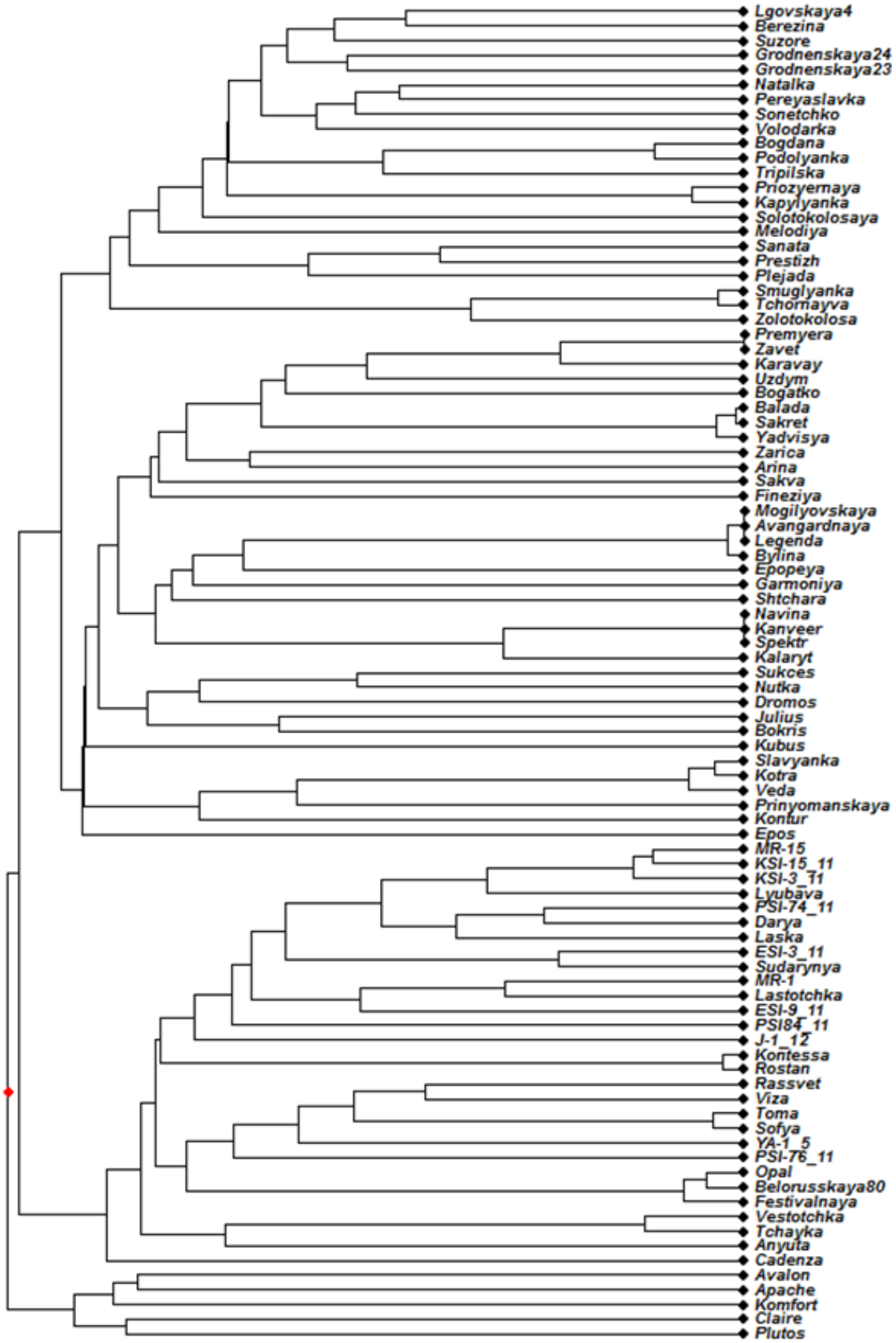


Fig. 3: TREE dendrogram of wheat genetic structure

The majority of roots of hierarchical tree have butstrap value more than 50%. Wheat accessions are grouped into 3 clusters. Mainly, we got topological differentiation that is in accordance with the type of vegetation (winter or spring).

The cluster 1 with winter accessions is subdivided into two subgroups (I-and, I-b). The first one (I-a) is presented by Belarusian winter wheat cultivars which had been introduced into agricultural production till 2000 year. This subgroup also involves Russian cultivar Lgovskaya4 and number of the Ukrainian winter wheat accessions which form topological discrete subclusters. The second subgroup (I-b) is formed by Belarusian cultivars (introduced into agricultural production after 2000 year), Polish (Sukcess, Nutka, Kobra, Bogatko, Sakva, Finezia) and German (Dromos, Bokris, Kubus) accessions. We were surprised with the fact of detection of genetically identical Belarusian accessions that could be caused of using closely related lines and probably siblings.

The cluster 2 includes a variety of spring wheat, mainly Belarusian selection. There are Scottish Cadenza and German cultivar Opal, which was used in Belarusian breeding for creation cultivars Beloruskaya 80 and Festivalnaya. Last one in result selection euploid progeny of monosomic plants of the wheat Opal.

The cluster 3 includes contemporary high-yielding cultivars of Brithsh, Scottish and German breeding and also one of the recently created Belarussian breeders cultivar Comfort.

Results of our study suggest a high genetic diversity of wheat, cultivated in Belarus, which is caused by some features of the local wheat breeding that began intensively develop only since 80s of the last century. Area under wheat in Belarus at those times was sowed by Russian and Ukrainian cultivars, which were actively used in breeding. This is confirmed by our results on the basis of which the local variety of early period (1985-2000) forms common sub-groups (I-a) with Ukrainian and Russian varieties. Period after 2000 year is characterized by active involvement in breeding of genetic variability from other European countries. However, there is a tendency of using closely related accessions and probably siblings, this follows from the presence of fully identical groups.

Really, to date breeding centers of Belarus have a good potential to wheat improvement, but variety needs to be systematized on platform DNA-typing, that can protect better management of genetic resources in breeding. Used SNP set are helpful in differentiating unique germplasm accessions and further allow us to validate allele-trait associations for wheat improvement in Belarus by genomic selection. Subsequently, our findings will contribute wheat improvement in Belarus through targeting selection for breeding.

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A comparative study of grain and flour quality parameters among Russian bread wheat cultivars developed in different historical periods and their association with certain molecular markers

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Food demands are constantly increasing along with growing of population in the world. The development of new more productive bread wheat cultivars is an actual problem. Many bread wheat accessions which are kept in gene banks still have no genotypic or phenotypic characteristics. It restricts their usage in breeding processes as a source of valuable alleles missing within modern wheat gene pool. Much effort is being made for the wide genotyping of the collected accessions (Roussel et al. 2005, Mitrofanova et al. 2009). Such investigations should be accompanied by a wide phenotyping for many traits.

Over 50 spring bread wheat cultivars selected by their ability to grow in Siberia were genotyped by Khlestkina et al. (2004) using microsatellite markers. These cultivars bred during dozens of years in USSR and Russia formed two distinct groups by microsatellite-based genetic similarity. The first group consisted predominantly of varieties bred before 1960th, the second group included varieties developed after 1960th, suggesting a qualitative shift in Russian spring bread wheat genetic diversity. In the former USSR the years around 1960 are known for a wide introduction of foreign cultivars in breeding programs. Analysis of pedigrees showed that the cultivars obtained before 1960 are mostly the old cultivars obtained by selection from the local varieties.

From the collection analyzed by Khlestkina et al. (2004) a smaller model collection (45 cultivars) was formed consisting of two groups – cultivars developed from 1911 till 1960 (23 cultivars designated as “old cultivars”) and from 1961 till 2006 (22 cultivars designated as “modern cultivars”). The aim of the work was to characterize the chosen cultivars for grain and flour quality; to describe the differences between groups for quality traits; to find out how the variability for these traits is associated with genotype variability determined previously with microsatellite markers. The quality traits were studied in 2007-2012 in West Siberia (Novosibirsk region).

Materials and Methods

The following quality traits were studied according the methods described in Anonymous (1988):

Thousand grains weight (TGW) - affects yield of flour during milling;

Vitreousness of grain (V) and Diameter of flour particles (particles size) (D) - determine the end use purposes of flour;

Raw gluten content in grain (RG) - determines the end use purpose of the flour as well as its nutritional value. It is directly related to the protein content in grain.

Flour analysis on Alveograph allows to determine strength of flour (W), dough extensibility (L) and stiffness (P) which determine the baking quality of the dough, P/L ratio determines dough elasticity.

Traits means, standard deviations and ANOVA analysis were calculated within MS Excel. Broad-sense heritability (h^2) estimates were obtained from the variance components, according to Dospekhov (1985). Principal component and cluster analyses were based on routines implemented in the statistics package PAST (Hammer et al. 2001). Relations between phenotype and the microsatellite markers were sought using Pearson's correlation analysis. In the most of cases the correlation coefficient not less than, 0.5 and the significant level not higher than 0.001 were considered.

Results

Identifying the differences between the groups of bread wheat varieties of different periods of creation

Table 1 presents the mean values of traits and their variation in groups. For TGW the modern cultivars gene pool showed higher average value and exhibited a larger variability. The average V values showed the larger variability among the old cultivars. But generally the modern cultivars had a higher vitreousness.

The D value correlated with V but was less dependent from environment. Generally, the modern cultivars possessed a higher D which is preferable for baking. The trait RG was characterized by a lower values in modern cultivars comparing to the old cultivars. The trait variability was larger within the old cultivars gene pool.

All alveograph parameters were higher among the modern cultivars. Variability for W among them was higher than among the old cultivars. P in the modern group was higher and its variation was wider. Modern cultivars showed a higher L but the variation limits were two times wider in the group of old cultivars. Differences for P/L ratio were not very clear but the variation range was slightly higher among the old cultivars.

ANOVA analysis was carried out to reveal the significant differences between groups using 4 year data. Table 2 presents the results of ANOVA analysis comparing two groups of cultivars created in different time. The significant differences were found between groups of old and modern cultivars for the most of quality traits. At the same time, Genotype x Environment interactions were also highly significant (data not shown). Insignificant differences were found only for P/L ratio. The modern cultuvars had a higher average meaning for all presented traits except RG. Among the old cultivars the heritability was found to be higer almost for all quality traits with the exception of P. This particular trait was better inherited in modern cultivars. No significant differences were found for parameters L and RG among the modern cultivars.

Multi-dimensional scaling was used to reciprocally arrange the cultivars in multidimensional space according their similarity for a complex of traits (Fig. 1). The closer to each other the points corresponding to genotypes the more similar is the distribution of traits among them. The regions of dispersion were determined for groups of cultivars for studied traits with 95% probability. It can be seen that the areas of determination of values were not coincided for two groups. It confirms the substantial phenotypical differences between two groups of cultivars obtained in different time-periods.

Identification of traits input in the differences between groups of cultivars

To determine which traits contribute more significantly to differences between groups of old and modern cultivars the Principal Component analysis was used. Two significant principal components were revealed (Table 3). The first component introduced more than 73% in variability and the second – more than 21%. The most significant inputs in two PCs were presented by (in descending order): L, V, RG, W, P and TGW.

The identification of markers related to the quality traits studied

Earlier, the frequency of occurrence of group-specific alleles was determined (Khlestkina et al. 2004). These frequencies were compared with the levels of expressions of quality traits. To do this, we used cluster analysis. All cultivars were grouped in the clusters in dependence of the expression of each trait. The found relationships with markers are presented in figure 2. We also searched for known quality traits QTLs found by other investigators in comparable positions. These literature data are presented to the right from the markers identified in our work.

On 1A chromosome *Xgwm357* was related with TGW, D and L. Significant correlation was found between TGW values and certain alleles of *Xgwm357*. On 1B chromosome the marker *Taglgap* (near *Glu-3* loci) was related with TGW, RG and Alveograph parameters. The second marker (*Xgwm18*) was related with RG, D, V, TGW, Alveograph parameters. Expression of the traits RG, L, W was associated with GWM length polymorphism. On 1D chromosome *Xgwm458* was related with TGW, RG, L. On 2A chromosome several relations were found with *Xgwm95* marker and quality traits. V and L levels correlated with the variability for the fragment length. Many relations were found on 2D chromosome around *Xgwm261*. RG and L showed a substantial polymorphism. Allele with length 185bp was group-specific and was connected with the highest RG gluten content. Allele with length 193bp was found only in the modern cultivars and was connected with a high L. The marker *Xgwm389* on 3B chromosome was related with TGW, RG and Alveograph parameters. The Relatedness of 120bp-allele was found with TGW and was detected only in 20% of modern cultivars. The allele of 140bp was group-specific for modern cultivars and was connected with high W, P and well-balanced P/L ratio. All these determine the good baking quality of flour. Many quality traits were connected with *Xgwm165* marker on 4B and 4D chromosomes. *Xgwm165* -4B showed the polymorphism associated with D and 262bp-allele was specific for old cultivars. High L was associated with 258bp fragment and was specific for modern cultivars. The allele of 199bp of *Xgwm165*-4D was associated with a high RG and was specific for old cultivars. On 6D chromosome the region of *Xgwm325* marker was related with many quality traits. High TGW was associated with allele 140bp and was group-specific for modern cultivars. The allele of 138bp was associated with high RG and low W and was found in 90% of old cultivars. On 7A chromosome many traits were connected with *Xgwm631* marker showing at the same time the polymorphism. High V and favorable for baking P/L were associated with the allele of 208bp. It was group-specific for modern cultivars. Allele fragment of 200bp was group-specific for old cultivars and was associated with a high RG.

Distribution of group-specific microsatellite alleles among the cultivars of different historical periods and their association with studied quality traits are presented in the Table 4. The number of group-specific markers is higher among the old cultivars which carry the group-specific alleles connected only with low values of TGW and V. At the same time, the modern cultivars carry alleles associated with high and medium values. For D, the old cultivars carry the alleles associated both with low and high values. For RG the group-specific markers were detected only among the old cultivars and all have high or medium values. For the trait P all specific alleles were associated with high values among modern cultivars while among old cultivars – with high and low. The largest number of microsatellite specific alleles related with L was detected among old cultivars – 23. Only 3 of them were associated with high and 5 – with low values. Among modern cultivars only 2 alleles were found associated with a high L. Out of 12 microsatellite alleles found among old cultivars and connected with P/L only 3 were associated with the values inapplicable for baking and 1 out of 3 among modern cultivars. The old cultivars do not carry the group-specific alleles associated with high W but 8 alleles were found associated with a low value. On contrary, the modern cultivars carry 3 specific alleles associated only with high W.

Our conclusions are as follows:

1. Between the groups of cultivars obtained in different time periods the largest differences were detected for L, V and RG.
2. The differences for expression level of the traits correlate with the variability for microsatellite group-specific alleles.
3. The old Siberian cultivars may be used as source of variability for the following traits: D, RG, Alveograph parameters.

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Table 1: Mean values with standard deviation (SD) of quality traits and their variation in groups

Groups	Statistical parameter	TGW	V, %	RG, %	D μk	W, u.a.	P, mm	L, mm	P/L
Modern	Mean±SD	27,5±2,7	87,7±3,9	32,9±2,2	21,4±3,3	271,6±110,4	117,2±35,3	74,0±10,9	1,7±0,5
	Limits	18,8-32,6	78,0-96,4	29,4-38,1	14,6-28,0	94,8-526,4	62,7-182,4	53,9-97,5	1,0-2,9
Old	Mean±SD	23,6±3,4	78,3±14,1	36,6±3,3	16,2±5,2	142,3±87,0	73,6±22,5	57,0±20,5	1,5±0,6
	Limits	16,8-29,1	54,2-93,8	31,7-43,8	9,9-26,5	40,6-359,2	46,1-150,3	19,8-87,9	0,7-2,9

Abbreviations: TGW - thousand grains weight; V - vitreousness of grain; D - diameter of flour particles (particles size); RG -raw gluten content in grain; W -strength of flour; L - dough extensibility; P - stiffness

Table 2: Analysis of variance (ANOVA) of grain quality traits for cultivars obtained in different time periods

Traits	Mean	Group	F_G	F critical	LSD_{05}	$h^{2\surd}$
TGW (g)	27,3	Modern	32,5***	3,9	2,8	0,48***
	23,6	Old				0,62***
V (%)	880	Modern	32,0***		7,4	0,34***
	78,3	Old				0,88***
RG (%)	29,9	Modern	28,2***		1,0	-
	33,6	Old				0,43***
D (µk)	22,1	Modern	58,0***		2,9	0,74***
	16,6	Old				0,79***
W (u.a.)	269,4	Modern	27,2***		72,3	0,43**
	141,1	Old				0,6***
P (mm)	117,2	Modern	29,4***		23,0	0,53**
	74,7	Old				-
L (mm)	74,0	Modern	14,7***		13,1	-
	56,8	Old				0,44*
P/L	1,7	Modern	1,8		0,4	0,4*
	1,5	Old				0,47**

[#] Data for TGW, V, RG and D obtained during 4 field seasons, for W, P, L, and P/L- during 2 field seasons

[√]The calculation of heritability (h^2) is performed within each group separately

***, **, * - P<0,001, P<0,01, P<0,05

Table 3: The input of significant principal components and quality traits in variability between groups

Principal components	Contribution to the total variation, %	Input of certain traits into principal component
1	73,5	L=-0,79; V=0,45; RG=0,23
2	21,3	L=0,53; V=0,43; RG=0,37; W=0,36; P=0,3; TGW=0,28

Table 4: The number of group specific markers associated with variability for quality traits among studied cultivars

Quality traits	Number of group-specific alleles in old cultivars			Number of group-specific alleles in modern cultivars		
	Total	Related with high value	Related low value	Total	Related with high value	Related with low value
TGW	9	-	1	5	2	-
V	7	-	5	3	3	-
D	14	2	8	5	-	-
RG	13	9	-	0	-	-
P	8	1	1	5	5	-
L	23	3	5	2	2	-
P/L	15	12	3	3	2	1
W	8	-	8	3	3	-

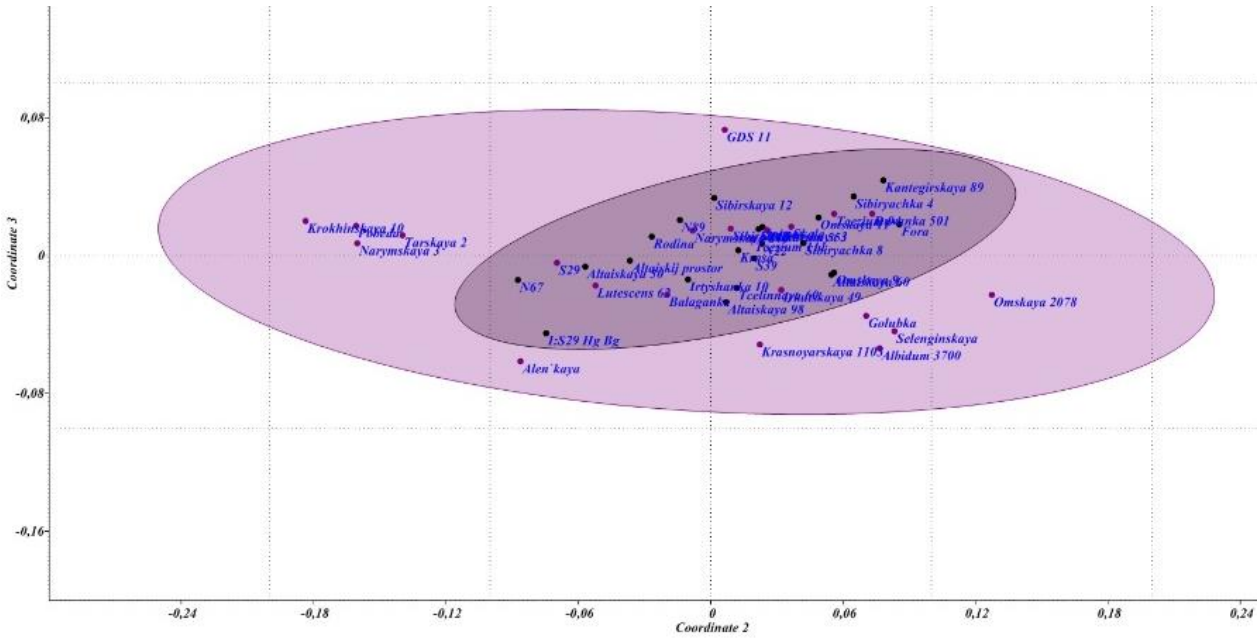

Fig. 1: Non-metric multidimensional scaling of studied cultivars based on total quality parameters of grain and flour. Grey and pink areas show 95% probability of dispersion for modern and old cultivars, correspondingly. Black or red dots on the left of each name indicate the cultivar of modern and old groups, respectively.

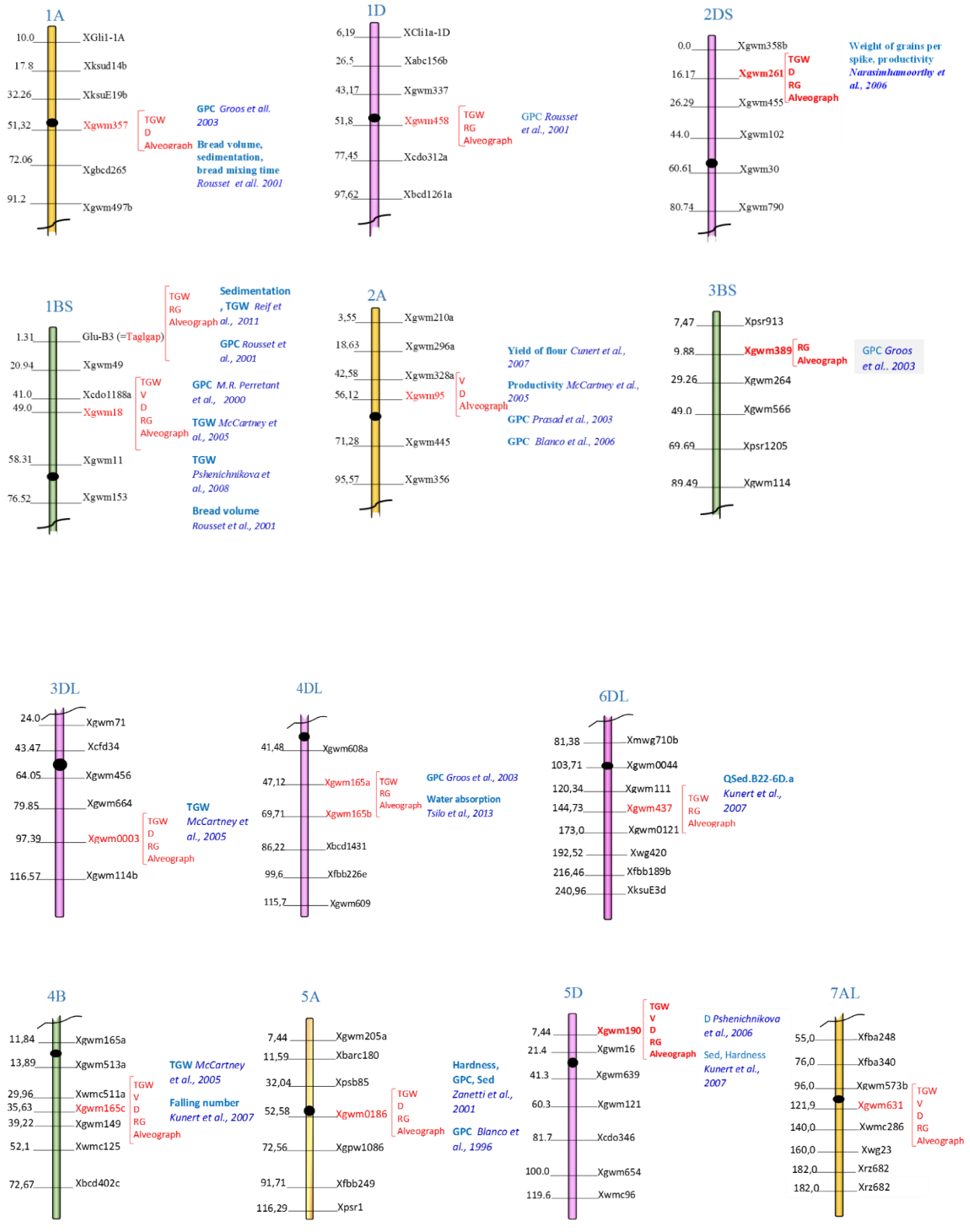

Fig. 2: The relatedness of quality traits with markers revealed in the present work (in red) and in other investigations (in blue).

Cereals genomics and molecular breeding - strong support for modern varieties development

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The growing population of the 21st century is placing increasing demand on our food production, with added pressures from diminishing resources and a more variable growing climate. During the last decade, the use of genomics and associated molecular marker technologies has made the leap from theory to practical breeding and is helping to deliver new cereal varieties which are better adapted to address these challenges.

From its origins in the dairy industry, genomic selection, has been modified and adopted for cereal breeding and is being used to accelerate the breeding process and when combined with the development of more sophisticated algorithms and improved computational power, this technology is able to capture both additive and epistatic effects (Jiang and Reif, 2015). In the near future, the use of Meta-data, trait-specific high-throughput phenotyping, such as that measured using drone technology, combined with genomic imputation, will provide the basis for further boosting the prediction accuracy and utility of genomic prediction (Mackey et al 2015).

The complete restoration of pollen fertility, reduced ergot infection in hybrid rye (Hackauf et al., 2012), and the enhancement of foliar disease resistances in wheat and barley, are also prime examples where selection intensity and accuracy can be accelerated by the application of molecular marker technology (Landjeva et al., 2007; Miedaner and Korzun, 2012).

Genomic tools have been applied to mutation breeding in cereals where they have proven to be powerful for forward and reverse genetic approaches with the products of this research making the jump to mainstream breeding programmes (Simmonds et al 2014). The increasing number of mapped markers, reduced redundancy, and lower price point has meant that whole genome SNP arrays can be routinely applied in breeding programmes for the accurate detection of marker-trait associations (Zanke et al 2015; Jiang et al 2015). Next generation tools such as customized genome capture arrays and long read sequencing are soon to make the leap from the lab to practical breeding.

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Recent advances in research at the CRI Prague on wheat flowering time genes and their primary and pleiotropic effects

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Wheat flowering time genes

The flowering time of plants is an important adaptive trait resulting from a complex action and interaction of gene families, primarily those of *Vrn, Ppd* and *Eps* responding to climatic conditions.

Vrn genes control vernalization requirement (a period of cold treatment) and are responsible for transition from vegetative to reproductive stage. Wheat can be classified as winter (requires vernalization) or spring type.

Comparative studies have shown that the vernalization pathways are not conserved between the model plant *Arabidopsis* and temperate grasses. In cereals, the *Vrn-1 (Vrn-A1, Vrn-B1, Vrn-D1)* genes code for a transcription factor (MADS box protein) responsible for the transition from the vegetative to the generative phase, and are equivalent to the *WAP1* gene. *Vrn-2* includes two completely linked zinc finger-CCT domain genes (*ZCCT1* and *ZCCT2)* acting as flowering repressors which are down-regulated during vernalization. *Vrn-3* is orthologous to *FT* of Arabidopsis: *FT* induction in the leaves results in a transmissible signal that promotes flowering. Transcript levels of the barley and wheat orthologues, designated as *HvFT* and *TaFT*, respectively, are significantly higher in plants homozygous for the dominant *Vrn-3* alleles (early flowering) than in plants homozygous for the recessive *vrn-3* alleles (late flowering). In wheat, a dominant *Vrn-3* allele is associated with the insertion of a retroelement in the *TaFT* promoter, whereas in barley, mutations in the *HvFT* first intron differentiate plants with dominant and recessive *Vrn-3* alleles (Yan et al. 2006, Higgins et al. 2010). *Vrn-D4* is a vernalization gene located in the centromeric region of chromosome 5D in hexaploid wheat. *Vrn-D4* is most probably active in the leaves, operating upstream or as part of the *VRN1/VRN2/VRN3* positive feedback loop.

***Ppd* genes** control photoperiod sensitivity, the response to daylength. *Ppd-1* (*Ppd-A1, Ppd-B1, Ppd-D1*) encode a type of protein with a CCT domain which belongs to the pseudo-response regulator (PRR) family (Turner et al. 2005). As in rice and *Arabidopsis*, this day-length flowering response is connected to activation of an *FT*-like gene, (*FT1*) which is induced in the leaves in long days (Turner et al. 2005). *Ppd-1* is a key regulator of inflorescence architecture (Boden et al. 2015) with its inhibiting effect on expression of *FT*.

***Eps* genes** generally act independently of environmental cues, but may respond to temperature changes. They affect the rate of plant development and fine tune flowering time, and thus can adapt plants to different environments. They are spread throughout the wheat genome, and their effects have been mainly detected as *QTL*.

Comparative genomics has revealed the divergence between flowering-time genes in their action in different species among tropical / temperate monocots and dicots, especially in the

vernalization pathway where major *Arabidopsis* flowering gene (*FLC-like*) seems absent in the genomes of monocots: Different genes regulate *FT* orthologues to elicit seasonal flowering-responses in *Arabidopsis* and the cereals.

More conserved is the photoperiodic pathway with genes analogous between *Arabidopsis*, tropical cereals (short-day plants with no vernalization requirement) and temperate cereals (long-day plants with a vernalization requirement. The autonomous and gibberellin pathways that are independent of photoperiod or vernalization also act similarly between groups.

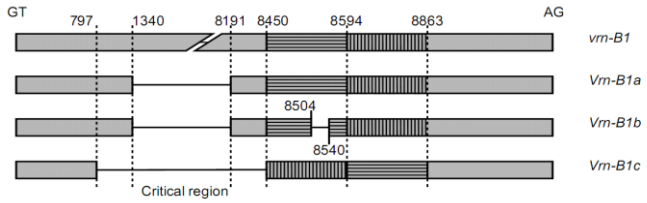
The role of the circadian clock and Phytochrome C in flowering time control

The convergence of circadian clock and phytochrome C (*PHYC*)-mediated light signals in the transcriptional regulation of *Ppd-1* is critical for the measurement of day length by the plant. In wheat and barley, *PHYC* is tightly linked to the *VRN1* gene, (=*WAP1*) which promotes the vegetative-to-reproductive transition of the shoot apical meristem. It is hypothesized that wheat *PHYC* modulates the expression of the circadian clock and clock output genes *CO1* and *CO2* in a way that is similar in *Arabidopsis* and rice. Also, most probably, the light activation of *Vrn-2* transcription is mediated by *PHYC*. This hypothesis is further supported by the drastic down-regulation of *Vrn-2* transcription in the mvp mutants with a deleted *PHYC* gene. *Vrn-2* and *Ppd-1* compete for the activation of *FT1*, but the repressing effect of *Vrn-2* is epistatic to the promoting effect of *Ppd-1*, preventing flowering in the autumn (Chen et al. 2014).

Detection and analysis of a new *Vrn-B1* allele

A new *Vrn-B1c* allele that considerably influences flowering time was recently described by our group and others (Shcherban et al. 2012, Milec et al. 2012, 2013):

This allele contains both deletion and duplication within the first intron and is associated with spring growth habit.

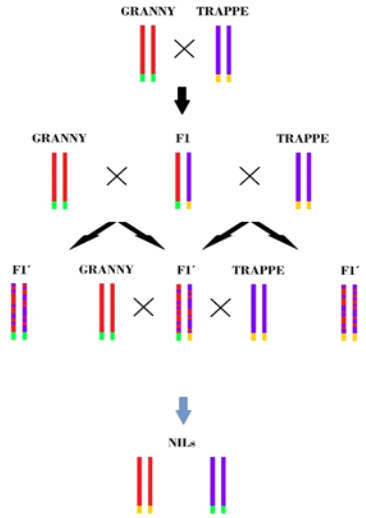


vrn-B1 allele from Triple Dirk C line
Vrn-B1a allele from Triple Dirk B line,
Vrn-B1b from spring variety Alpowa
Vrn-B1c (novel) allele

A recent study by our group has compared the effects of the different alleles *Vrn-B1a* and *Vrn-B1c:*

Two wheat varieties, Granny and Trappe, were revealed to carry the same alleles at the major flowering time loci apart from *Vrn-B1*, yet differ by 4.6 days in their flowering time under experimental conditions. The allelic status of the varieties is given below:

Trappe FT 70,4 d.	*ppd-D1b*	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*vrn-B3*
Granny FT 65,8 d.	*ppd-D1b*	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*vrn-B3*



NILs were produced for a comparison of the effects of *Vrn-B1a* and *Vrn-B1c* using the following scheme:

The following analyses will be carried out on the derived lines:

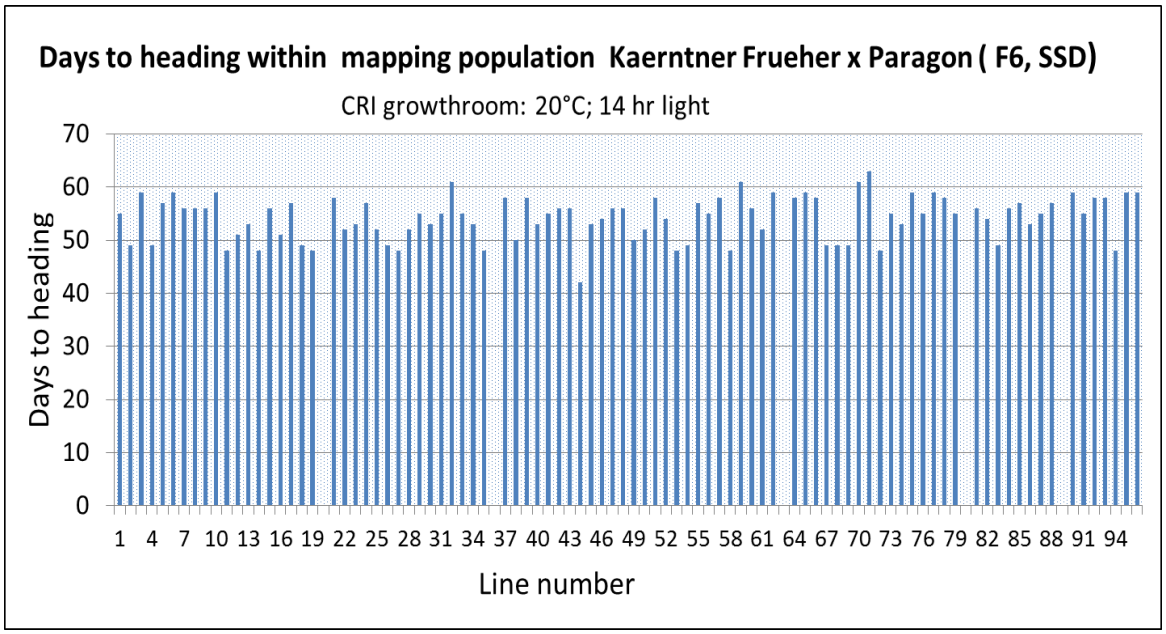
- The presence of the alleles *Vrn-B1a, Vrn-B1c* will be detected using multiplex PCR.
- The effects of the *Vrn-B1c and Vrn-B1a* alleles on growth and development, and on yield components of the NILs will be evaluated.
- Expression analysis of transcripts will be carried out.

Studies of the effects of *Ppd* genes

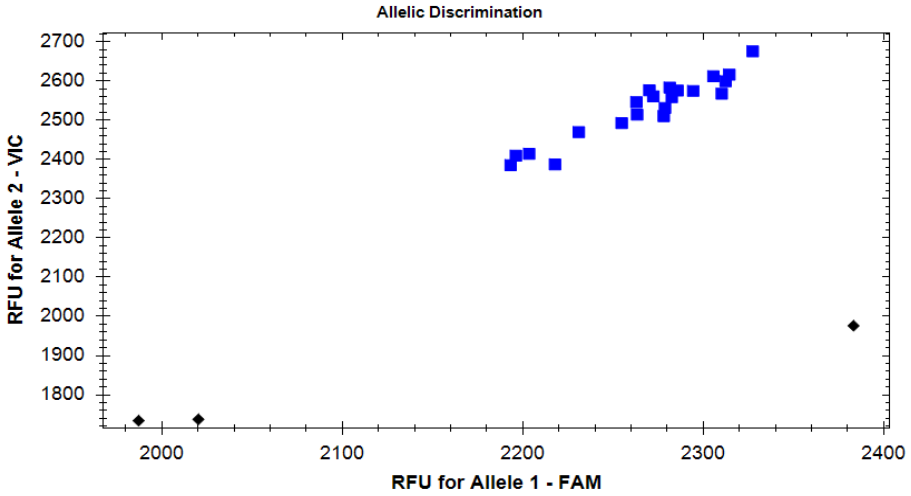
Two spring wheat cultivars, Kaerntner Frueher and Paragon were found to differ in heading time by 10.7 days in a field experiment. DNA marker analysis showed an identical allelic constitution at all the major flowering time loci apart from, possibly, *Ppd-B1*. Allelic constitutions are given below:

Kaerntner Frueher FT57.8 d.	*Ppd-B1?*	*ppd-D1b*	*Vrn-A1a*	*Vrn-B1a*	*vrn-D1*	*vrn-B3*
Paragon FT 68.9 d.	*ppd-B1b*	*ppd-D1b*	*Vrn-A1a*	*Vrn-B1c*	*vrn-D1*	*vrn-B3*

A single seed descent mapping population was produced after a cross between Kaerntner Frueher and Paragon to analyse the effect of possible alternative *Ppd-B1* alleles, see below for heading time variation in a growth room experiment:



- Flowering times of each line were analysed in a growth-room experiment.
- DArT markers were used to develop a genetic map.
- QTL analysis from the obtained data was performed.
- Differences were localized as a QTL in the *Ppd-B1* region.
- To confirm/reject *Ppd-B1* allelic variation as the source of HT variation:
- The sequence of DArT marker 985149 was BLASTed against the 2BS survey sequence.
- The *Ppd-B1* gene of Kaerntner Frueher was sequenced.
- KASP analysis was performed to identify the presence of the same/different alleles at the *Ppd-B1* locus.



The results of KASP analysis of KF and P with allele specific primers is shown above. The dot plot shows the presence of the same sequence of the *Ppd-B1* allele in both lines.

The analyses could not prove that variation in *Ppd-B1* sequence was present between the lines or that the variation was due to a linked QTL. However, another explanation of the flowering time difference could be copy number variation (CNV) at the *Ppd-B1* locus. In 2012, Díaz et al. identified early, day neutral heading time that was caused by increased CNV at the *Ppd-B1* locus. Their presumption coincides with our observations in terms of identification of Paragon as a late flowering variety and the observation of the ability of KF to flower under short days.

Thus, the next aim of this study is to determine differences in CNV between Paragon and Kaerntner Frueher by quantitative PCR.

Growth and developmental stages were also studied in Paragon, Paragon *Ppd-1* isogenic lines, and Kaerntner Frueher, grown under short days. The lines used are shown below:

Variety /Line		**Ppd-A1**	**Ppd-B1**	**Ppd-D1**
Kaerntner Frueher	[KF]	*ppd-A1a*	*ppd-B1?*	*ppd-D1a*
Paragon	[Par]	*ppd-A1a*	*ppd-B1a*	*ppd-D1a*
Paragon (GS100 2A)-1	[Par_2A]	*Ppd-A1a*	*ppd-B1a*	*ppd-D1a*
Paragon (Son 64 2B)-1	[Par_2B]	*ppd-A1a*	*Ppd-B1a*	*ppd-D1a*
Paragon (Son 64 2D)-1	[Par_2D]	*ppd-A1a*	*ppd-B1a*	*Ppd-D1a*

The experiment carried out had the following structure:

Growing conditions: After 3 days treatment to allow germination (20°C, in the dark), the plants were sown into pots and placed under growth room conditions: temperature 17.5°C, light/dark: 10/14h = short days (SD); PAR 250 µmol m-2 s-1 (Philips Agro 400W High Pressure Sodium Lamp + incandescent bulb).

Plant development was monitored by dissecting apexes at regular intervals. From measurements on the Waddington scale, the rate of development decreased in the genotype order: KF, Par_2D, Par_2A, Par_2B, to Par; the rate of development of Par was very slow from stage W2 through to stage W4, and accelerated only after this period.

Plants of Par, transferred from SD to LD, began to develop very quickly, unlike those of Par plants remaining on SD.

Using the Zadoks scale, genotypes could be classified into 3 rates of development:

From the beginning of growth stage Z40, the genotypes KF, Par_2D, Par_2A, and Par_2B developed very similarly; then their rates of development differentiated up to stage Z70. From this stage onward they developed at the same rate.

Up to stage Z70 (flowering), Par plants (sensitive to SD) developed very slowly at first; thereafter, their rate of development was similar to the other genotypes.

Precise mapping of an *Eps* locus on wheat chromosome 3B

Flowering time locus, *QRt.CRI-3B.1* was previously genetically mapped to chromosome 3B of the wheat alternative variety, Ceska Presivka (Pánková et al. 2009). Further studies have been carried out to fine map this locus:

- NIL mapping populations have being developed.
- Several closely linked polymorphic markers in the *QRt.CRI-3B.1* locus region have been developed.
- To date, 274 polymorphic DArT, SSR and STS markers were identified. Sequencing of the 3B chromosome from Česká přesívka (CP) and Sandra (S) has led to the identification of 27,388 SNPs within the *QFt.cri-3B.1* region.
- Identified SNPs have been used for sequence-based and KASP marker development and subsequently to saturate the region of interest.
- An F_5 NIL mapping population combined with SNP genotyping will be used for precise fine mapping of the gene which will be eventually followed by positional cloning.
- Candidate gene analysis, and their detailed characterization, will provide novel information which could lead to a deeper understanding of how fine tuning of flowering time in wheat is achieved.
- The effect of the analysed gene could be useful in future breeding programs to create the most suitable varieties for local environments.

Although these genetic studies are continuing, phenotyping of the different genotypes under different conditions of temperature, daylength can give different results, and it is still necessary to develop a reliable and reproducible protocol for differentiating the *QFt.cri-3B.1* allelic variation.

Acknowledgements

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Importance of flowering time loci for NS wheat breeding program

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Introduction

The transition from vegetative to reproductive growth is critical developmental switch and a key adaptive trait in both crop and wild cereal species that ensures that plants set their flowers at an optimum time for pollination, seed development and dispersal. First Martiníc (1975) and then Hunt (1979) indicated that winter wheat varieties grown in countries of more northern latitudes were usually highly sensitive to photoperiod, whilst those grown in more southern latitudes such as Italy and Yugoslavia were normally highly insensitive. Later, in the paper of Worland et al. (1998), the results of 10 years' trials showed that southern European wheat breeders have, by breeding photoperiod insensitive varieties, gained adaptability through avoiding summer desiccation. The early flowering was associated with the Ppd-D1 gene which was introduced to southern European wheat breeding programs, likewise NS wheat breeding program, from Japanese variety Akakomugi (Strampelli, 1932).The early genotypes also showed a height reduction of around 18 cm, a reduction in number of spikelets developing in the ear, and a net increase in grains per ear due to higher levels of spikelet fertility in the remaining spikelets. As a result, in Yugoslavia yield was increased about 30% (Worland et al., 1998). Recent study of Langer et al. (2014) revealed that flowering time in European winter bread wheat cultivars is mainly controlled by *Ppd-D1* (which explains 58% of the genotypic variance), while the fine tuning to local climatic conditions is achieved through *Ppd-B1* Copy Number Variations (3.2%) and a larger number of QTLs with small effects. QTLs for heading and flowering time in wheat have been identified in several linkage mapping and association mapping studies (Hanocq et al., 2007; Griffiths et al., 2009; Reif et al., 2011; Rousset et al., 2011; Kamran et al., 2014).

The objective of this study was to assess the allelic diversity at marker loci potentially associated with flowering time in a set of wheat core collection of Institute of Field and Vegetable Crops, Novi Sad, Serbia, as well to detect stable QTLs for flowering time in the Serbian agro-climate region.

Materials and methods

A set of 283 wheat accessions originated from 24 countries worldwide assembled from a larger core collection of Small Grains Department of the Institute of Field and Vegetable Crops, Novi Sad, Serbia, was used for phenotype evaluation. The accessions were chosen according to their contrasting expression for yield and yield related traits.

The genotypes were sown in a randomized block design, in ten growing seasons, from 2000 to 2009 at locality Rimski Šančevi, Novi Sad, Serbia. Flowering time (Fl) was recorded as the number of days after January 1st when 50% of the spikes within a given plot had reached the anthesis.

Genomic DNA from all varieties was extracted from fresh young leaves using the CTAB protocol described by Doyle & Doyle (1990). The accessions were genotyped with 30 microsatellite markers positioned along almost all three genomes and located near previously

detected important QTLs. The fragment analysis of PCR products was carried out using capillary electrophoresis on an ABI Prism 3130 genetic analyzer (Applied Biosystems, Foster City, CA, USA).

The population structure was revealed using genotypic data which were processed by the Structure software, version 2.3.4 (Pritchard et al., 2000). All subpopulations derived from structure analysis were also evaluated by the principal coordinate analysis (PCoA) implemented by the Excel add-in GenAlEx 6.5 (Peakall and Smouse 2006, 2012).

The marker-trait associations were analyzed in the Tassel software, version 2.1. (Bradbury et al., 2007) using two models: GLM and MLM (Yu et al., 2006). The magnitude of QTL effects was explained by the R^2 parameter. The descriptive statistics of phenotypic data was performed in the Statistica software, version 12 (Statsoft, Tulsa, OK, USA).

Results and discussion

The analyzed wheat population offered large phenotypic differences for flowering time which varied from mid-april to early June with mean value for mid-may.

A total of 349 alleles was detected at 30 analyzed SSR loci. The PIC values ranged from 0.297 (GWM157-2D) to 0.896 (CFA2086-2A), with an average of 0.688. The microsatellite markers from A and B genome revealed high level of polymorphism, while polymorphism of D genome was moderate.

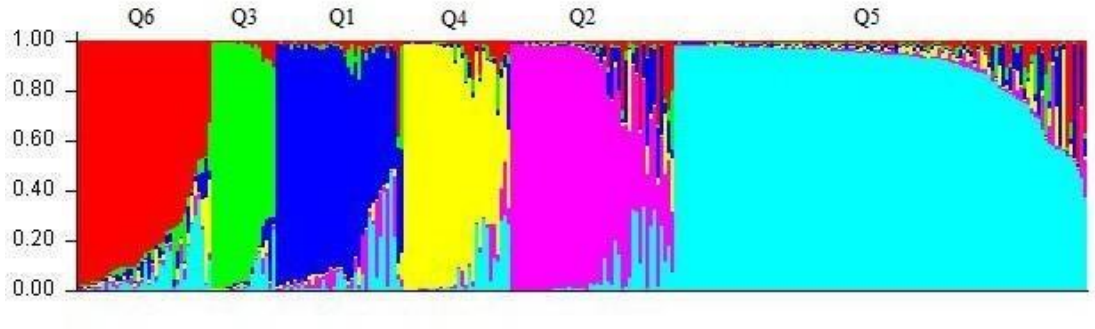

Fig. 1: Population structure of 283 wheat (*Triticum aestivum* L.) genotypes estimated using the model-based Bayesian algorithm implemented in the Structure software (Pritchard et al., 2000) performed with 30 microsatellite loci. Q1 to Q6, genotype clusters on the Q matrix.

Using log probability of data obtained by the Structure software genotypes were distributed into six subpopulations (Fig. 1). The largest group (Q5) consisted of 114 genotypes, with 80 % originated from Serbia, whereas the smallest group (Q3) included 18 cultivars, mostly from the USA. Results of PCoA were consistent with grouping by the Structure software for all subpopulations except Q1 and Q6 which showed dispersed distribution in the coordinate system (Fig. 2).

The total number of significant marker-trait associations (MTAs) for the 10 growing seasons was 42 using the GLM and 40 using MLM. 39 MTAs were common for GLM and MLM. A total of 8 microsatellite markers were associated with Fl in at least 3 analyzed seasons (Table 1).

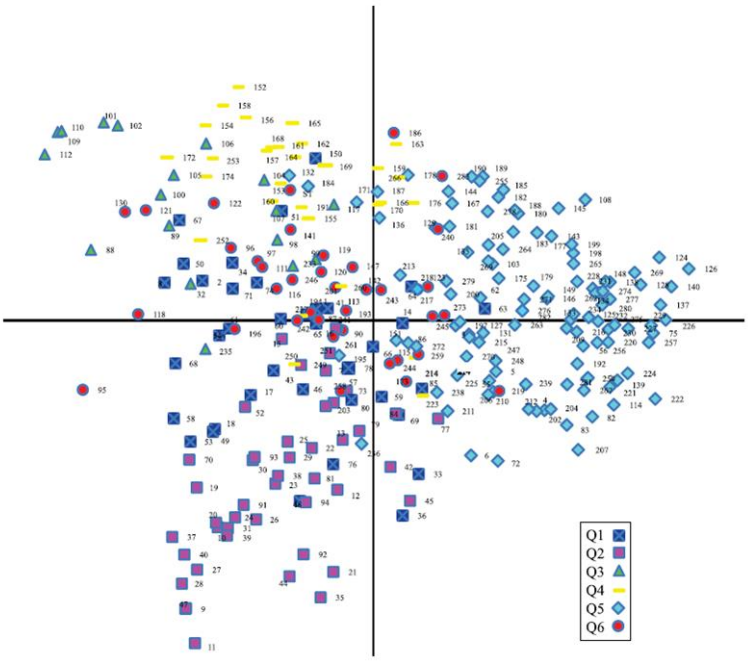

Fig. 2: Principal coordinate analysis of the 283 wheat (*Triticum aestivum*) varieties. Each mark represents a sample obtained by the Structure software (Pritchard et al., 2000). Q1 to Q6, genotype clusters on the Q matrix.

Table 1: Markers associated ($p \leq 0.05$) with flowering time in wheat using the general and mixed linear model, the mean value of phenotypic variation (%) and reference.

Chrom.	Locus	Year	Average R^2	MTAs previously reported
1B	GWM11	2003, 2005, 2006, 2007, 2008	5.88	Brbaklic et al., 2013
	WMC44	2005	12.97	
1D	WMC216	2000, 2001, 2003	8.38	Roy et al., 2006
2A	GWM294	2007, 2008, 2009	8.79	
2B	BARC101	2006, 2007, 2008, 2009	7.14	
	GWM148	2006, 2007, 2008	8.08	Lukman, 2003
2D	WMC144	2002, 2005	5.49	
	WMC167	2003, 2008	7.81	Roy et al., 2006
4A	GWM160	2008	4.77	Zanke et al., 2014
5B	GWM271	2006, 2007	3.90	
	WMC28	2000, 2001, 2004	8.16	
6A	WMC333	2006, 2007, 2009	2.93	
6D	PSP3200	2001, 2005	6.27	Quarrie et al., 2003; Dodig et al., 2012
7A	CFA2257	2005	5.90	
	WMC83	2000, 2001, 2009	6.82	Roy et al., 2006

Conclusions

According to results of our study high genetic polymorphism of analyzed material was revealed by molecular evaluation. The population structure distributed genotypes into six subpopulations consistent with their origin. 16 SSR markers out of 30 analyzed were in significant associations with flowering time. 8 markers (GWM11-1B, WMC216-1D, GWM294-2A, BARC101-2B, GWM148-2B, WMC28-5B, WMC333-6A and WMC83-7A) were stable in at least 3 years. 7 marker-trait associations (GWM11-1B, WMC216-1D, GWM148-2B, WMC167-2D, GWM160-4A, PSP3200-6D and WMC83-7A) previously reported in studies conducted in our or different agro-climate regions were confirmed. 4 potential QTLs for Fl were detected (GWM294 -2A, BARC101 -2B, WMC28-5B, WMC333 – 6A).

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Interactive effects of water and salt stress on wheat growth and productivity under greenhouse condition

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Abstract

The objective of the study was to use the HAS-Shoot Stress Diagnostic System to analyse salt tolerance of wheat genotypes under well-watered and drought conditions. The experiment was conducted with 14 wheat (*Triticum aestivum* L.) cultivars, from Serbia (5), Austria (4) and Azerbaijan (5), which were chosen on the basis of data available for their salt and drought tolerance.

Plants were grown under 4 watering/salt conditions:

1. Well-watered (60 % field capacity) and no salt (NaCl) added (control 1),
2. Water limited (20 % field capacity) and no salt (NaCl) added (control 2),
3. Well- watered (60 % field capacity) and saline conditions (0.2% NaCl),
4. Water limited (20 % field capacity) and saline conditions (0.2% NaCl).

Various morphological, physiological, and biochemical parameters involved in salt and drought tolerance processes were investigated at different developmental stages. The results showed large difference among the studied cultivars, and revealed tolerant and sensitive genotypes. The best performance in total grain yield under salt stress alone was observed in the NS-Avangarda, Gobustan and Tale-38 cultivars, while under water stress alone the NS 40S, Gyrmyzy gul-1 and Gobustan showed the highest grain yield. Under conditions of combined water and salt stress the Capo and Tale-38 showed the best performance. The obtained results can help the breeders from three different geographic regions in the selection and crossing programs to achieve good level of drought/salt tolerance.

Introduction

Soil salinization is caused by natural processes or anthropogenic activity, such as irrigation in the arid or semi-arid regions. There are over 800 million hectares of saline soils worldwide, which is approximately 6% of the total world land area. When it comes to global cultivated area, the percentages rise much higher, reaching 23% of total arable land and around 20% of irrigated land affected by secondary salinization (El-Hendawy et al., 2005). Moreover, ratio of saline soils is projected to increase even more in the time to come, due to inadequate irrigation drainage, rising sea level and global climate changes (Munns and Gilliham, 2015).

Development and productivity of most crops is at risk under high soil salinity, but different adaptations can enable some crop cultivars to continue development and yield satisfactorily under moderate soil salinity. Negative effects of salts are even more profound when coupled with drought, which is a common natural setting. One of the possible ways to alleviate the negative effects of salinization and drought on the global food industry is to improve salt/drought tolerance in crops or to introduce new species into cropping systems (Zhang et al., 2014).

Salt and drought tolerance of crops may vary with their growth stage. Identifying the multiple parameters associated with drought/salt tolerance during different growth stages is important for evaluation of crop genotypes (Arzani, 2008; Langridge and Reynolds, 2015). Given the rapid development of high-throughput genotype screening in plant breeding and genomics for related growth, yield and tolerance to different biotic and abiotic stresses (Tuberosa 2012; Langridge and Reynolds, 2015;), there is a call for more effective and reliable phenotyping data to support modern genetic crop improvement. Effective, high-throughput phenotyping platforms have recently been developed in growth chambers or greenhouses to solve this problem. These platforms use robotics, precise environmental control and imaging technologies to assess plant growth and performance (Li et al., 2014). Such precise phenotyping data obtained by using these technologies may facilitate improvement of drought/salt tolerance in breeding programs.

The objectives of the study were:

- To use the HAS-Shoot Stress Diagnostic System to analyse salt tolerance of selected wheat cultivars under well-watered and drought conditions.
- To analyse the synergistic effect of salinity and drought stress on wheat growth and development.
- To obtain information on salt tolerance of cultivars originating from different geographical locations (Austria, Azerbaijan, Serbia).

Material and methods

Fourteen genotypes of winter wheat (*Triticum aestivum* L.), originating from different countries (Serbia -5, Austria-4 and Azerbaijan-5) were used in the study. The cultivars were chosen on the basis of data available for their salt and drought tolerance.

The experiment was carried out in a greenhouse of CR Ltd., Szeged, from December 2013 to the middle of June 2014. Soils with defined nutrition levels were used for planting and water was provided by computer controlled protocols.

Four watering/salt conditions in the soil were applied:

- Well-watered (60 % field capacity) and no salt (NaCl) added (control-C),
- Water limited (20 % field capacity) and no salt (NaCl) added (treatment 1-T1),
- Well-watered (60 % field capacity) and saline conditions (0.2% NaCl)(treatment 2-T2),
- Water limited (20 % field capacity) and saline conditions (0.2% NaCl))(treatment 3-T3).

The shoot growth parameters were analysed during the whole life cycle of the chosen wheat cultivars by using semi-automated Complex Stress Diagnostic System, constructed by HAS-BRC and CR Ltd. These measurements provided information on total green biomass change during the cultivation period. Water use profiles were recorded at the level of individual plants during the whole cultivation period from which the efficiency of water usage, as well as the effect of NaCl on water utilization was determined.

At the end of the experiment grain production parameters (over ground mass, plant height, no. of spikelets and seed per spike, thousand kernel weight, etc.) were also determined.

Results

The results have shown that salt stress (0.2 NaCl/kg soil) and drought stress (20 % field capacity) when applied separately caused a reduction of all parameters examined, but the negative effects of these stresses were different (Table 1). In general, the negative effect of drought stress on most of the parameters was higher than effect of salt stress. Only in case of net photosynthesis rate (NPR) the effects of both stress treatments were very similar in most of the genotypes. However, when both stresses were applied together the negative effect was dramatically increased, except in case of NPR.

The green biomass (GB) was significantly affected by salt stress under well-watered conditions (60% field capacity). In average for all the genotypes, the GB was reduced by salt stress around 20% in relation to the control. But the effect of drought stress was significantly higher (around 50% average reduction), and also when salt and drought were applied together GB decreased drastically to the capacity of 10-30% of the well-watered no salt control. All stress treatments had very similar effects to the water usage (WU) as well (Table 1).

Table 1. Responses of wheat cultivars to different stress treatments in relation to the well-watered no-salted control.

Cultivar	**GB (%)**				**WU (%)**				**TGY (%)**			
	C	**T1**	**T2**	**T3**	**C**	**T1**	**T2**	**T3**	**C**	**T1**	**T2**	**T3**
Tale 38	100	50,7	86,8	26,9	100	40,0	92,5	23,7	100	51,3	93,5	24,8
Azamatli 95	100	44,4	81,9	13,1	100	36,7	92,1	16,6	100	43,8	82,0	10,4
Giymatli 2/17	100	44,0	60,3	9,8	100	39,8	72,2	13,9	100	48,0	60,4	18,0
Gobustan	100	53,8	82,9	16,3	100	45,5	92,0	19,6	100	49,1	85,6	15,3
Gyrmyzy gul	100	56,4	78,7	20,9	100	41,0	78,4	19,0	100	52,4	77,0	18,4
Balkan	100	54,2	78,6	18,6	100	40,5	80,3	17,6	100	53,4	78,5	16,8
NS 40S	100	52,8	75,4	18,1	100	44,7	73,1	19,1	100	51,3	72,4	16,3
NS Avangarda	100	41,9	82,9	14,3	100	32,3	79,4	14,7	100	39,5	85,5	12,6
Suboticanka	100	58,2	81,4	16,8	100	43,3	85,9	19,2	100	49,5	72,4	13,2
Renesansa	100	39,1	74,2	13,8	100	33,8	80,5	17,1	100	37,4	75,8	12,6
Donnato	100	48,6	92,8	16,1	100	36,4	78,7	15,6	100	41,9	92,3	15,7
Midas	100	53,5	75,6	22,6	100	48,9	89,7	23,4	100	50,7	72,3	21,0
Gallio	100	65,6	103,8	16,7	100	56,6	117,3	17,4	100	62,7	107,2	12,7
Capo	100	46,3	86,6	29,3	100	42,0	97,6	25,0	100	37,2	83,0	28,4
Average		**50,7**	**81,6**	**18,1**		**41,5**	**86,4**	**18,7**		**47,7**	**81,3**	**16,9**

GB-green biomass; WU-water usage; TGY-total grain yield; C – well-watered (60 % field capacity) and no salt (NaCl) added; T1 - water limited (20 % field capacity) and no salt (NaCl) added; T2 – well-watered (60 % field capacity) and saline conditions (0.2% NaCl); T3 - water limited (20 % field capacity) and saline conditions (0.2% NaCl)

Comparison of values for grain yield per plant has shown that the best performance under salt stress alone was observed in the cvs. NS-Avangarda (5,3 g), Gobustan (5,2 g) and Tale-38 (5,2 g), while under water stress alone the NS 40S (3,1 g), Gyrmyzy gul-1 (3,0 g) and Gobustan (3,0 g) showed the highest grain yield. In case when both stresses were applied the best grain yield had cvs. Capo (1,5 g) and Tale 38 (1,4 g), while the cv. Azamatli-95 (0,4 g) had the lowest yield.

The reduction of total grain yield in relation to the well-watered and no-salted control varied significantly among the treatments and the cultivars. Total grain yield (TGY) was reduced to the capacity of 37-63% in response to the drought stress, changed from 60-107% in response to salt stress and reduced dramatically to the ca. of 10-28% of its control value when salt stress was combined with limited water availability (Table 1).

Regarding the all analysed traits the cultivars had different reactions to the applied treatments. According to the values shown in Table 1, it can be concluded that the most salt sensitive cultivar was Azerbaijan cv. Giymatly-2/17, because it had the minimal values for three out of four analysed traits. The most salt and drought tolerant was Austrian cultivar Galio, with the lowest reduction of grain yield at the stress treatments in relation to the control. All other cultivars had very similar reactions to salt and drought stresses. In case when both stresses were applied the best grain yield had cvs. Capo and Tale 38, while the cv. Azamatli-95 had the lowest yield.

The results are in accordance with previous findings that a salinity-tolerant genotype could also be drought tolerant, and has largely identical mechanisms to deal with these stresses (Ashraf and O'Leary 1996; Farooq and Azam 2001, Zhang et al. 2014). It happens because there are some common features between drought and salinity stresses in plants. Both stresses impose cellular dehydration, which causes osmotic stress and removal of water from the cytoplasm into the intercellular space. Early responses to drought and salt stress are also similar to each other, except for the ionic component in the cells of plants under salt stress. However, the responses of crops to a combination of these stress factors appear to differ from the responses to a single stress (Pinto et al. 2010). Therefore, more attention should be paid to the joint impact of a combination of stresses of drought, salinity and high temperature. Exploiting the genetic variability in crop species may be a useful strategy to improve climatechange-related stress tolerance (Zhang et al. 2014).

Conclusion

On the basis of all presented results it can be concluded that:

- Drought stress had significantly higher negative effect on wheat growth and development then salt stress alone, while the combination of two stresses had additional adverse effect on wheat productivity.
- The results showed large difference among the studied cultivars, and revealed tolerant and sensitive genotypes.
- The best performance in total grain yield under salt stress alone was observed in the NS-Avangarda, Gobustan and Tale-38 cultivars, while under water stress alone the NS 40S, Gyrmyzy gul-1 and Gobustan showed the highest grain yield.
- Under conditions of combined water and salt stress the Capo, Tale-38, and NS-40S showed the best performance. Grain yield stability was also the highest in the Capo and Tale-38.
- The obtained results can help the breeders from three different geographic regions in the selection and crossing programs to achieve good level of drought/salt tolerance.

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Germplasm development to study rare alleles at the *Xgwm261* locus

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Introduction

In bread wheat, there is a general correlation between reduced height and reduced yield (Law et al., 1978). The most influential breeding strategy of the 20th century was the introduction of the major semi-dwarfing genes *Rht-B1b* and *Rht-D1b* into Mexican (CIMMYT) germplasm by Norman Borlaug. These genes break the height/yield correlation. *Rht-B1b* and *Rht-D1b* are gibberellin (GA) insensitive and in optimal conditions, reduce plant height by 15-35% (Gale and Youssefian, 1985, Trethowan et al., 2001) whilst increasing yield to similar levels (Worland and Law, 1986). Originally derived from the Japanese cultivar 'Norin 10', these genes became prevalent in CIMMYT wheat varieties and are now found in the majority of modern wheat cultivars (Hedden, 2003). However, *Rht-B1b* and *Rht-D1b* are not universally beneficial. Where heat stress occurs during ear emergence, interactions between these dwarfing genes and the environment have been shown to reduce fertility resulting in a yield penalty (Worland and Law, 1986). Furthermore, poor seedling emergence due to reduced coleoptile length and maladaptation to dry environments are other problems associated with *Rht-B1b* and *Rht-D1b* (Botwright et al., 2005, Rebetzke and Richards, 1999, Trethowan et al., 2001). In these conditions, the GA-responsive semi-dwarfing gene *Rht8* produces a semi-dwarf phenotype without the undesirable effects of the Norin 10-derived genes (Ellis et al., 2004, Rebetzke and Richards, 1999). Pre-dating Borlaug, the Italian wheat breeder Strampelli introduced *Rht8* to Europe from the Japanese variety 'Akakomugi'.

Using a chromosome substitution line between Cappelle-Desprez and the Strampelli cultivar Mara, Korzun et al. (1998) reported a tight linkage of 0.6cM between *Rht8* and a 192-bp allele at the microsatellite locus *Xgwm261*. A screen of over 800 wheat varieties revealed that 90% of varieties carried the three most common alleles of 165-bp, 174-bp or 192-bp at this locus (Worland et al., 1998, Worland et al., 2001). A height-reduction of 7-8cm was attributed to the 192-bp allele relative to the 174-bp allele; a 3cm reduction was found in varieties carrying the 174-bp relative to 165-bp, and the 165-bp was found to be neutral for height. It was therefore suggested that genotyping at *Xgwm261* represents a simple method to assay for variants at the *rht8* locus, and that a 192-bp allele was diagnostic for *Rht8* (Worland et al., 1998, Worland et al., 2001).

However, it was reported by Ellis et al. (2007) that the 192-bp allele at this locus is not always diagnostic for the height-reducing *Rht8*. Instead, the *Xgwm261*-192-bp allele is only indicative of *Rht8* in wheat cultivars that have inherited this allele from Akakomugi or a Strampelli-wheat ancestor. The authors found that Norin-10 derived material has an identical 192-bp allele at the *Xgwm261* locus which is not associated with *Rht8* and suggested that this alternative haplotype evolved prior to the *Xgwm261*-192-bp linkage with *Rht8*. Furthermore, it was reported that *Xgwm261* maps further away from *Rht8* (1.95 cM) (Gasperini et al., 2012) than previously described (0.6 cM) (Korzun et al., 1998). Taken together, the linkage between the 192-bp allele at *Xgwm*261 and *Rh8* can be broken, thus a 192-bp allele at this locus is insufficient to unequivocally determine whether a particular cultivar carries *Rht8*.

Despite the more complex relationship between the *rht8* locus and *Xgwm261* than initially believed, genotyping at *Xgwm261* is likely to remain a popular method to assess the allelic variant at *rht8*, at least in conjunction with other information, such as pedigree or height-reducing effect. This is for two main reasons. First, *Xgwm261* is multi-allelic whereas *DG279* and *DG371*, previously reported flanking markers to *Rht8*, are bi-allelic and showed very low polymorphism across a diversity wheat panel (Gasperini, 2010). The markers developed in the PhD thesis building on this work (Kowalski, 2015) remain untested for the extent of their multi-allelism. Further, novel KASP markers closely linked to *Rht8*, developed by Kowalski, 2015, have been provided to breeders to test the performance of these markers relative to *Xgwm261*. One breeder reported 100% match with the 192-bp allele in Akakomugi-derived material (personal communication). Therefore, breeders will likely use these new high-throughput markers in addition to the well-established *Xgwm261* screen. Second, *Xgwm261*, in addition to the SSR-markers developed by Kowalski, 2015, will still be used by breeders in countries where SNP markers and the associated technology is not yet prevalent.

Despite the importance of understanding the variants at the *Xgwm261* locus, almost all research, analysing allelic distribution or otherwise, has focused on height-related effects only. Therefore germplasm development to enable better understanding is crucial. The 192-bp allele at *Xgwm261* is found in Bulgaria, Greece, former Yugoslavia, Ukraine, China, North America and more recently Australia (reviewed in Asplund et al., 2012). Other than the most common 165-, 174- and 192-bp alleles, multiple genotypic screens have reported distinct and less prevalent ('rare') *Xgwm261* alleles ranging from 180- to 220-bp (Ahmad and Sorrells, 2002, Asplund et al., 2012, Bai et al., 2004, Bakshi and Bhagwat, 2012, Chebotar et al., 2001, Ganeva et al., 2005, Liu et al., 2005, Worland et al., 1998, Worland et al., 2001, Yediay et al., 2011). The precise number of these rare alleles remains uncertain, since it has been demonstrated that variations of 2-5bp are 'stutter' as a result of polymerase slippage during amplification of the alleles (Schmidt et al., 2004), and the same authors speculated that many scientists did not adjust allele sizes to produce uniformity of results in line with previous investigations. For these reasons, the reporting of 'novel' *Xgwm261* alleles, varying by only two bp from previously reported alleles, should be treated with caution (e.g. as in Bakshi and Bhagwat, 2012). Despite ambiguities about the precise number, 'rare' alleles at *Xgwm261* exist and their adaptive significance remains poorly understood. This is despite 'rare' alleles (in terms of global distribution) being highly prevalent in certain germplasm collections e.g. Argentinian wheat with 42% of varieties reported to contain a 210-bp allele (Worland et al., 2001), which would indicate non-random selection by breeders or founder effects.

In order to determine the agronomic significance, including height and yield components, of allelic variants at *Xgwm261*, rather than only cataloguing diversity at the locus, the alleles need to be studied in a common genetic background. Work to achieve this was first mentioned in Worland et al., 2001 (p.159). A range of alleles at *Xgwm261* were selected and backcrossed into a UK-adapted winter wheat, Mercia, used by Worland and colleagues to study other genes such as *Ppd*. Since this first mention in 2001, adaptive significance of the alleles at the *Xgwm261* locus remains poorly studied. One analysis of distribution of 192- and non-192-bp genotypes showed no advantage of the 192-bp allele to coleoptile elongation in 135 US and Chinese winter wheat cultivars (Bai et al., 2004). Further, a screen on a 19th century wheat collection revealed no correlation between genotype at *Xgwm261* and plant height, but the authors cited height measurements taken from small, non-replicated plots as a possible reason for this result (Asplund et al., 2012). Another study found that all Bulgarian cultivars carrying the rare 203-bp allele were the earliest in heading and also had increased yield due to increased spikes per area (Ganeva et al., 2005), but these effects were not dissected away from other genes determining

earlier flowering on 2D, since the allele was studied in different genetic backgrounds. Clearly, isogenic lines grown in yield-size plots in replicated conditions are required in order to unambiguously determine the pleiotropic effects of the *Xgwm261*-allelic variants. The germplasm first mentioned in Worland et al. (2001) was recovered from the JIC. The development of this germplasm is described here.

Our current knowledge of the adaptive significance of variants at *Xgwm261*, the extent to which they reveal variation at *Rht8* and the pleiotropic effects of *Xgwm261* variants is poor. The importance of studying the agronomic performance in a comprehensive way, other than just height effects, has been demonstrated (Kowalski, 2015). The *Rht8* allele from Mara has great agronomic importance in reducing lodging and has no yield penalty (and a non-significant higher mean yield) in certain agronomic conditions (Kowalski, 2015). Additionally, it was shown that an interesting spike morphology closely segregates with *Rht8*. The allelic diversity of the *Rht8* flanking markers developed, mapping closer to *Rht8* than *Xgwm261*, *DG279* and *DG371* remains untested, but they could be used by breeders in conjunction with typing for *Xgwm261* in worldwide breeding programmes. With the aim of filling this gap in our knowledge of variants at *Xgwm261*, the isogenic lines first described by Worland et al. (2001), were developed here and will provide the basis for ongoing work.

Recovered germplasm and development pipeline

Rare *Xgwm261* alleles, as well as the 192-bp Mara-derived allele were introgressed into Mercia in 2006 by Liz Sayers at JIC, in multiple streams. Mercia carries the 174-bp allele at *Xgwm261* and also *Rht-D1b* (GRIS, 2015). The alleles which were introgressed were derived from Maringa, a Brazilian wheat, Pliska, of Bulgarian origin (reported initially as 201-bp by Worland et al., (2001) and later as 203-bp (Ganeva et al., 2005), Klein 157 and Klein 49 (Argentinian wheats, reported originally as 210-bp and 215-bp, respectively, by Worland et al., (2001)). Extant allele sizes as described in the initial screen were used here for continuity (Sayers, personal communication), even though the actual sizes detected were larger due to the tailed primer used in conjunction with a labelled adapter (shown in Appendix). Four streams produced fertile heterozygous seed (Fig. 1). The F_2 seeds from each stream were planted and genotypes collected. The segregation patterns for the introgressed *Xgwm261* allele did not significantly deviate from 1:2:1 Mendelian ratios (Table 1). The plants homozygous for the parent and donor alleles were grown to maturity in the glasshouse and bagged in order to bulk seed.

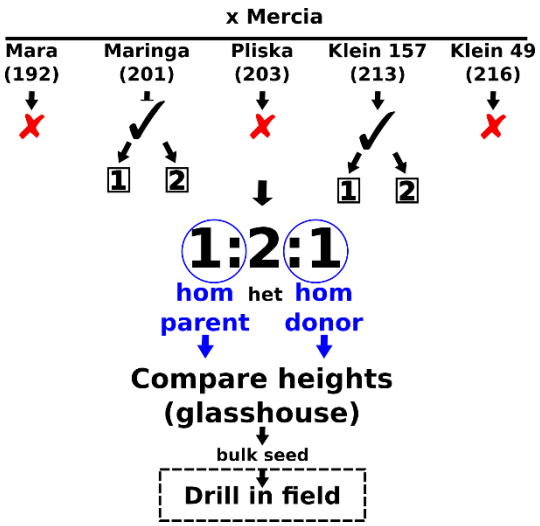


Fig. 1: Germplasm development pipeline for rare *Xgwm261* variants. Material was recovered from the introgression of rare *Xgwm261* alleles (sizes indicated in brackets) into a common Mercia background. Four successful streams are highlighted from which the homozygous plants for donor and parent allele were selected to be grown in the glasshouse. This seed has been bulked and will next be drilled in replicated plots in the field in order to assess agronomic performance.

Table 1: Segregation for *Xgwm261* in the F_2 germplasm in the Mercia background . The P-value at two degrees of freedom was calculated for each Chi-square value. The test value for Chi-square tests is: $H_0 = 5.99$, N = 48.

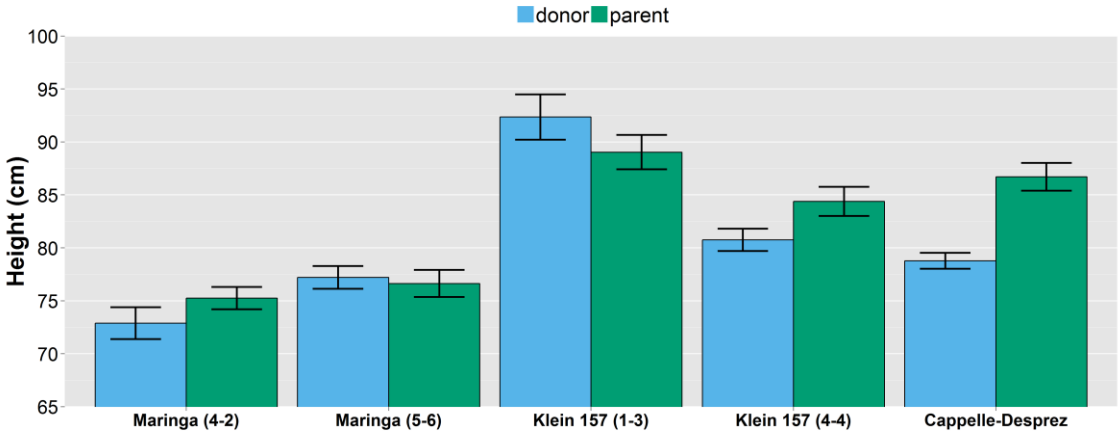
Cross and stream	Size		Frequency	%	χ^2 1:2:1	*P* 2 d.f.
Maringa x Mercia 4-2	174 and 201	het	27	56	0.792	0.673
	174	hom parent	10	21		
	201	hom donor	11	23		
Maringa x Mercia 5-6	174 and 201	het	24	50	0.000	1.000
	174	hom parent	12	25		
	201	hom donor	12	25		
Klein 157 x Mercia 1-3	174 and 213	het	28	58	2.833	0.243
	174	hom parent	13	27		
	213	hom donor	7	15		
Klein 157 x Mercia 4-4	174 and 213	het	19	40	3.125	0.210
	174	hom parent	17	35		
	213	hom donor	12	25		

Preliminary height measurements

In order to determine the effect of the donor allele on height, the plant height and internode lengths were measured at maturity. The homozygotes for the donor versus parent (*Mercia*) allele were compared using the Student's T-test. Cappelle-Desprez and RIL4 were grown concurrently as controls in order to compare the effects of the rare *Xgwm261* alleles to the 192-bp allele. In

glasshouse conditions, the Mara-derived 192-bp allele in RIL4 had an 8-cm height-reducing effect and consistently highly significant reduction in spike length and internodes measured, relative to the wild-type Cappelle-Desprez (Fig. 2). Neither streams from the Maringa introgression into Mercia showed a significant difference in heights. The Maringa allele was also neutral for reduction in the spike and internode length. In the Klein 157 x Mercia streams, there were two significant differences between the donor- and Mercia-allele. In one stream (4-4), the donor allele had a significant overall height-reducing effect of 3.6cm, but no further differences in height components. The other stream (1-3), had no difference in overall height, but the donor allele had a length-promoting effect in the first internode (Table 2).

Fig. 2: Plant height at maturity of homozygous individuals within each stream, contrasting for



donor and parent allele at the Xgwm261 locus. Cappelle-Desprez parent is wild-type, donor is RIL4. Data represent means, error bars represent standard error. N shown in Table 1 and for Cappelle-Desprez and RIL4, N = 16.

Table 2: Plant height and height components of Xgwm261 allele introgressions. P-values to student's T-test comparing the donor and parent alleles within each stream are shown. Means are based on N shown in Table 1. All units are in centimetres.

lowest highest

		Total height		Spike length		Internode-1		Internode-2	
		mean	p-val	mean	p-val	mean	p-val	mean	p-val
Cappelle-Desprez	parent	86.7	***	10.2	**	32.4	***	17.9	***
RIL4	donor	78.8		9.5		26.4		16.4	
Maringa x Mercia 4-2	parent	75.3	NS	9.2	NS	26.0	NS	16.7	NS
	donor	72.9		9.4		25.6		16.3	
Maringa x Mercia 5-6	parent	76.6	NS	9.7	NS	26.3	NS	17.0	NS
	donor	77.2		9.7		24.7		17.3	
Klein 157 x Mercia 1-3	parent	89.0	NS	8.5	NS	28.9	*	21.7	NS
	donor	92.4		8.9		33.5		22.8	
Klein 157 x Mercia 4-4	parent	84.4	*	8.5	NS	29.2	NS	22.2	NS
	donor	80.8		9.4		32.4		22.4	

NS P>0.05
* P<0.05
** P<0.01
*** P<0.001

Discussion

The work here progresses the development of germplasm which will enable the agronomic effects of rare *Xgwm261* alleles to be assessed in the field. Measurements from plants grown in the glasshouse revealed an overall height-reducing effect of the Maringa allele (201-bp) in only one of the streams by 3.6cm. This is approximately comparable to the reduction of the 174-bp allele relative to 165-bp allele described previously (3cm) (Worland et al., 1998), but modest with respect to the 8cm reduction from the 192-bp allele. The isogenic lines contrasting for the rare donor and common background parent allele have been bulked which will enable replicated plots to be drilled in the field, and other agronomic traits to be measured.

It was shown in Kowalski (2015) that it can be crucial to verify height effects observed in glasshouse-grown plants in the field. In the same thesis, a reported 8cm difference in heights was not observed in the glasshouse due to confounding factors. Therefore, the even more modest height effects reported here require verification in the field.

Germplasm development is a crucial resource, since advancing generations requires time and often limits projects in crops. At the same time, germplasm development can be perceived to be an unglamorous task and usually continued over time by different people. Often, continuity and records are lacking. The work here in advancing a germplasm resources was therefore included for transparency.

Cloning *Rht8* will be an assured way of understanding the adaptive significance of variants at *Xgwm261* and will also likely identify new alleles at the *Xgwm261* locus.

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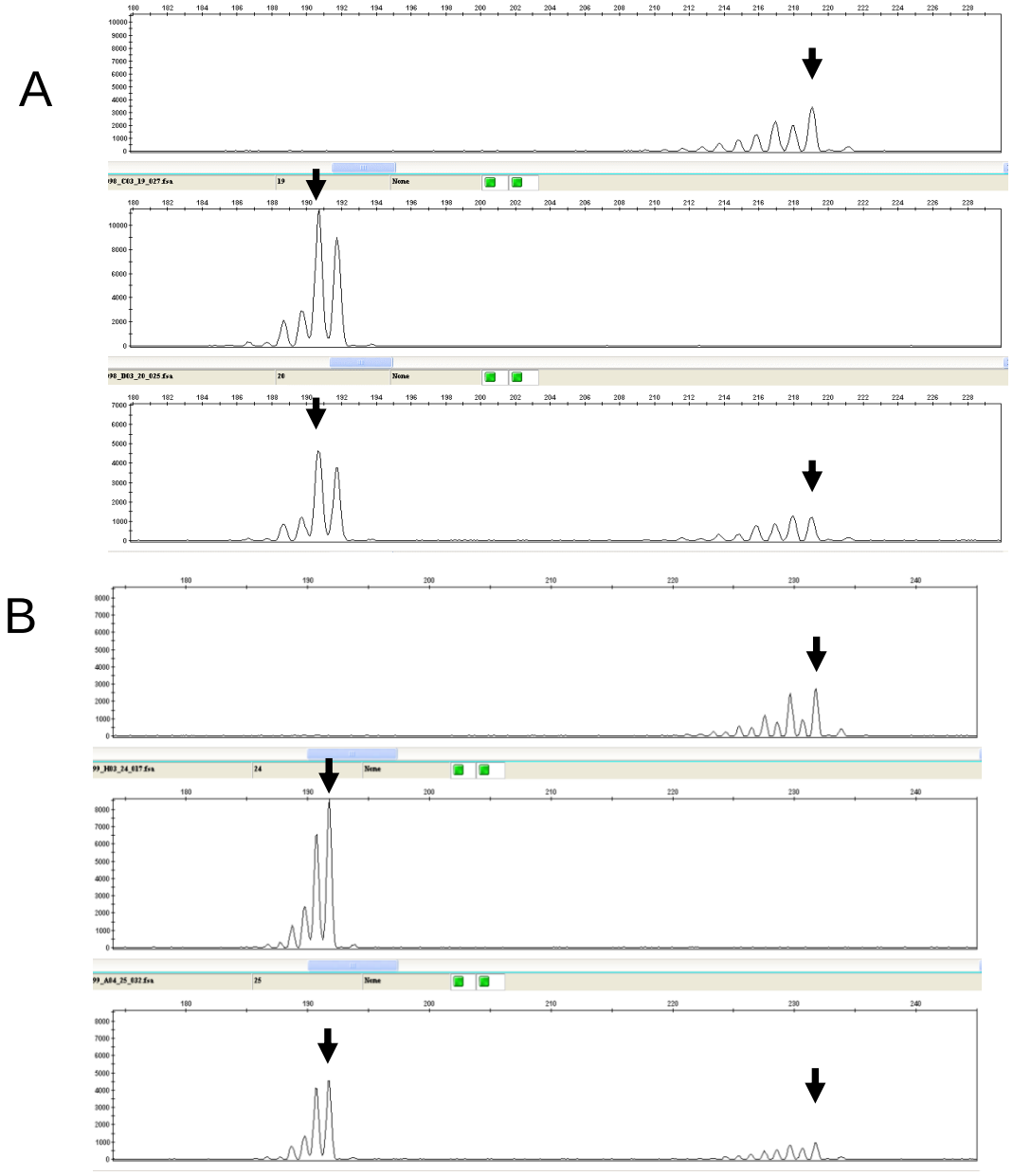
Appendix



Allelic variation at *Xgwm261*. The arrows show the calculated size of each DNA fragment. (A) Maringa x Mercia 4-2: top = donor (Maringa, 219-bp), middle = parent (Mercia, 191-bp), bottom = heterozygous. (B) Klein 157 x Mercia 4-4: top = donor (Klein 157, 231-bp), middle = parent (Mercia, 191-bp), bottom = heterozygous.

Molecular approach to validate the transfer of APR-*Lr* genes into Romanian adapted wheat genotypes

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Abstract

The continuous and unpredictable evolution of rust diseases, under global and local climate changes, emphasized resistance as one of wheat breeding priorities. In Romania, leaf rust (LR) caused by *Puccinia triticina* has been for many years the most frequent rust in wheat crop, causing yield losses. To date, 74 leaf rust resistance genes have been catalogued in wheat, but only a few of these are race non-specific and confer adult plant resistance (APR) quantitatively expressed in terms of *slow-rusting*, which is considered to be more durable. The *Lr34*, *Lr46*, *Lr67* and *Lr68* genes with pleiotropic adult plant resistance (PAPR) to leaf rust, stripe rust, powdery mildew, and stem rust are the most studied.

The aim of this study was to validate the transfer and pyramiding of *Lr34*, *Lr46* and *Lr67* genes into the adapted Romanian bread wheat genotypes Glosa and Miranda, using molecular markers. Analysis of F3 lines from crosses developed at NARDI Fundulea revealed the transfer of targeted *Lr* genes. Out of the 224 analysed genotypes, 45 genotypes presented the *Lr34* and *Lr46* genes and one genotype the *Lr34* and *Lr67* genes. Phenotypic distribution analysis of leaf rust severity and AUDPC, under field artificial inoculations, showed generally a higher resistance in carriers of pyramided *slow-rusting Lr* genes. Additional presence of other, still unknown, *Lr* genes could not be excluded. Deployment of more APR *Lr* genes, validated by molecular markers will allow the successful development of pre-breeding Romanian bread wheat genotypes with improved durable resistance to leaf rust.

Introduction

In Romania wheat plays an important role in the national economy, being grown on about 2 million hectares and its production was 7.5 million tons in 2014 (Anonymous 2015). A limiting factor in the world wheat production is represented by foliar and head diseases such as rusts, powdery mildew, fusarium head blight, smuts, etc. In Romania the leaf and stripe rusts represent a threat for wheat production, and so, a continuous challenge for breeders is to obtain new cultivars with improved resistance, particularly of durable (APR) type to these pathogens. Developing and growing of resistant cultivars is an effective and friendly to environment way of protecting crops against pathogens.

Most of leaf rust resistance genes confer race-specific resistance, acting in "gene for gene" manner as seedling plant resistance genes, but the resistance provided by these genes can be short-lived, as new races of the pathogen appear. There are a few *Lr* genes that confer non- race-specific resistance, acting at the adult plant stage (APR- Adult Plant Resistance), as partial resistance. This small group of leaf rust resistance genes is named "slow-rusting" group; although, their effect is smaller than that of the race-specific genes they had remained durable in time (Singh et al., 2003; Lagudah, 2011). The effect of slow-rusting genes is also more dependent on environmental conditions. The best known slow-rusting *Lr* genes are *Lr34* (Dyck, 1977), *Lr46* (Singh et al., 1998), *Lr67* (Hiebert et al., 2010 and Herrera Foessel et al., 2011) and *Lr68* (Herrera-Foessel et al., 2012).

The aim of this study was to validate the transfer and pyramiding of ***Lr34, Lr46*** and ***Lr67*** genes into the adapted Romanian bread wheat genotypes **Glosa** and **Miranda**, using molecular markers.

Materials and methods

A total of 224 lines were used for ***Lr34, Lr46*** and ***Lr67*** genes detection by molecular markers system.

Donor parents for target slow rusting genes:

-*Lr34*: RL6058 (Thatcher*6/PI158548), by the courtesy of Dr. M. Csösz (Cereal Research Non-profit Ltd. Company, Szeged, Hungary and Prof. Beat Keller, UZH-IPB, Switzerland).

-*Lr46*: Pavon 76-(Source: CIMMYT);

-*Lr67*: RL6077 (Thatcher*6/PI250413), by the courtesy of Dr. C. Hiebert (CRC, Agri-Food Canada).

Romanian Adapted genotypes:

- Glosa (released in 2005; obtained by DH system) - described as MR to LR, carrier of *Lr34* (Ciuca et al., 2015);

- Miranda (released in 2010; obtained by DH system) - characterized as susceptible to LR.

In these two cultivars presence of effective seedling resistance genes is unknown.

Field test

The experimental field was placed in Fundulea (44°27′10″N), plots - each 1.20 m row alternating with spreaders rows. Artificial inoculations were made with *P. triticina* isolate from the local population virulent to: *Lr1, Lr2c, Lr3, Lrbg, Lr10, Lr14a, Lr15 and Lr37 (at seedling stage).*

Disease severity (DS) was scored using the Modified Cobb Scale (Petterson et al., 1948)

The area under disease progress curve for leaf rust severity (LR AUDPC) was calculated according to the equation in Roelfs et al. (1992).

DNA isolation and PCR analysis for *Lr34*, *Lr46* and *Lr67*genes

Genomic DNA was isolated from leaves, harvested from field, using CTAB method by Sagai-Maroof et al., 1984. PCR was conducted using functional and SSR markers presented in table 1.

Table1: Molecular markers used for LR genes detection

APR Gene	Marker	Gel electrophoresis	Reference
Lr34	Functional – cssfr5	Agarose - 1.5%	Lagudah et al., 2009
Lr46	SSR-wmc44	Agarose - 2.5%	http://maswheat.ucdavis.edu/protocols/Lr46/index.htm
Lr67	SSR-CFD71	6% Non-denaturing Polyacrylamide - gel – silver stain	Hiebert et al., 2010

Results and discussion

MAS-Marker Assisted Selection approach was applied in winter wheat breeding program at NARDI Fundulea to check the transfer of single and multiple leaf rust resistance genes, as well as *Lr34*, *Lr46* and *Lr67*. For this purpose known markers were employed.

In 2014, 224 F3 lines coming from 4 crosses (Glosa x Pavon76; Miranda x Pavon76; Miranda x RL6077 and Glosa x RL6077) were analyzed. The multiplex PCR based on cssfr5 marker for *Lr34* gene showed 53% of lines belong to resistant haplotype, 18% to heterozygous lines and 29% to susceptible haplotype. Regarding to *Lr46* gene detection, molecular assay with wmc44 marker showed that, in our material, 35% of lines were homozygous resistant, 17% heterozygous and 44% homozygous susceptible, but, we found 4% of lines with different PCR products. This result suggested, in our case, that this marker is not so appropriate for markers assisted selection, because the SSR locus Xwmc44 was mapped distal to gene locus (http://maswheat.ucdavis.edu/protocols/Lr46/index.htm).

Among F3 lines from Glosa (*Lr34*) x Pavon76 (*Lr46*) cross, pyramiding events were also found: 45 samples combined both leaf rust resistance genes *Lr34* and *Lr46*. The *slow rusting* phenotypic effect, of pyramiding the *Lr* genes *Lr34* and *Lr46* in F3 derivatives of the cross Pavon76 (*Lr46*) x Glosa (*Lr34*), expressed as AUDPC, was higher in derivative lines carrying both R genes in a homozygous status (Fig.1).

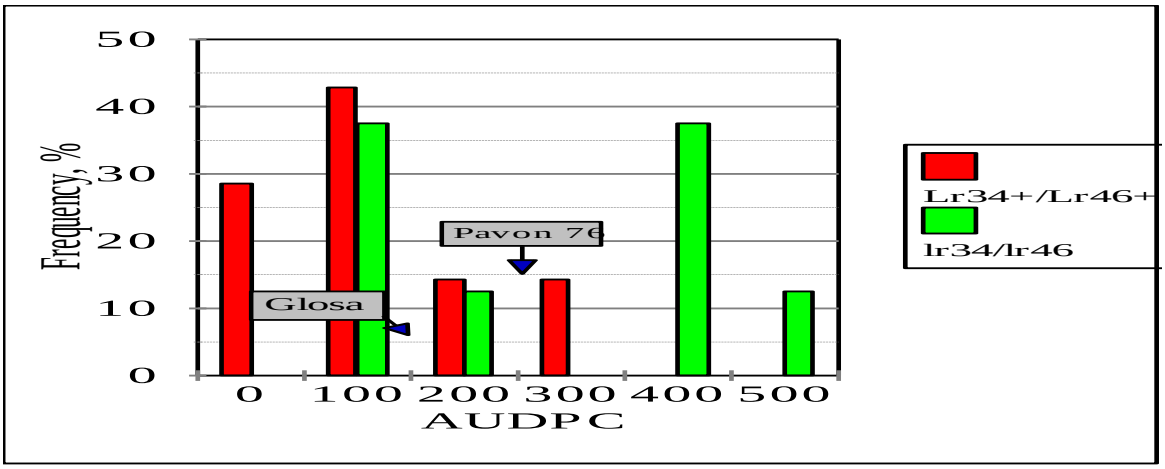

Fig.1: Effect of gene combinations on LR AUDPC

On the other hand, in lines derived from the cross Glosa/Pavon76, homozygous for each *Lr* gene, the effect of gene *Lr34 (Lr34+/lr46-*) on AUDPC was relatively more efficient as compared to *Lr46 (lr34-/Lr46)* (Fig.2).

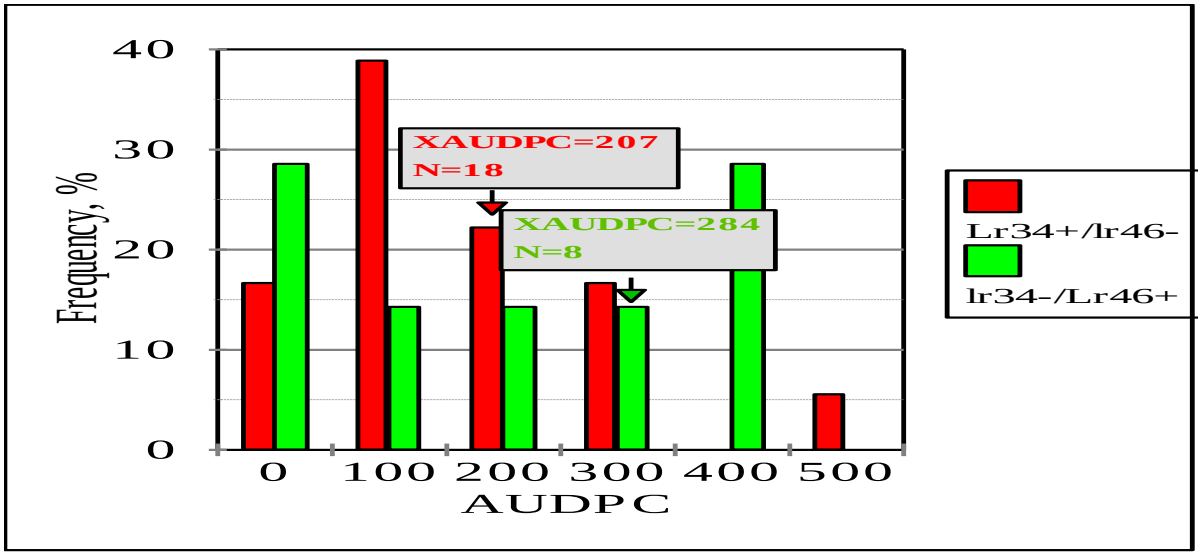

Fig. 2: Effect of single gene on LR AUDPC

Our study was conducted for Lr67 gene detection in Miranda X RL6077 and Glosa x RL6077 crosses. Molecular analysis with SSR marker cfd71 showed 54% homozygous resistant (*Lr67Lr67*), 9% heterozygous (*Lr67lr67*) and 37% homozygous susceptible (*lr67lr67*). A very low number of samples (five) from Glosa x RL6077 cross were analyzed and the results presented one line, from this cross, combined both *Lr34* and *Lr67* genes.

Conclusions

Results suggest the relevance of combining *Lr34* and *Lr46* slow rusting genes and markers assisted selection. MAS proved to be a reliable method to select the genotypes with durable resistance to leaf rust and accelerate the breeding of this type of resistance in wheat.

Acknowledgements

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The study of interaction of genes for spike shape located in 5A chromosome of common wheat

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Introduction

The division of hexaploid wheats on separate species and the subspecies is based on some characteristics of the ear. For example, wheat with a lax elongated spike with poor threshability is attributed to species *T. spelta*. Compact dwarf wheat refers to as species *T. compactum*. Wheat with compresed spike and spherical grains belongs to species *T. sphaerococcum*. The wheat not having such features is a common wheat (*T. aestivum*). These traits are controlled by single genes with pleiotropic effect, called in the Catalogue of Gene Symbols for wheat (McIntosh et al. 2013) "Gross morphology genes" (Table 1).

These genes have been localized and mapped to particular chromosomes. The only full homeological series was found for *S* genes that cause compressed spike and spherical grains are located around the centromere of the chromosomes of the third group.

The gene *Q* was mapped to 5AL chromosome (Kato et al., 1998) and determines the difference between *T. spelta* and *T. aestivum*. Sears, and Muramatsu in 50-60th (Sears, 1954; Muramatsu, 1963) in experiments with a dose of chromosome 5A found that monosomy and nullisomy for 5A chromosome causes speltoidy in the varieties Chines Spring. On the contrary, trisomy and tetrasomy compress the ear. From this, it was concluded that the gene *Q* in wheat inhibits speltoidy. Compressed ear was also observed at saturation of the genome with 5-6 doses of chromosome 5A from *T. spelta*. Accordingly, it was concluded that the *q* allele of *T. aestivum* is less effective and it was identified by a small letter. This designation is included now in the Catalogue of Gene Symbols for Wheat. However, from the usual crosses between *T.spelta* and *T.aestivum* it follows that speltoidy is dominant under normal form of spike. F_1 hybrids between them were always like speltoid (McFadden, Sears, 1946; Filipchenko, 1979). The gene Q^S also causing the speltoidy of spike in bread wheat (Simonov et al. 2009) was introgressed from a diploid species *Aegilops speltoides* Tausch. This speltoidy was dominant and was inherited monogenically; introgression occurred in chromosome 5A (Simonov, Pshenichnikova, 2012).

Gene *C* that causes the characteristic dwarfism and compact spike of species *T. compactum*, was localized to chromosome 2D (Unrau, 1950; Rao, 1972). Later the loci were found in other wheat chromosomes (1AL, 2BS, 2DS, 4AS, 5DL and 6DL) that effect spike compactness to a certain extent (Sourdille et al., 2000, 2003). The independently derived dwarf mutants of common and durum wheat carry mutations in chromosome 5AL and show the allelism (Kosuge et al., 2008; Kosuge et al., 2012). One of such mutant genes, C^{17648}, was introduced into isogenic line of the bread wheat cultivar Novosibirskaya 67 (Kosuge et al., 2012).

The aim of this study was to study of the possible interaction of speltoid genes *Q* and Q^S with the gene C^{17648}, with the opposite effects on the spike shape and located in the same chromosome.

Materials and methods

We used the compact isogenic line ANWB-5A carrying C^{17648} gene and three speltoid samples: introgressive line 84/98^{w} and i:Rodina Q^S both carrying Q^S gene and *T. spelta* Grey carrying the wild allele of *Q* gene (Fig. 1). The compact sample was obtained from Dr. N. Watanabe, College of Agriculture, Ibaraki University, Japan. The isogenic line was created on the basis of the variety Novosibirskaya 67. The donor of the gene was a mutant of durum wheat MA-17648 (Kosuge et al., 2008). The winter speltoid sample 84/98^{w} was obtained from the «Arsenal» collection (Lapochkina, 2001). The line i:Rodina Q^S is the spring speltoid analogue of the line 84/98^{w}. *T. spelta* Grey is from the collection of John Innes Center, Norwich, England.

The following hybrid populations were used in the experiment:

84/98^{w} x ANBW-5A: F_1 – 13 plants, F_2 – 103 (only spring) plants;

i:Rodina Q^S x ANBW-5A: F_1 – 5 plants, F_2 – 130 plants;

T. spelta Grey x ANBW-5A: F_1 – 6 plants, F_2 – 49 plants.

Length of the spike and stem, number of spikelets in the ear and the index of the spike density (number of spikelets per 10 cm spike length) were determined. Experiments were carried out in a hydroponic greenhouse of the Institute of Cytology and Genetics (Novosibirsk) during the autumn and spring seasons of 2014 and 2015.

To statistically analyze the results the observed segregation into phenotypic classes in F_2 was checked for correspondence to the expected segregation by the χ^2 test. We studied the phenotypic distribution in F_2. The analyses were carried out using the MS Excel and Past software packages.

Results and discussion

The plants of the first generations were characterized by a dense spike, like in ANBW-5A in all combinations. The average number of spikelets and ear lengths significantly differed from both parents and were intermediate between ANBW-5A and 84/98^{w} (Table 2). Therefore, the gene C^{17648} partially suppressed Q^S gene. However, the average stem length was nearer to the speltoid parent.

In the second generation (Fig. 2) the phenotypes that are similar to the parents, compactoid and speltoid, were presented, with density indexes of less than 19 and 28, respectively (Table 3). Also, the plants were presented with compressed ear tip and sparse ear base having the high density indexes. They were attributed to the compactoids. The plants with the normal shape of the ear with an average density index which is characteristic for bread wheat also segregated. As the majority of F_2 populations were presented by compactoids the epistatic interaction was suggested and 12 : 3 : 1 theoretical ratio was used for calculations of distributions of the above described phenotypes. The segregation for phenotypical classes was different in each population (Table 4).

In the F_2 population of *T. spelta* Grey x ANBW-5A the segregation practically fitted the theoretical ratio (12:3:1, χ^2 = 5,45). In general, it may be stated that the genes $C^{17648}$and *Q* are practically not linked. The population F_2 84/98^w x ANBW-5A segregated inconsistently with theoretical ratio. Therefore, the recombination, between genes under study was difficult. Earlier, the line 84/98^w was studied in other combinations of crossing and the inversion was suggested resulting in linked inheritance of awns, winter habit and speltoidy (Simonov et al., 2009). In addition, some recombinants were not obtained due to a selective elimination of certain recombinant chromosomes.

The F_2 population i:Rodina Q^S x ANBW-5A segregated in the consistence with theoretical segregation (12:3:1). So, in this cross the genes C^{16487} and Q^S were inherited independently. It may be supposed that the big part of the introgression from *Ae. spetloides* in 5AL chromosome including *vrn-s1* gene was eliminated during the development of isogenic line. This facilitates the recombination between the genes $C^{17648}$and *Q*.

Table 1: Allelic state of "Gross morphology genes" in hexaploid *Triticum* species

Species (subspecies)	Chromosomes and alleles of genes		
	5AL	2DL	3D, 3B, 3A
T. aestivum	*Q*	*c*	*S*
T. compactum	*Q*	*C*	*S*
T. sphaerococcum	*Q*	*c*	*s*
T. spelta	*q*	*c*	*S*

(Based on Catalogue of Gene Symbols for Wheat, McIntosh et al. 2013)

Table 2: Average values of spike and stem parameters of parental forms and F_1 hybrids: 84/98^w x ANBW-5A, i:Rodina Q^S x ANBW-5A and *T.spelta* Grey x ANBW-5A

Genotypes	Stem length	Spike length	Spikelet number	Density index
ANBW-5A	26,9	3,1	11,2	37,3
T.spelta Grey	53,3	9,2	14,2	15,5
F_1 *T.spelta* Grey x ANBW-5A	**46,0*****	**4,4*****	*14,8****	*34,2****
84/98^w	69,4	9,9	15,2	15,4
F_1 84/98^w x ANBW-5A	**59,2*****	**5,6*****	*16,8****	**29,9*****
i:Rodina Q^S	54,9	8,0	13,8	17,2
F_1 i:Rodina Q^S x ANBW-5A	**51,2*****	**5,2*****	**15,5*****	**29,8*****

In bold: the significant differences from both parents; in italics: the significant differences from one of the parent

Table 3: Average values with errors for the parent forms and average values with limits of variability for F_2populations compared to a respective parental averages

Genotypes	Stem length	Spike length	Spikelet number	Density index
ANBW-5A	41,4±4,7	4,1±0,3	14,1±1,3	34,3±2,3
84/98^{w}	95,4±10,4	13,5±1,2	19,4±1,9	14,4±0,8
i:Rodina Q^S	78,0±3,3	9,8±0,5	15,1±0,8	15,3±1,0
T. spelta Grey	93,1±7,7	11,9±1,2	18,3±1,2	15,7±1,7
F_2 84/98^{w} x ANBW-5A	66,6 12 – 114	6,4 2,0 – 14,6	16,6 10 – 27	28,3 12,6 – 50,0
parental averages	67,0	8,6	16,5	24,3
F_2 i:Rodina Q^S x ANBW-5A	54,7 8 – 99	5,5 1,5 – 11,3	13,6 7 – 18	27,3 13,3 – 48,0
parental averages	59,8	7,0	14,6	24,7
F_2 *T.spelta* Grey x ANBW-5A	66,1 30 – 102	6,0 3,0 – 11,8	14,7 12 – 20	29,1 12,7 – 46,9
parental averages	65,7	8,0	16	24,9

Table 4: Segregation of F_2 hybrids for spike shape according the digenic inheritance scheme under epistatic interaction of genes C^{17648} and Q^S

Phenotype / Segregation	Compactoid	Speltoid	Normal	Σ	χ^2 12:3:1	P
F_2 *T.spelta* Grey x ANBW-5A						
expected	37	9	3	49	5,449*	<0,05
actual	33	15	1	49		
F_2 84/98^{w} x ANBW-5A						
expected	67	19	7	103	130,23	Not significant
actual	47	22	34	103		
F_2 i:Rodina Q^S x ANBW-5A						
expected	97	24	8	129	1,6**	<0,5
actual	91	30	8	129		

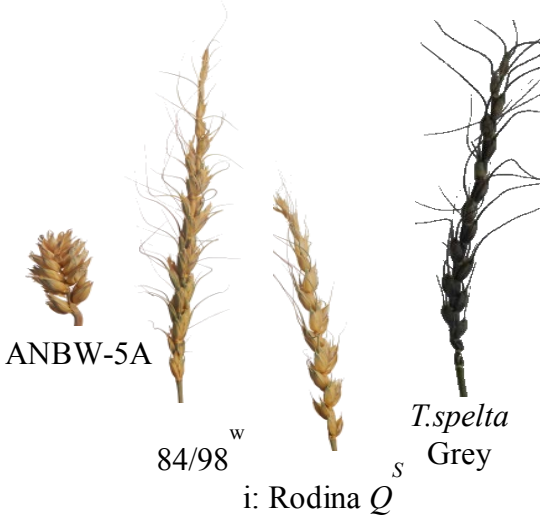

Fig. 1: Parents with compact and speltoid ears

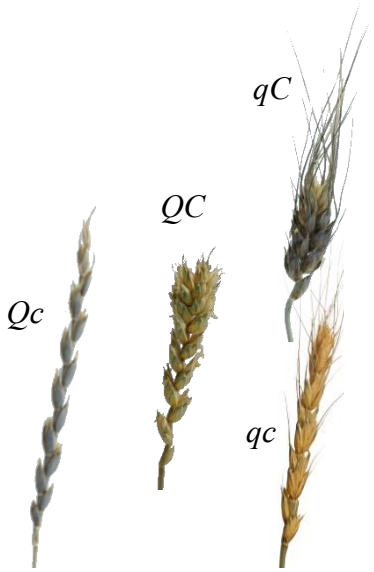

Fig. 2: Representative examples of spike phenotypes in F_2 hybrids

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Improvement of agronomic traits and disease resistance in triticale by interspecific and intergeneric hybridization

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Introduction

Interspecific and intergeneric hybridization is a very useful tool in the breeding of cultivated species from *Triticeae* tribe. This technique has been widely used to introduce desirable traits from wild relatives to cropped species and to increase the genetic variation among the species by developing new varieties. The breeding programs are looking for genetic sources of additional traits including resistance to disease, lodging or various unfavorable environmental conditions such as salt and drought tolerance, as well as starch profile, higher nutritional value or root improvement. Wide crossing is also used to new genera development, one such example is triticale, which has been obtained by cross wheat (*Triticum ssp*.) as the female parent and rye (*Secale ssp.)* as the male parent. However, as a synthetic crop it is not genetically as diverse as naturally evolved crops. Hence, the lack of inherent genetic diversity may be overcome by the varied spectrum of gene pool derived from wild species. The development of wide crosses will also enable to gain some knowledge about crops evolution by their resynthesis, that will be valuable base material in plant breeding (Hills et al. 2007, van Ginkel and Ogbonnaya 2007, Mares and Mrva 2008).

In Institute of Plant Genetics, Breeding and Biotechnology many various interspecific and intergeneric hybrids have been obtained. To develop these hybrids many wild relatives have been used, inter alia, species from *Aegliops*, *Dasypyrum* or *Agropyron* genera. *Aegilops* species is known to be donors to resistance to serious cereal diseases such as leaf rust (*Puccinia recondita f. sp. tritici, P. graminis f. sp. tritici*), septoria blotch (*Septoria tritici*), powdery mildew (*Puccinia graminis f. sp. tritici*) [Monneveux et al. 2000]. In addition, *Dasypyrum spp.* is known to be donor of resistance to cereal diseases: powdery mildew, leaf and stem rusts, take-all, cereal eyespot, wheat streak mosaic virus (WSMV) and its vector wheat curl mite, as well as donor of resistance to abiotic stress (salt and drought tolerance, winter hardiness). Commonly used species is *Dasypyrum villosum* (De Pace et al. 2011). Another synthetic genera is *Agrotriticum sp*. It was obtained by crossing *Triticum aestivum* and *Agropyron spp.* It is used to improve root system and thus drought tolerance development (Gruszecka and Czerwińska 2004).

Moreover, many primary triticale have been received by wide crossing employment, where various cultivars of spring and winter wheat and rye with desirable traits were used.

The aim of this study was to obtain intergeneric and interspecific hybrids with improved resistance to important cereal diseases, that could be base material in plant breeding programs for serious wheat and triticale diseases.

Material and methods

The plant material used in project: Broadening of Triticale genetic variation by wide hybridization (2003-2014) were cultivars and lines of triticale, wheat and rye. There were also used materials derived from collection of winter triticale, wheat and rye forms maintained by

Institute of Plant Genetics, Breeding and Biotechnology at University of Life Sciences in Lublin. Wild relatives of common wheat, that were collected by Project Team from germplasm banks all around the world, were also included as forms for hybridization.

All combinations of primary triticale, secondary triticale, as well as intergeneric hybrids with *Aegilops spp.*, *Dasypyrum spp*. and *Agrotriticum sp*. were performed in Experimental Farm of University of Life Sciences in Felin. Intergeneric and interspecific hybrids were obtained by flowers emasculation and hand pollination. Moreover, primary triticale (8×) was obtained as a result of crossing hexaploid common wheat and diploid rye, then the number of chromosomes in hybrids (F_1) was doubled by colchicine treatment. Secondary triticale was obtained by hybridization primary triticale after colchicne treatment with cultivars of wheat and triticale, as well as crossing triticale with other forms of wheat and triticale. Intergeneric hybrids were obtained by hybridization forms belonging to *Aegilops spp.*, *Dasypyrum spp*. or *Agrotriticum sp*. genera as female parent and different cultivars of triticale and wheat as a male parent.

Obtained hybrids were also assessed with regard to resistance to plant diseases. In the years 2012 and 2013 hybrids evaluation of resistance to leaf rust and powdery mildew has been done in Plants Breeding Ltd. Company IHAR Group in Strzelce division. Hybrids were tested by a 9-point assessment scale in field conditions.

Results

In the years 2006-2014 733 grains of primary triticale were obtained by crossing winter common wheat as a female parent and winter and spring rye as a male parent. Hybrids of winter common wheat and spring rye were obtained in 2010 and number of grains of these hybrids counted 64. The highest number of grains of primary triticale, hybrids of winter common wheat and winter rye, were obtained in 2008 and total 130. Between 2006 and 2014 21 616 grains of secondary triticale were obtained. Number of grains of hybrids of winter and spring triticale with winter and spring wheat total 12 210. The highest efficiency was in 2012, when 2 569 grains hybrids of winter triticale and winter wheat and 205 grains of hybrids of spring triticale and spring wheat were obtained. In addition, crosses of both forms spring and winter were obtained and number of their grains counted 9 056. The highest number of grains was 2 512 in 2012. Secondary triticale was also obtained as a result of crossing octoploid triticale (after colchicine treatment) with triticale or wheat, as well as total number of grains was 922. The most successful year was 2007, when 572 grains was obtained. There were also made many intergeneric crosses with *Aegilops spp.*, *Dasypyrum spp*. and *Agrotriticum sp*. Number grains of intergeneric hybrids with *Aegilops spp.* total 227, however the highest efficiency was in 2012, when 79 grains were obtained. Total number grains of intergeneric hybrids *Dasypyrum villosum* with wheat and triticale was 42, as well as intergeneric hybrids with *Agrotriticum sp.* was 659 grains.

All obtained hybrids are also used in practical breeding. Moreover, received crosses are sent into Plant Breeding Companies. Results of hybrids evaluation of resistance to diseases show that received crosses could be valuable resource of favourable traits. In the years 2012 and 2013 hybrids evaluation of resistance to leaf rust and powdery mildew has been done in Plants Breeding Ltd. Company IHAR Group in Strzelce division, where obtained crosses were tested by a 9-point assessment scale. Consequently, 61% of tested hybrids were characterized by low degree of plant infection by leaf rust, even though only 16% hybrids were characterized as high infested by these disease (Fig. 1). There has also been done the evaluation of infection by powdery mildew. As a result 79% of tested hybrids were characterized by medium rate of

infection (5 and 6 points), approximately 10% were characterized by low degree of infection and 9% were highly infested (Fig. 2).

Conclusions

- Wild relatives of common wheat could be donors of agronomically important traits for common wheat, as well as triticale.
- Most of intergeneric and interspecific hybrids could be used in practical breeding.
- Most of intergeneric hybrids were characterized by good resistance to leaf rust.

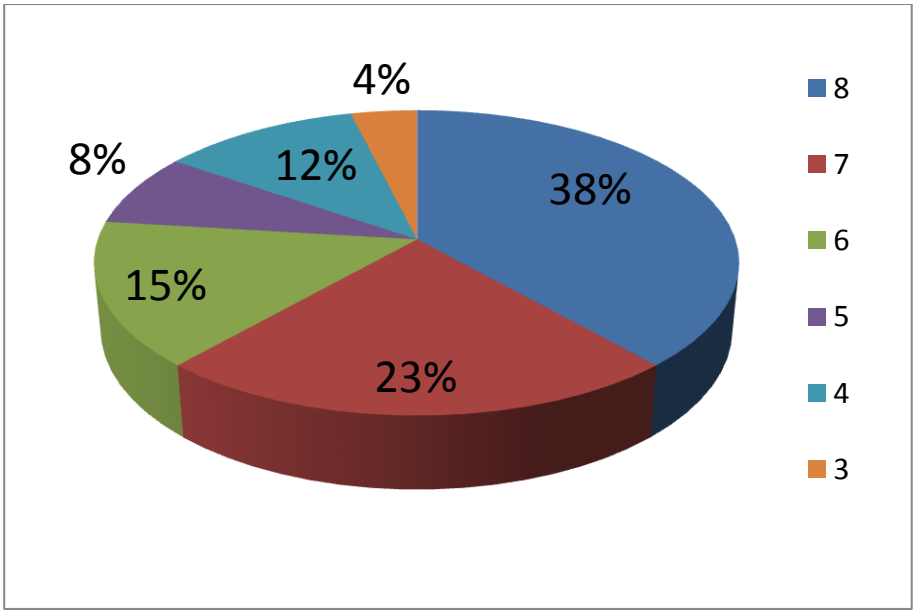

Fig. 1: Hybrids resistance to leaf rust evaluated by 9-point assessment scale.

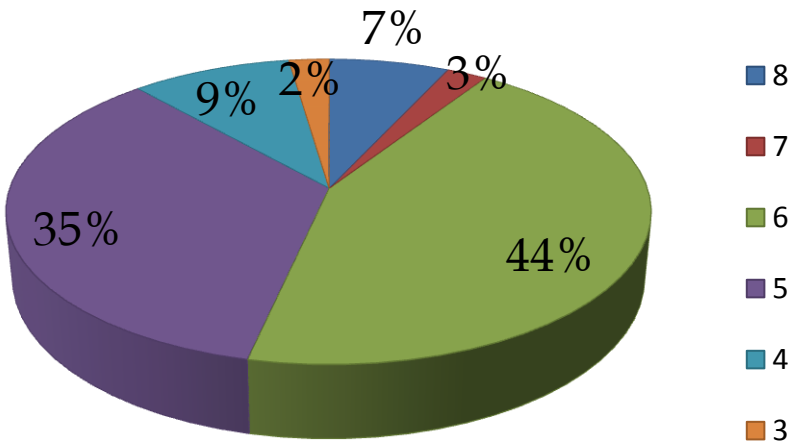

Fig. 2: Hybrids resistance to powdery mildew evaluated by 9-point assessment scale.

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Analysis of gibberellins biosynthesis genes expression alteration in response to plant growth regulators application in barley (*Hordeum vulgare* L.) plants with different dwarfing genes

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Introduction

Lodging is one of the most common problem in cereal crops that causes inhibition of plant development and changes in life function. Significant decrease of grain yield can be occurred as an effect and leads to high economic losses. There are two approaches in plant prevention against lodging. The most effective method is designing resistant plants by introduction of the dwarfing genes into cereal genome. Other technique concerns inhibition of gibberellins biosynthesis by plant growth regulators application.

Gibberellins (GAs) are plant hormones responsible for regulation of plant growth and development, particularly important for stem elongation. Biosynthesis of bioactive gibberellins in plant cell is complex process catalyzed by several enzymes. Regulation of bioactive gibberellins content in tissue is also dependent on GA 2-oxidase activity, which transforms bioactive gibberellins into inactive forms.

The purpose of this study was the analysis of influence of three growth regulators (chlormequat chloride, ethephon and trinexapac-ethyl) application on alteration of the expression of genes encoding gibberellins biosynthesis enzymes (CPS, KS, GA20ox, GA3ox, GA2ox) in barley (*Hordeum vulgare* L.).

Material and methods

Analyzed plant material comprised genotypes with different dwarfing genes: Triumph with *sdw1*, Hv287 with *sdw3* and Falk Mutant with *Dwf2*. As a control, tall form without dwarfing genes (Morex) was used. For determination of analyzed dwarfing genes transcript level in plant tissue qPCR method based on SYBR Green dye was performed.

Five-day-old barley seedlings were treated with three commercial retardants based on CCC, ethephon and trinexpac-ethyl and after two days the response on the transcription level was analyzed. RNA concentration and purity was measured with spectrophotometer (NanoDrop 2000). Moreover, quality of the RNA was analyzed by means of electrophoresis in 2% agarose gel. After reverse transcription of 1μl of total RNA, 80 ng of the obtained cDNA was used for quantitative PCR as a template. For amplification reaction the sequence specific primers for selected dwarfing genes were developed.

Results

Our analysis proved that application of plant growth regulations caused modification of selected genes expression profiles in tested plants. Significant increase of *CPS* gene expression was observed after CCC, ethephon and trinexpac-ethyl application (Fig. 1a). For majority of tested genotypes significant enhance of *KS* gene expression was proved after growth regulators

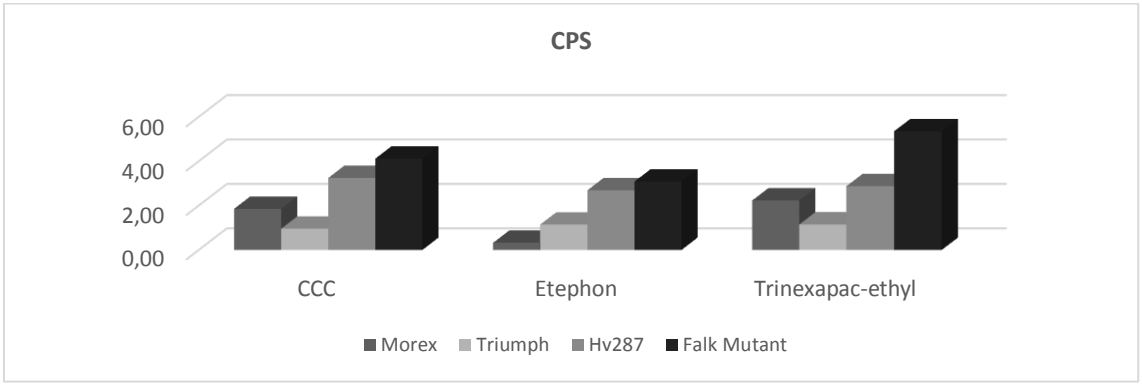
treatment. The strongest upregulation was noticed for Falk Mutant (4,66x after CCC; 3,69x after ethephon and 5,67x after trinexpac-ethyl treatment) (Fig. 1b).

Application of all three analyzed growth regulators caused increase of *GA20ox* gene expression for Morex and Triumph. Additionally, enhanced expression was also observed for Hv287 after ethephon treatment. For the Falk Mutant decrease of gene expression was noticed after CCC, ethephone and trinexpac-ethyl treatment (Fig. 1c).

CCC and trinexpac-ethyl application showed similar effects on *GA3ox* gene expression. Decrease of expression was observed for all tested genotypes. However, ethephon application caused upregulation of expression for Morex (3,75x) and Triumph (1,83x) but for Falk Mutant significant inhibition was observed (2,19x). For Hv287 the changes in gene expression profile were not significant (Fig. 1d).

After CCC and ethephon treatment significant decrease of *GA2ox* gene expression was noticed for all genotypes. However, trinexpac-ethyl caused enhanced *GA2ox* expression in Morex, Triumph and Hv287. Those results can be an effect of trinexpac-ethyl structure which is similar to GA20ox cofactor. For Falk Mutant the changes in expression profile were not significant after trinexpac-ethyl application (Fig. 1e).

a)



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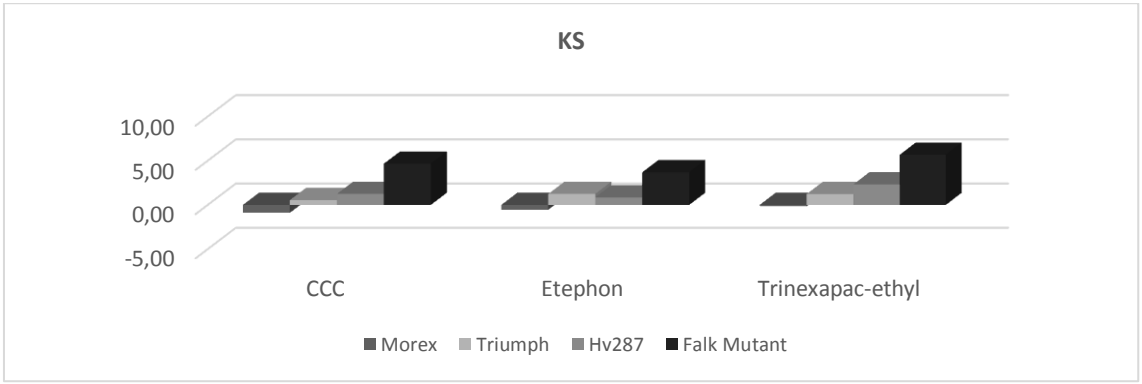

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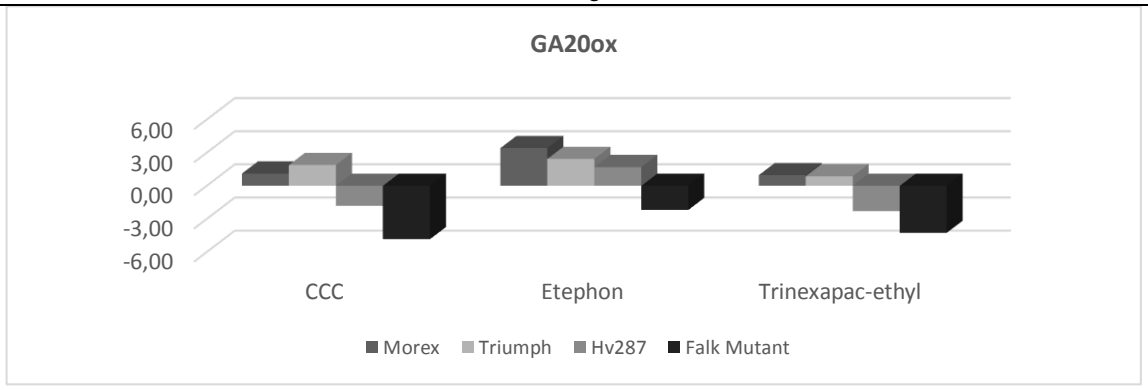
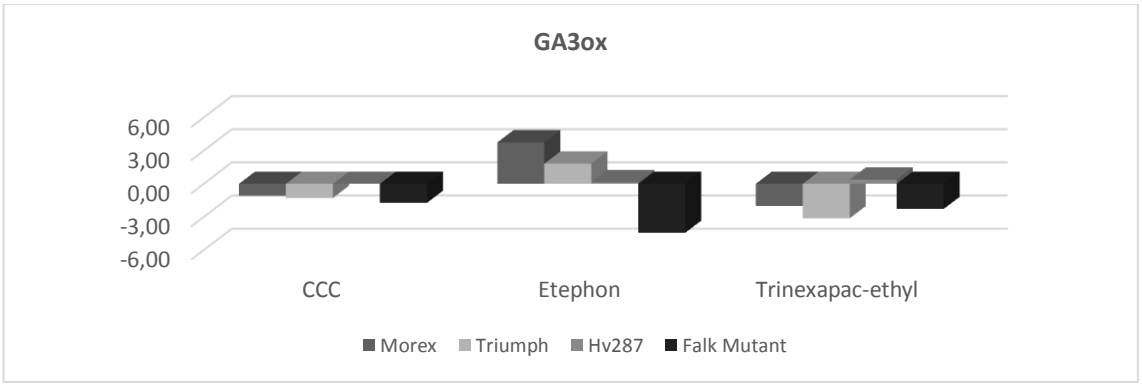


d)



e)

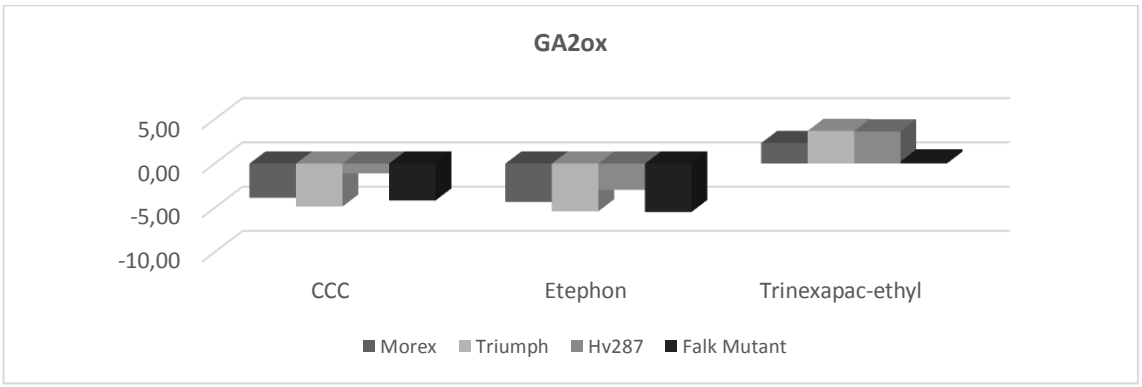


Fig 1: Relative expression of analysed genes after PGRs treatment in comparison to respective untreated control forms.

Conclusions

This study demonstrated that regulation of gibberellins biosynthesis pathway is a component of complex plant systems which is dependent on specific enzymes activity. After growth regulators application modification of analyzed genes expression was observed. The patterns of reactions were different and comprise both inhibition and activation of genes expression. Those results suggest there are active mechanisms that lead to overcoming gibberellins deficiency caused by retardants through enhanced expression and increased formation of active enzymes. Variant effects of growth regulators application on genes expression indicated on species-specific molecular response. Results of that study proved similar effects of CCC and trinexpac-ethyl application. However, ethephon treatment showed different changes in most cases. Detailed characterization of these differences will be a subject of further examinations.

Acknowledgment

Presented results were obtained within the framework of the project #N N310 774140 granted by Polish National Science Centre.

Effect of plant growth regulators on transcript levels of genes encoding enzymes involved in gibberellin biosynthesis pathway in common wheat isogenic lines with different *Rht* genes

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Introduction

Lodging is defined as the tendency of cereal crops to bend over, so that they lie more or less flat on the ground. Lodging is often the result of adverse weather conditions, such as rain, wind, and/or hail combined with inadequate standing power of the crop. Lodging alters plant growth and development. Severe lodging interferes with the transport of nutrients and moisture from the soil, reduces photosynthetic capabilities of the plant thus affecting carbohydrate assimilation. It is serious problem affecting plants' yield and grain quality (Berry et al. 2004; Kashiwagi and Ishimaru 2004).

The ability of a crop to withstand lodging depends on the length of the stems. The introduction of the dwarfing genes into wheat genetic background has become one of the most effective ways of defence against lodging. Several of the *reduced height* (*Rht*) genes of wheat encode DELLA proteins, which are growth-suppressing transcriptional regulators. The presence of bioactive gibberellins (GAs) stimulate growth by initiating the degradation of DELLAs. The *Rht* dwarfing alleles, such as *Rht-B1b* and *Rht-D1b*, cause a reduced response to GAs and work as constant growth repressors, due to the point mutation resulting in the production of the N-terminally truncated DELLA proteins (Mutasa-Göttgens and Hedden 2009, Pearce et al. 2011). Another approach in lodging prevention is the application of plant growth regulators (PGRs) that inhibit gibberellin biosynthesis by blocking the activity of enzymes involved in the process, such as chlormequat chloride (CCC), ethephon and trinexapac-ethyl (Rademacher 2000, Berry et al. 2004).

The biosynthesis of GAs can be separated into three stages: 1) the formation of *ent*-kaurene, catalyzed by *ent*-copalyl diphosphate synthase (CPS) and *ent*-kaurene synthase (KS) 2) the formation of gibberellin A12-aldehyde catalyzed by KAO (ent-kaurenoate oxidase) 3) further oxidation of gibberellin A12-aldehyde to the different GAs. The enzymes involved in the process of catalysing the final steps of bioactive GAs formation are GA 20-oxidase (GA20ox) and GA 3-oxidase (GA3ox). The inactivation of bioactive GAs is performed by GA 2-oxidase (GA2ox) [Sponsel, Hedden 2010; Hedden, Thomas 2012].

The aim of the research was the expression analysis of the genes encoding enzymes involved in gibberellin biosynthesis pathway (*CPS, KS, GA20ox, GA3ox* and *GA2ox*) in wheat isogenic lines with different *Rht* genes exposed to plant growth regulators.

Material and methods

Analysed plant material consisted of common wheat (*Triticum aestivum* L.) near isogenic lines of the variety 'Bezostaya 1' carrying *Rht-B1b*, *Rht-B1d*, *Rht-B1e* (gibberellic acid-insensitive) and *Rht12* (gibberellic acid-sensitive) dwarfing genes. As a control the tall form 'Bezostaya 1 *rht*' line was used.

The 5-day-old seedlings of wheat isogenic lines, growing in controlled environment, were sprayed with the solution of three different PGRs, namely: ethephon, chlormequat chloride (CCC) and trinexapac-ethyl (TE). The concentrations of the growth retardants were as follows: 5,600 mg l^{-1} for ethephon, 6,750 mg l^{-1} for CCC and 500 mg l^{-1} for TE. For plant spraying, commercial agents based on the aforementioned active substances were applied. Two days after the treatment the plant tissue was collected and immediately frozen in liquid nitrogen.

The RNA was extracted with the 'RNeasy Plant Mini Kit' (Qiagen) according to manufacturer's protocol. The concentration and purity of RNA was assessed with NanoDrop 2000 spectrophotometer (Thermo Scientific). The integrity of RNA samples was analysed by the means of electrophoresis in 2% agarose gel stained with ethidium bromide.

The reaction of reverse transcription was performed on 1 µg RNA with SuperScript VILO cDNA Synthesis Kit (Life Technologies) according to the supplier's recommendations. Obtained cDNA was used as template in the qPCR analysis.

The real-time PCR was used to determine transcript levels of CPS, KS, GA20ox, GA3ox and GA2ox genes. The previously obtained cDNA (80 ng) was used as a template in qPCR reactions. Each sample was analysed in three technical replications. Reactions were prepared with the use of sequence specific primer sets and SYBR Select Master Mix (Life Technologies). As a reference E3 ubiquitin ligase (Ta.35546) gene was utilized. Sequences of primers used in experiment were based on the sequences of genes of interest available in TIGR Plant Transcript Assemblies and UniGene (NCBI) databases and defined with the use of QuantPrime and Primer3 software. The relative quantification of transcript levels was performed. The analysis of the results was based on the $2^{-\Delta\Delta C}{}_{T}$ algorithm (Livak and Schmittgen 2001). The amplification was carried out on Mx3005P apparatus with MxPro software (Stratagene).

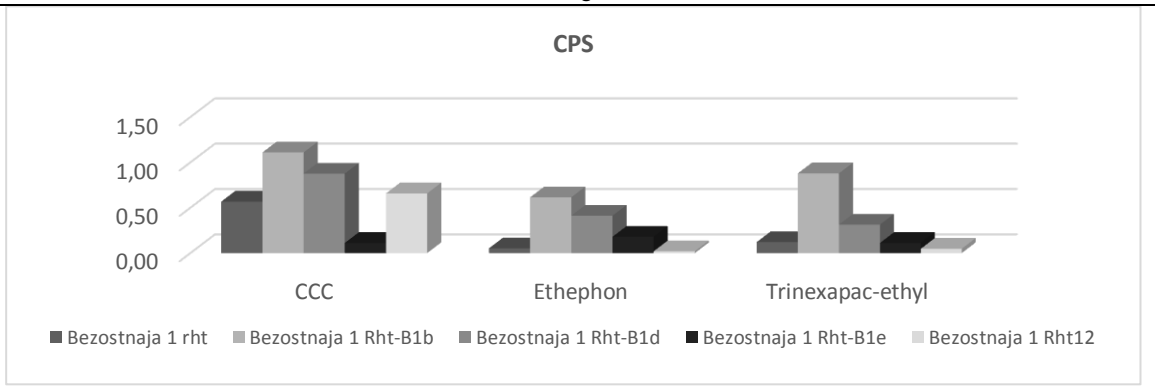
Results

The application of plant growth regulators resulted in an increase of CPS transcript level in all analysed lines. Moreover, obtained profiles of expression were similar for all three plant growth retardants (Fig. 1a). Plant growth regulators induced the expression of KS, particularly in *Rht-B1b* and *Rht-B1d* lines. The *Rht12* line reacted to the application of each growth inhibitor with a strong decrease in the transcript level of studied gene. In the control line a significant decline (1,06×) in KS transcript level in response to ethephon occurred (Fig. 1b).

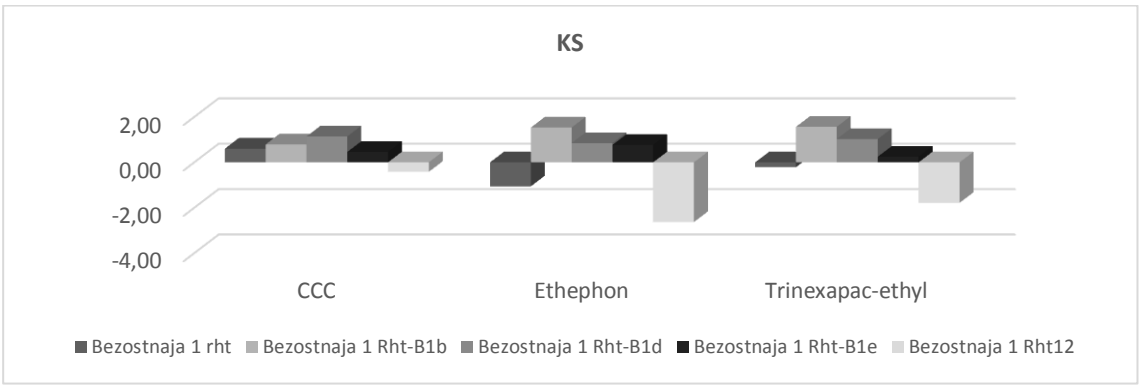
A significant, increase in a GA 20-oxidase transcript level was observed in *Rht-B1d* line upon the CCC and ethephon application. In other isogenic wheat lines CCC and ethephon had no significant effect on the transcript level of this gene. Trinexapac-ethyl enhanced the *GA20ox* expression in all tested wheat genotypes (Fig. 1c). In all GA-insensitive lines the expression of GA 3-oxidase was downregulated by CCC. Two other genotypes showed enhanced gene expression. Opposing response was observed in all GA-insensitive lines treated with ethephon, where an increase in the *GA3ox* transcript level was noticed. Ethephon had no significant effect on *GA3ox* expression in *Rht12* and control lines. Trinexapac-ethyl greatly reduced (2,52×) the expression of GA 3-oxidase in control line. The *Rht-B1d* line, on the contrary, reacted to trinexapac-ethyl with almost two-fold increase in the transcript level of analysed gene (Fig. 1d).

Both CCC and ethephon downregulated the expression of GA 2-oxidase in all analysed lines. The strongest reduction of *GA2ox* transcript level was observed in the Bezostaya *Rht-B1d* line. Trinexapac-ethyl enhanced the *GA2ox* gene expression. The effect of trinexapac-ethyl was the strongest in control line and line with *Rht12* dwarfing gene (Fig. 1e).

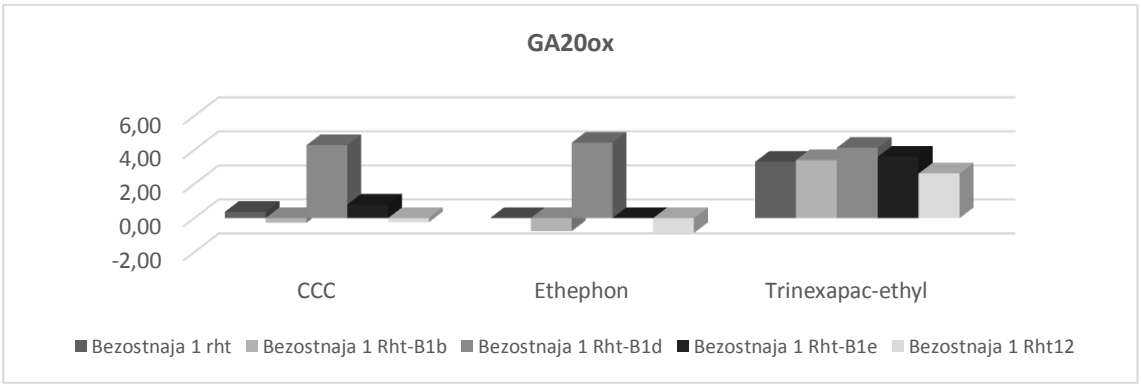
a)


CPS
1,50
1,00
0,50
0,00
CCC
Ethephon
Trinexapac-ethyl
Bezostnaja 1 rht
Bezostnaja 1 Rht-B1b
Bezostnaja 1 Rht-B1d
Bezostnaja 1 Rht-B1e
Bezostnaja 1 Rht12

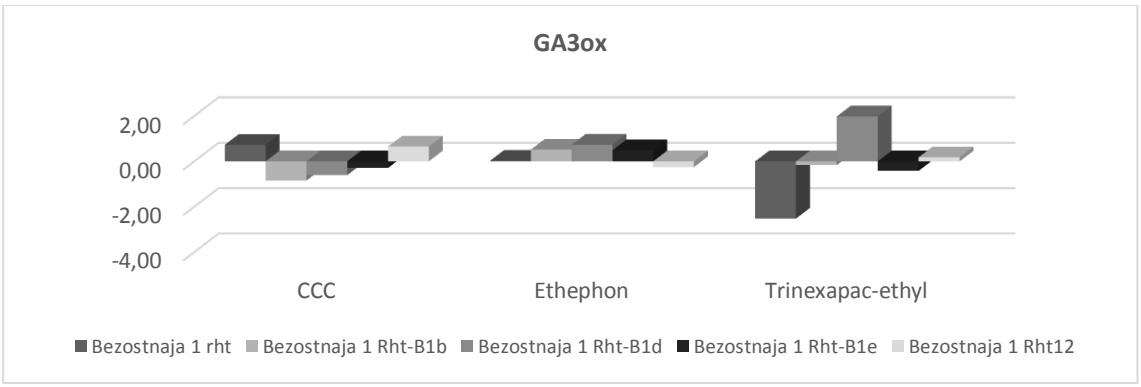
b)


KS
2,00
0,00
-2,00
-4,00
CCC
Ethephon
Trinexapac-ethyl
Bezostnaja 1 rht
Bezostnaja 1 Rht-B1b
Bezostnaja 1 Rht-B1d
Bezostnaja 1 Rht-B1e
Bezostnaja 1 Rht12

c)


GA20ox
6,00
4,00
2,00
0,00
-2,00
CCC
Ethephon
Trinexapac-ethyl
Bezostnaja 1 rht
Bezostnaja 1 Rht-B1b
Bezostnaja 1 Rht-B1d
Bezostnaja 1 Rht-B1e
Bezostnaja 1 Rht12

d)


GA3ox
2,00
0,00
-2,00
-4,00
CCC
Ethephon
Trinexapac-ethyl
Bezostnaja 1 rht
Bezostnaja 1 Rht-B1b
Bezostnaja 1 Rht-B1d
Bezostnaja 1 Rht-B1e
Bezostnaja 1 Rht12

e)

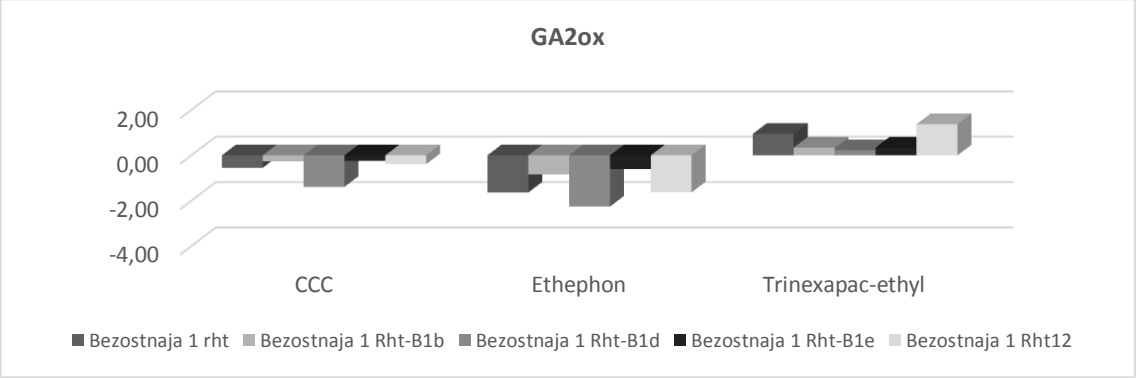


Fig. 1: Relative expression of analysed genes after PGRs treatment in comparison to respective untreated control forms.

Conclusions

The study revealed that the application of plant growth regulators has an effect on the expression of genes encoding enzymes involved in in the first steps of gibberellin biosynthesis (*CPS* and *KS*), catalysing final steps of bioactive gibberellins formation (*GA20ox* and *GA3ox*) and responsible for their inactivation (*GA2ox*). In most cases, the profiles of response obtained for plants treated with CCC and ethephon were similar. Trinexapac-ethyl brought about distinct, specific changes in the expression of genes encoding 2-oxoglutarate dependent dioxygenases (*GA20ox, GA3ox, GA2ox*). Our results can indicate that the short-stemmed phenotype caused by the application of PGR generally associated with the changes on the biochemical level, may also be the result of the changes in the functioning of plant genome.

Acknowledgement

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Cumulative effects of *Lr34*, or genes, and 1AL/1RS translocation on some agronomic traits in a set of wheat mutant/ recombinant DH lines

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Mutation breeding technology was considered and has proved to be a complementary and valid tool to classical breeding, contributing substantially to diversification of genetic base and development of better cultivars in more than 170 cultivated species, including bread wheat (Lagonda 2009).

Currently, rapid increasing interest in molecular biology is also reflected in mutagenesis programmes by gene discoveries and function studies through the association of changes in their DNA sequences to modified phenotype expressions. The particular advantages of induced mutation are that progenies with desired traits can often be produced with high frequencies, in short time, in a chosen genetic background without disrupting the original genetic constitution of the crop. It was also proved that mutation efficiency can be improved by combining recurrent irradiation with hybridization. Moreover, by using DH- technology it is possible to fix in only one generation any variability generated by genetic recombination and mutagen factors, too.

A wheat mutagenesis programe developed at NARDI Fundulea was started in 2008 by using two modern winter wheat genotypes (Izvor and F00628-34), each having valuable but some contrasting agronomic traits, and two irradiation cycles application (Seibersdorf Centre - International Atomic Energy Agency, Vienna), hybridization and DH- technology using *Zea* system (Fig. 1).

Up to now, more than 500 mutant/ recombinant DH-lines were generated. Cultivar Izvor, released in 2009 at NARDI- Fundulea is drought tolerant, with high yielding ability in dry years, carrying *or* recessive allele (controlling osmotic adjustment) on 7A chromosome (Banica et al. 2008 a,b). Although, cultivar Izvor carries *Lr34* resistance allele (Ciuca et al. 2015), still expressed the typical "slow" rusting level of resistance. The advanced breeding line F00628-34, manifested a good resistance to foliar pathogens, higher yielding potential in areas without water stress and carries 1AL/1RS translocation (Saulescu et al. 2011).

In 2011, under an uncommon epiphytic condition, the *Lr* unknown resistance gene, probably located on the short rye arm 1RS, have been overcome.

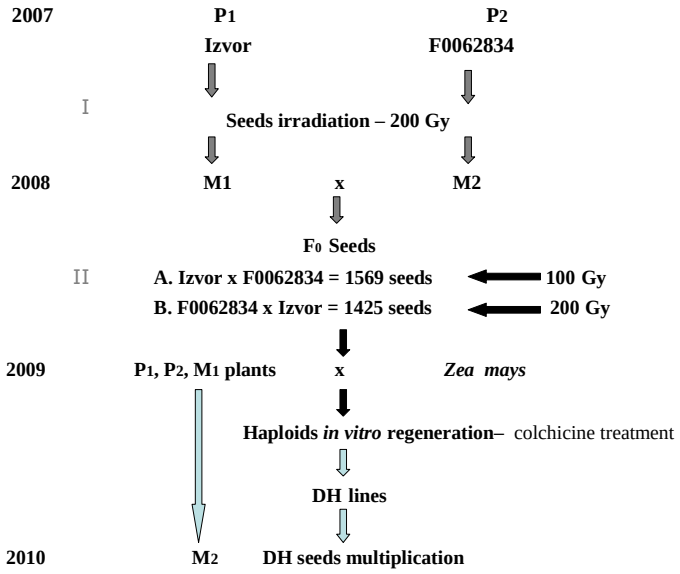

Fig. 1: Obtaining mutant wheat genetic stocks (*after Giura A., adapted*)

In the same time, a whole spectrum of symptoms as response to fungus from high susceptibility to immunity was noticed in mutant/recombinant DH-lines populations. It is worth to mention that ear productivity parameters of resistant DH-lines, including kernel weight, were higher compared to parental forms or to other susceptible/medium susceptible DH-lines (Giura 2013).

A similar situation was encountered in 2014 when alongside of leaf rust, yellow rust and *Septoria sp*. manifested, too. For Fundulea site, the winter 2013-2014 was much warmer and dried on long term average and the months of April- July were slightly cooler than usual but the weather for that period was unusually wet, just suitable for yellow rust development.

In such circumstances, a part of DH-lines had a reduced TKW values. However, many lines proved to be less affected being noticed with high TKW values.

The paper reports some results regarding cumulative or individual effect(s) of the *Lr34* and *or* genes and of *1AL/1RS* translocation on some important agronomic traits.

Material and methods

According to field observation data, a number of 28 mutant/recombinant DH lines (Table 1), selected mainly for their high TKW values characterized for their susceptibility to foliar pathogens, especially leaf rust, together with parental forms, were analyzed at molecular level for presence/absence of the two genes, *Lr 34* and *or*, and for *1AL/1RS* translocation.

Genetic analyzes included:

- DNA isolation from seeds, using CTAB method by Mohammadi Mohsen et al. (www.shigen.nig.ac.jp/ewis/article/html/118/article.html).

- DNA amplification (PCR) : - All amplification reactions were carried out in a 25µl volume.

We used:

- *Lr34/Yr18/Pm38/Sr57/Ltn1* –cssfr5 markers (Lagudah at al. 2009);
- *or* – wmc603 marker (Ciucă and Petcu 2009);
- *1AL/1RS* translocation –SCM9 marker (Saal and Wricke, 1999);
- secaline (locus *Sec-1*) –o- sec5'/a and o- sec3'/r markers (Shimizu at al. 1997);
- *Glu-A3* –SSR- PSP2999 marker (Devos at al. 1995).

- The PCR products were separated on 1.2-1.5-2-2.5% agarose for routine use, in 0.5X TBE buffer, stained with ethidium bromide and photographed under ultraviolet light with Vilber Lourmat system.

Results

Among the 28 wheat DH mutant/ recombinant genotypes we found plant material without favorable alleles, or pyramiding of two genes *Lr34- 1AL/1RS; 1AL/1RS - or*, even lines in which we found all the two genes and rye translocation (Table 1). Between analyzed lines, one stood out (genotype 70 - Table 1) because it contains the rye translocation (1AL.1RS) with *Sec-1* locus but a distal rye segment was removed and replacement with wheat chromatin, *Glu-A3* locus.

Howell et all., 2014, reported similar results, NILs -1BL.1RS with the removal and replacement of two interstitial rye segments with wheat chromatin (to remove *Sec-1* locus and to introduce *Glu-B3/Gli-B1* loci). So, our results showed presence of the *Glu-A3* locus on 1RS arm using mutagenesis and then hybridization.

The design of analyzed lines regarding pyramiding of *Lr34, or* genes and *1AL/1RS* translocation has conducted to grouping in 6 classes, and the variety Izvor was framed, the only one, in the seventh class (Fig. 2). The highest proportion (42.9%) is represented by the lines that contain the *Lr34* gene, from the cultivar Izvor, and the *1AL/1RS* translocation, from the F00628-34 line that give them a better resistance to foliar diseases and a high TKW under stress conditions.

Table 1: TKW values and resistance/susceptibility to leaf rust in 2014 and distribution of allelic variants of *Lr34*, *or*, secaline, *GluA3*, *1AL/1RS* translocation in DH- lines and parents

Genotype	TKW (g)	Brown rust resistance qualificative	*LR34*	Rye *1AL/1RS*	Secaline	GluA3	*or*
31	36.83	R	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
68	40.85	MR	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
70	39.35	R	Lr34+	1AL.1RS	SEC1	GluA	nonor
73	38.13	RHy	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
40	36.23	RHy	lr34-	1AL.1RS	SEC1	nonGluA	nonor
50	36.11	RHy	**Lr34+**	**1AL.1RS**	**SEC1**	**nonGluA**	**or**
62	39.78	R	lr34-	1AL.1RS	SEC1	nonGluA	or
72	35.15	R	lr34-	1AL.1RS	SEC1	nonGluA	nonor
79	35.6	R	lr34-	1AL.1AS	nonSEC1	GluA	nonor
82	36.21	RHy	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
85	37.11	RHy	lr34-	1AL.1AS	N.A.	GluA	or
98	39.14	RHy	**Lr34+**	**1AL.1RS**	**SEC1**	**nonGluA**	**or**
128	36.94	R	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
136	34.04	R	**Lr34+**	**1AL.1RS**	**SEC1**	**nonGluA**	**or**
20	39.84	R	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
26	36.8	R	lr34-	1AL.1AS	N.A.	GluA	nonor
50	36.71	R	lr34-	1AL.1RS	SEC1	nonGluA	or
100	35.87	MS	**Lr34+**	**1AL.1RS**	**SEC1**	**nonGluA**	**or**
175	38.04	R	lr34-	1AL.1RS	SEC1	nonGluA	or
192	41.8	R	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
198	37.5	R	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
220	39.26	R	lr34-	1AL.1RS	SEC1	nonGluA	or
224	37.04	R	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
251	38.27	R	lr34-	1AL.1AS	N.A.	GluA	nonor
258	34.34	R	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
272	35.53	R	lr34-	1AL.1RS	SEC1	nonGluA	nonor
277	39.61	R	Lr34+	1AL.1RS	SEC1	nonGluA	nonor
286	34.76	R	**Lr34+**	**1AL.1RS**	**SEC1**	**nonGluA**	**or**
290	36.11	R	**Lr34+**	**1AL.1RS**	**SEC1**	**nonGluA**	**or**
IZVOR	25.97	R	**Lr34+**	**1AL.1AS**	**nonSEC1**	**GluA**	**or**
F0062834	27.59	S	**lr34-**	**1AL.1RS**	**SEC1**	**nonGluA**	**nonor**

* R – resistant, MR – Medium resistant brown rust, RHy – Resistant by hypersensibility, MS – Medium sensible, S – Susceptible, ***** N.A. – not analyzed

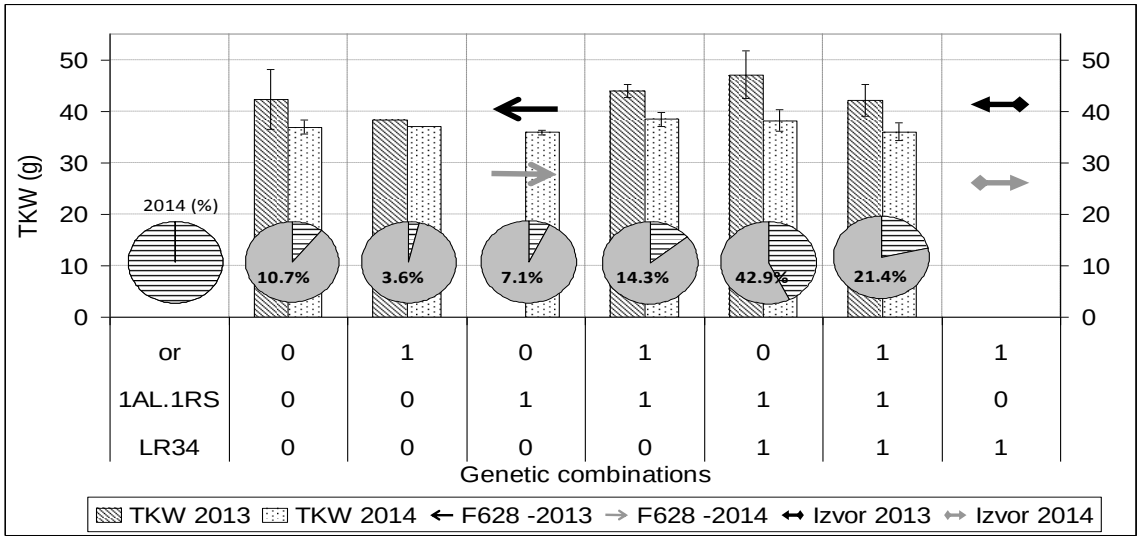

Fig. 2: DH lines distribution (percentages) according to TKW in 2013 and 2014 and for presence/absence of *Lr34*, *or* alleles and *1AL / 1RS* translocation

Following the molecular analysis of the 28 wheat DH mutant/recombinant lines in 21.4% of them, both genes, *Lr34* and *or*, and *1AL / 1RS* translocation were cumulated; 14.3% were

highlighted by the presence of *or* gene and *1AL / 1RS* translocation, but most of them, 42.9% of lines contains the *Lr34* gene and *1AL / 1RS* translocation, and of the data presented in Table 1 suggests that this last genetic structure significantly influenced the TKW values.

Taking into account the favorable climatic condition of 2014, and the values of TKW, it seems that *or* gene had not any involvement in TKW expression.

Conclusions

Identifying a mutant/recombinant line (line-70) that has the rye chromatin, but possesses wheat glutenin locus *Glu-A3*, could help improve the quality of bread in the presence of favorable genes from 1R.

Lr34 gene and *1AL/1RS* translocation cumulated in analyzed lines, gives them the ability to obtain productivity even in biotic stress conditions but the high productivity may be also determined by other chromosomal segments.

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Monosomic analysis of leaf hairiness in isogenic lines of bread wheat Novosibirskaya 67

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Leaf pubescence of bread wheat is represented by straight non-secreting trichomes. N.I. Vavilov (1922-1923) pointed out the predominant contribution of the genetic component compared to the environmental conditions in the formation of this feature. Various forms of leaf pubescence were found for both diploid, tetraploid and hexaploid species of wheat (Krupnov, Tsapaikin, 1990). In bread wheat, the dominant gene *Hl1* which determines leaf pubescence in a number of Soviet spring varieties was identified for the first time in 4B chromosome (Maystrenko, 1976). Later it was mapped to the long arm of this chromosome (Dobrovolskaya et al. 2007). The second dominant gene, *Hl2*, was found in the pubescent Chinese variety Hong-Mang-mai and localized in the short arm of 7B chromosome at 14.3% of the centromere (Taketa et al. 2002). The great phenotypic diversity of leaf pubescence in wheat and its relatives allow assuming the existence of other genes determining this trait. However, their identification is difficult due to the complications of the phenotypic analysis of this trait.

Recently, we developed a method to obtain digital information about the structure of leaf pubescence from the computer image processing of a wheat leaf fold. Simultaneously with improving the methodological aspect of the pubescence analysis it is necessary to expand the genetic base of investigations including the donors with different types of pubescence. In the present work, we performed the genetic and monosomic analyses of the pubescence in isogenic lines ANK 7B and ANK 7C produced on the base Novosibirskaya 67 variety (Koval, Koval, 2001). The lines have a well-defined leaf pubescence that differs from the parent variety. The aim of this work was to establish the chromosomal location of coding gene and genetic relatedness of this pubescence to the known genes.

Materials and methods

The isogenic lines ANK 7B and ANK 7C, its parental cultivar Novosibirskaya 67 (N67) and monosomic lines of cv. Diamant 2 (Dm2) for chromosomes of 7^{th} homoeological group were used as a genetic material. The recipient cultivar has a soft and uniform pubescence on leaf surface while the lines carry long and tough trichomes (Figure 1) inherited from Chinese and Soviet cultivars (Koval, Koval, 2001). Hairiness was studied on detached leaves using the method of high-throughput phenotyping LHDetect2 (Genaev et al, 2012). It allows separating the phenotypic classes on the basis of quantitative characteristics of leaf hairiness among segregates in crosses. To produce F_1 hybrids the monosomic plants that carry a univalent in MI of meiosis (20″ + 1′) were selected as maternal forms. These plants were crossed with ANK 7B and ANK 7C isogenic lines. Using again cytological analysis the monosomic plants were selected from the first generation and self-pollinated. The offspring of these plants formed monosomic F_2 populations for chromosomes 7B and 7D. About 110-120 plants were examined in every monosomic population. All the material was grown under greenhouse conditions.

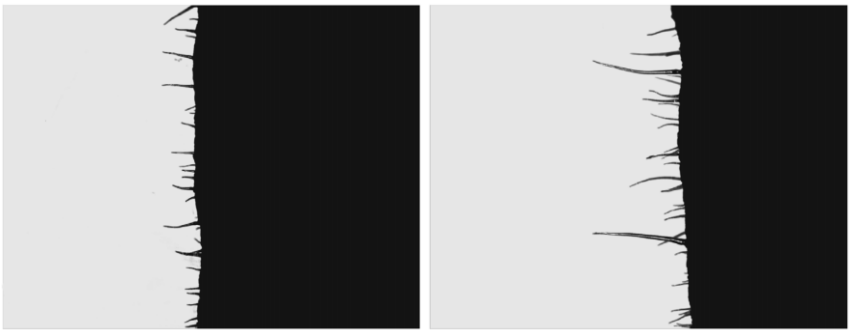

Fig. 1: Typical images of leaf folds of N67 and ANK-7B used for obtaining the quantitative characteristic of pubescence

The Pearson $\chi 2$ criterion was used in order to estimate the correspondence of obtained and theoretically expected values in monosomic analysis. The significance of differences of average length and number of trichomes between genotypes was estimated using the Student's t-test.

Results and Discussion

Earlier, it was found that the long pubescence introgressed in the genotype of the N 67 isogenic lines is controlled by a single gene (Doroshkov et al. 2014). In the previous work of Taketa et al. (2002) the gene for a similar pubescence phenotype of Chinese cultivar was localized in 7B chromosome. Therefore, the monosomic lines for 7th homoeological group of poorly pubescent cv. Dm2 were used. The digital descriptions of pubescence in cvs. N67, Dm2 and isogenic line ANK-7B are presented in Figure 2.

On the first stage, the three monosomic populations were examined visually. The plants both with and without long trichomes were found in the populations for 7A and 7D chromosomes. The segregation of traits was significantly valid for the monogenic ratio (3 : 1) (Table 1). For chromosome 7B, there was a significant difference from the monogenic segregation. Four plants without long trichomes were nullisomic. This proves that the gene involved in the control of this trait is localized in chromosome 7B. At the same time, between pubescent plants of monosomic populations for 7A and 7D chromosomes the visual differences in density of pubescence were found. However, this method did not allow for a clear description of these differences. They were clearly identified using LHDetect2 program (Figure 3). Plants that form long pubescence exceeding 450 μm were placed into class 1 which phenotypically corresponded to ANK 7B and ANK 7C isogenic lines. Plants that form pronounced pubescence with lengths that do not exceed 450 μm but are greater than 100 μm were placed in class 2 which phenotypically corresponds to N67 recipient variety. Plants without trichomes longer than 100 μm were placed in class 3 with the lowest degree of leaf pubescence. In 7B and 7D monosomic populations (Table 2) segregation deviated from digenic (15 : 1). The plants from 3rd class were absent. In 7B population it is explained by the presence of the dominant gene from the isogenic line. Reduction of this class from 7D population indicates that this chromosome of N67 substituted the homologue of Dm2 also carry the dominant gene influencing pubescence length and density. This is indirectly confirmed by the fact that, in Table 3, the number of plants of class 2, which is phenotypically similar to the N67 variety, is higher than in populations for chromosomes 7A and 7B. Based on the results of the previous studies and the present work, we can assume the existence of a homoeoallelic series of leaf pubescence genes on chromosomes 7 of homoeologous group of bread wheat (Table 3).

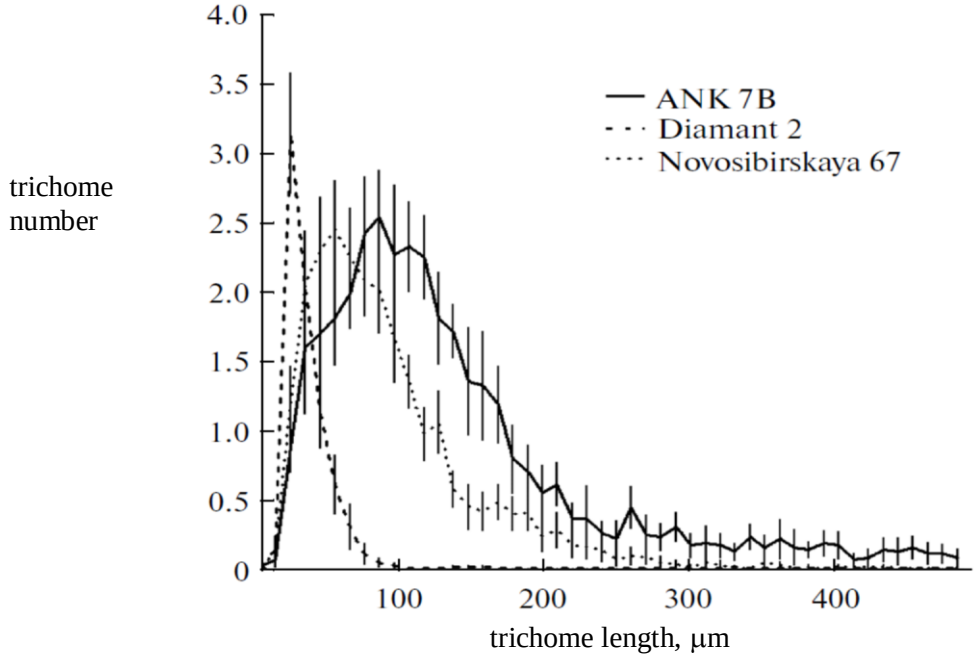

Fig 2: Comparison of trichome length distributions obtained using LHDetect2 program among parental monosomic cultivars Dm2, parent of isogenic line N67 and isogenic line ANK 7B

Table 1. Segregation of F2 hybrids by presence of long trichomes on the leaf surface in monosomic populations for 7th homoeological group of wheat variety Diamant 2 (Dm2)

Hybrids combinations F_2	Presence of long trichomes		χ^2 (3 : 1)	P
	Yes	No		
mono 7A Dm2 × ANK 7B and 7C	78	31	0,97	0,50-0,25
mono 7B Dm2 × ANK 7B and 7C	112	4*	30,6	<< 0,05
mono 7D Dm2 × ANK 7B and 7C	83	30	0,19	0,75-0,50

* - nullisomics (20″)

Table 2. Segregation of F2 hybrids by different pubescence classes selected by the computer LHDetect2 program in monosomic populations for seventh homoeological group of Dm2 wheat variety

Hybrid combinations F_2	Types of pubescence		χ^2 (15 : 1)	P
	Class 1 and 2 (strong and intermediate)	Class 3 (weak or absent)		
mono 7A Dm2 × ANK 7B and 7C	102 (80 + 22)	8	0,15	0,75-0,50
mono 7B Dm2 × ANK 7B and 7C	116 (98 + 18)	0	-	-
mono 7D Dm2 × ANK 7B and 7C	112 (80 + 32)	1	5,44	<< 0,05

Table 3. Hypothetical homoeoallelic set of genes for leaf pubescence in bread wheat and the genetic germplasm

Gene	Chromosome	Genetic material
Hl-B2	7B	cv. Hong- Mang-mai (Taketa et al. 2002)
- " -	- " -	ANK isogenic lines (present work)
Hl-D2	7D	cv. Novosibirskaya 67 (present work)
Hl-S2	7B-7S substitution	Introgressed from *Ae. speltoides* into cv. Rodina (Pshenichnikova et al. 2007)

It was found that chromosome 7B of all near-isogenic lines carry the gene determining the presence of long trichomes and chromosome 7D of cv. N67 carry the gene enhancing trichome density. The obtained data allowed formulating the hypothesis about the presence of homoeoallelic gene series controlling leaf hairiness in chromosomes of the seventh homoeological group of bread wheat and its relatives.

The work was supported by grant №14-14-00734 from Russian Scientific Foundation.

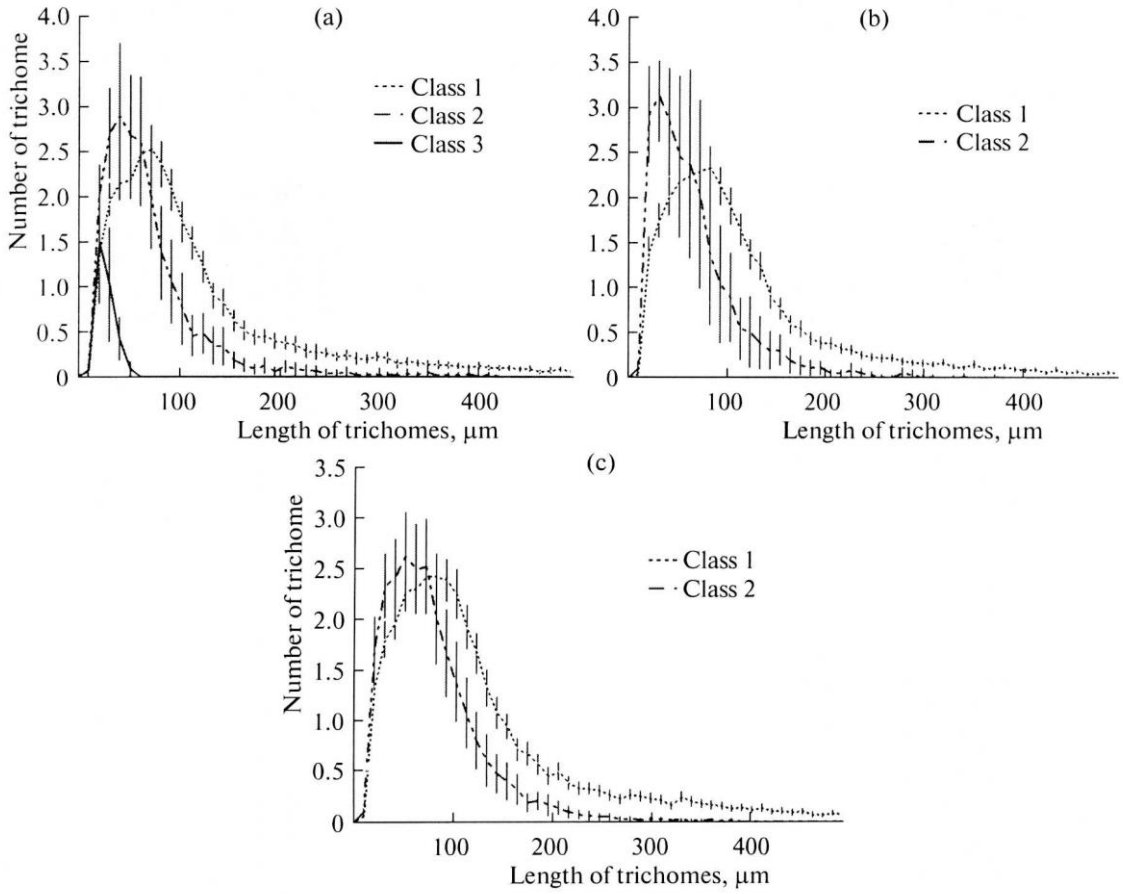

Fig. 3: Distribution for trichome length in the phenotypic classes found in F2 monosomic populations for chromosomes of 7th homoeological group: A – mono 7A Dm 2 × ANK (7B and 7C); B – mono 7B Dm 2 × ANK (7B and 7C); C – mono 7D Dm 2 × ANK (7B and 7C)

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Characterization of HMW-GS alleles associated with D and B genome of wheat *Triticum aestivum* L.

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Introduction

Bread wheat quality is mainly correlated with high molecular weight of glutenin subunits (HMW-GS) of endosperm. The number of HMW-GS alleles with good processing quality is limited in bread wheat cultivars, but there are plenty of HMW-GS alleles in wheat-related grasses to exploit high-molecular-weight glutenin subunits are important determinants of wheat dough quality as they give the dough visco-elastic properties required for mixing and baking performance. Because of that HMW-GS alleles are key markers in breeding programs. In this study, we describe the use of the PCR markers *Glu-1Bx7*, *Glu-1Dx2*, *Glu-1Dx5*. The examined sequences that were derived from different cultivars of *Triticum aestivum* L. appear to be highly homologous whereas polymorphic sites identified in them are primarily of single nucleotide polymorphisms (SNPs). These small differences in nucleotide sequences make huge differences in the quality of wheat.

Material and methods

Plant material

The study presented here examines the gene integrase in 26 old cultivars of wheat *(Triticum aestivum ssp vulgare* L.). All the material was obtained from the collection of the Institute of Plant Breeding and Acclimatization in Radzików.

DNA purification

Genomic DNA was extracted from fresh 5-6 days old ethiolated coleoptyles from 28 cultivars of wheat. The isolation was performed with Genomic DNA from tissue kit (Macherey-Nagel)

PCR-STS analysis

The oligonucleotides used as primers were designed based on the literature data and synthesized in the Institute of Biochemistry and Biophysics, Polish Academy of Sciences. It was used following primers sequences (tab.1)**.** PCR analyses were performed in a T100 ^{TM} Thermal Cycler (BIO-RAD) with a heated lid in the final volume of 25µl. The single PCR reaction mixture contained: 1xbuffer (Novazym), 2mM $MgCl_2$, 2 mM dNTP, 5 mM each primer, 50ng genomic DNA and 0,4U Allegro Taq DNA Polymerase (Novazym). These reaction samples were detected by agarose gel electrophoresis of the 10µl of the PCR products, staining by ethidium bromide (EtBr), and photographing under UV light.

Results and Discussion

In this study using STS-PCR method, after performing sequencing, made it possible to obtain sequences having the following lengths: *Glu-1Bx7*- 287bp, *Glu-1Dx2*- 357bp *Glu-1Dx5*- 414bp (Table 1, Fig. 2). Using an appropriate pairs of primers facilitated the amplification of the desired variations of the HMW-GS allelic genes. A strong resemblance was observed among the analyzed allelic variations of the *Glu-1* genes both when directly comparing the obtained sequences: *Glu-1Bx7, Glu-1Dx2, Glu-1Dx5* and when comparing them to the sequences derived from the gene bank. It is understandable since all these sequences encode allelic variants high-molecular-weight of glutenin subunits. Despite enormous homology, polymorphic sites were also observed in these sequences, predominantly single nucleotide polymorphisms (SNPs). Analysing the obtained *Glu-1Bx7* sequences, single SNP mutations were found. In the case of *Glu-1Dx2,* in addition to the SNP indel mutation was found while in the *Glu-1Dx5* only very few substitutions were observed (Fig. 1). In the pool of the studied ordinary wheat cultivars, the *Glu-1Bx7: 1a* allele was located only in two cultivars, the *Glu-1Dx5: 1d* in 13, and the *Glu-1Dx2: 1a* in 15 cultivars (Table 2).

Table 1: Primers names, sequences and references

Allele	**Primer sequences 5'-3'**	**Products lenght [bp]**	**Reference**
Glu1Dx5	F:TGCCTGGTCGACAATGCGTCGCTG R: GAAACCTGCTGCGGACAAG	414	D'Ovidio, Anderson 1994
Glu1Dx2	F: GCCTAGCAACCTTCACAATC R:GAAACCTGCTGCGGACAAG	357	Anderson, Green 1989
Glu1Bx7	F: CGCAACAGCCAGGACAATT R: AGAGTTCTATCACTGCCTGGT	287	Schwarz et al. 2004

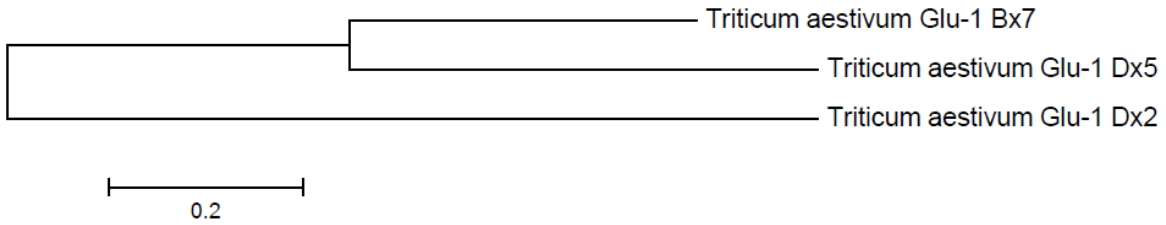

Fig. 1: Relationships between the obtained sequences: *Glu-1Bx7, Glu-1Dx2, Glu-1Dx5* of wheat (*Triticum aestivum* ssp *vulgare* L.) , generated in the data MEGA 6.0

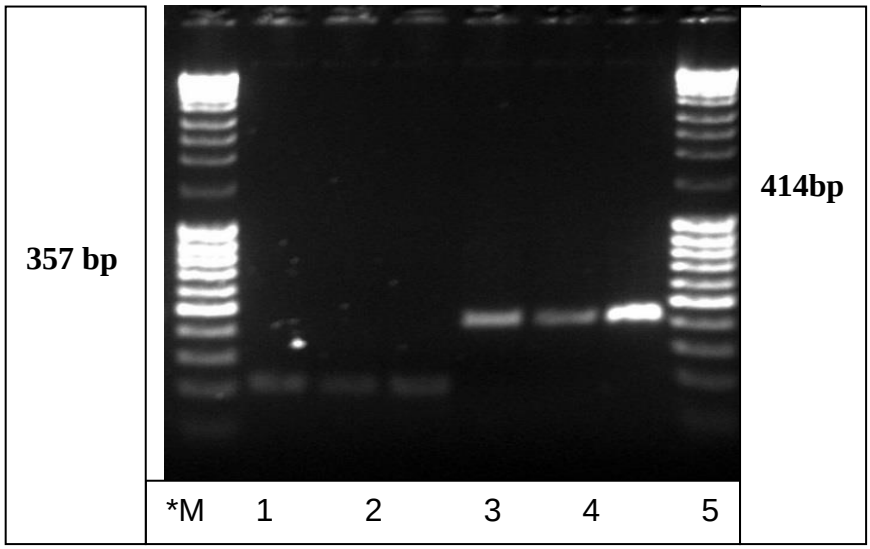

Fig. 2: PCR-STS analysis for the *Glu-D1a: Dx2:*357 bp. Lanes: *M-Mass Ruler^{TM}DNA Ladder Mix, 1-Antonińska S.46, 2-Balta, 3-Biała Kaszubska end *Glu-D1d: Dx5:*414bp, 4-Dańkowska Biała, 5-Murzynka Lipińskiego, 6- Sobótka

Table 2: The presence of product of aplification *Glu-1* genes in studied the cultivars of wheat

Cultivars	Genome D		Genome B	Cultivars	Genome D		Genome B
	Glu1Dx5: 1d	***Glu1Dx2: 1a***	***Glu1Bx7 : 1a***		***Glu1Dx5: 1d***	***Glu1Dx2: 1a***	***Glu1Bx7: 1a***
Antonińska S46	0	1	0	**Mydlniczanka**	1	0	0
Balta	0	1	0	**Niewylegająca**	1	0	0
Biała Kaszubska	0	1	0	**Ostka Czerwona Łopustka**	1	0	0
Choryńska	0	1	0	**Ostka Górczańska**	1	0	1
Dańkowska Biała	1	0	0	**Ostka Kazimierska**	0	1	0
Eka 132	0	1	0	**Ostka Mikulic Selit**	0	1	0
Magnatka Rogalińska	0	1	0	**Ostka Nadwiślańska**	0	1	1
Mudczanka Czerwona	0	1	0	**Poznańska**	0	1	0
Murzynka Lipińskiego	1	0	0	**Sielecka Genetyczna**	1	0	0
Sobieszyńska 44	1	0	0	**Tryumf Mikulic**	1	0	0
Sobótka	1	0	0	**Udyczanka Czerwona**	1	0	0
Srebrzysta	1	0	0	**Wysokolitewska Antoninska**	0	1	0
Ślązaczka	1	0	0	**Wysokolitewska Sztywnosłoma**	0	1	0
Squarehead Grodkowicka	0	1	0	**Żelazna**	0	1	0

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New winter wheat DH lines with high intergeneric crossability

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Introduction

Enhancing genetic variability of cultivated wheat gene pool by alien useful gene introgression can greatly contribute to genetic progress in wheat breeding. However, the success of wide hybridization methods aiming to transfer desirable new alien genetic variability from more distant related species i.e. secondary or even tertiary reservoirs is difficult and depends firstly upon cross compatibility (crossability) between wheat and donor species. Unfortunately, the great majority of modern wheat cultivars carry dominant restrictive alleles at *Kr* loci located on group 5 homoeologous chromosomes (Riley and Chapman, 1967; Sasaki and Wada, 1966; Krolow, 1970) and on 1A (Luo et al., 1989)- thereby reducing the chances of their use as recipient parent. Recessive kr alleles promoting crossability were reported in several old spring cultivars and landraces from China, Japan, Iran and Eastern Siberia (Zeven,1987). Since these primitive genotypes are not suitable for breeding purposes because of their lower agronomic values, transfers of recessive *kr* alleles into advanced wheat cultivars was accomplished using simple backcross procedures or by intervarietal substitution method of individual chromosomes. Gay and Bernard (1994) reported the transfer of recessive *kr* alleles from cultivars Norin 29 and Fukuhokomugi into cultivar Courtot by substituting chromosomes of homoeologous group 5. Similarly, Marta Molnar et al. (1996, 2010) transferred recessive *kr1* and *kr2* alleles from Chinese Spring into Martonvasari 9 cultivar by backcrossing. Chromosome method of substitution was also used to replace chromosome 5B of the cultivars Favorit and Bezostaia 1 by its homologue from Chinese Spring carrying *kr1* allele (Giura, 2001). Although, the crossability values for Favorit(Chinese Spring-5B) and Bezostaia1(Chinese Spring-5B) substitution lines was increased three-four times registering 10.30% and respectively 12.47% by comparisons with recipient cultivars Favorit with 2.98% seed set and Bezostaia 1 (3.77% seed set), their involvement in wide hybridization was insignificantly. Moreover, both intervarietal substitution and backross methods are time consuming so that the recipient genotype is often outdated and surpassed from the agronomic point of view by other new genotypes.

Subsequently, another locus *SKr1* controlling crossability was identified and located on short arm of 5B chromosome. The effect of *SKr1* was stronger that the minor QTL located on the 5B long arm, supposed to be *Kr1* (Tixier et al.1998; Lamoureux et al.2002; Alfares et al.2009).

A new opportunity have emerged by casually identification of an advanced semi- dwarf (*RhtB1*) breeding line F.132 showing a good but variable cross compatibility with several rye genotypes. This line has in ascendance Pecking 8 genotype of Chinese origin from which inherited crossability feature. It carries also *Lr34* and *Lr67* genes that confer good resistance to leaf rust, one of the most important diseases seriously affecting wheat yield in Romania. Separating the component biotypes by DH technology, it was possible to identify several of them with acceptable 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%. DH F.132-1-30, one of these lines, was then selected as donor for kr allele (probably kr1) in a program aiming to develop crossable genotypes more adapted to local conditions.

Material and methods

Two sub-lines of Martonvasari 9 genotype (Mv3/1 and Mv4/3) both carrying kr1kr1 kr2kr2 alleles (kindly provided by Marta Molnar-Lang, Martonvasar Institute, Hungary) were crossed with DH-line F.132-1-30. The F1`s plants resulted from crosses Mv3/1 x DH.132-1-30 (group A) and Mv4/3 x DH.132-1-30 (group B) were then crossed by maize under greenhouse conditions, haploid plants regenerated by *in vitro* haploid embryos culture, colchicine treatment applied on plantlets at three-five tiller stage and DH0 harvested. Following seed multiplication, parental forms and a number of 38 DH-lines previously selected for high values of seed set from more than one hundred were crossed with *Secale cereale*, cv. Harkovskaia in four consecutive years (2010-2014). As supplementary checks in group B were included also two DH lines proved to be equal crossable to parent DH line F.132-1-30 in a preliminary test. Five spikes (20- 22 florets/spike) per each DH- line and parents were emasculated and 2-3 days latter pollinated with fresh collected rye pollen once the stigmas had become receptive. Before and post-pollination, spikes were covered with special bags to avoid uncontrolled pollination. All crosses were carried out under field conditions, each season in May. Hybrid state of the resulting seeds was determined at maturity firstly upon their size and if uncertain, in next spring period, in field conditions, at heading time, after spike morphology. The degree of crossability was evaluated as percentage of total hybrid seed obtained/total pollinated florets. An average seed set percentage was then calculated for each genotype and parents each season and averaged over years.

Results and discussion

As expected from the allelic constitution at *Kr* loci the average crossability percentages of parents was largely different. For DH line F.132-1-30 the average seed set of seasons was lower of only 22.40% but with more uniform values between years (SD=2.70). Conversely, the two sub-lines of Martonvasari 9 with higher average over seasons of 48.7% for Mv3/1 and 45.3% for Mv4/3 presented a large variation between years evidenced by SD values of 6.25 and respectively 9.8 (Fig. 1).

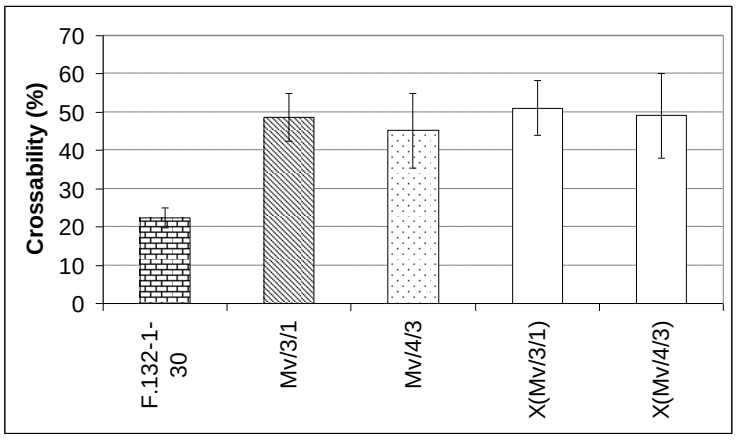

Fig.1: Percent crossability of parental genotypes and groups of DH-lines (X and SD)

This variation was probably caused by susceptibility to biotic and abiotic stress factors. So, the limits of variation for parental sub-line Mv3/1 that ranged in the limits 39.04- 52.90% and for sub-line Mv4/3 between 33.52-56.68% become plausible.

Similarly, the majority of tested DH lines presented distinct annually variation. Nevertheless the average values of two groups are not significantly different. Many DH lines of the two groups were noted as having superior average values in one or even in two specific seasons, but significant low crossability in the other seasons in circumstances of severe foliar diseases, especially brown and yellow rusts and Septoria sp.

However, as valuable exception, some DH lines presented constantly high seed set levels with smaller differences between years. Moreover, these lines exceed the crossability levels of parental forms too.

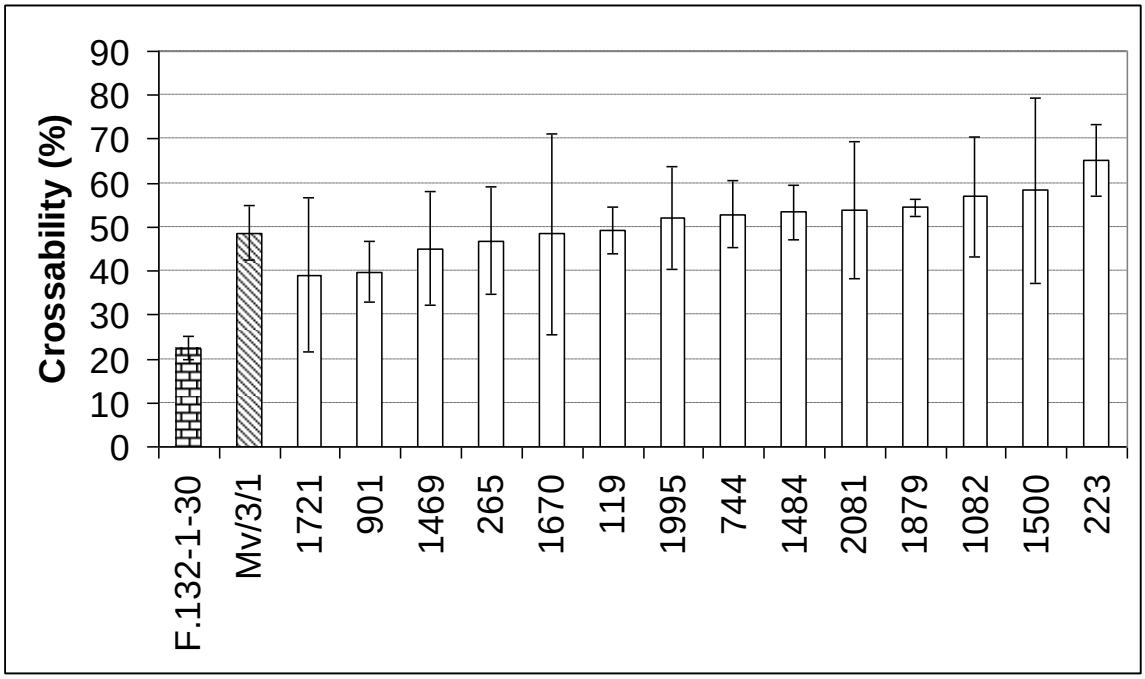

Fig.2: Distribution of the crossability (%) in the group A of DH lines. Standard deviation and confidence intervals calculated for each line.

Among the 14 DH lines of group A derived from the cross Mv3/1 x DH F.132-1-30, two sublines coded by numbers 223 and 1879 were noted for a constant higher crossability ranging from 57.34 to 77.60 (SD=2.70) and respectively 53.5-56.60 (SD=1.81). Other two lines 1500 and 1082 with high average seed set, still presented larger seasonal differences (Fig. 2).

A quite similar dispersion for crossability was observed in group B of 26 DH lines derived from the cross Mv4/3 x DH F.132-1-30. Three lines, 1751, 889 and 1773, were evidently noted for their constant percent crossability over the four seasons, with individual average over 60 (Fig. 3). Other lines showed a variable seasonal crossability. The eventually connection between high/low crossability and resistance/susceptibility to foliar diseases, in due course, will provide more information on that subject in relationships to wheat genetic pool enrichment with valuable alien genes.

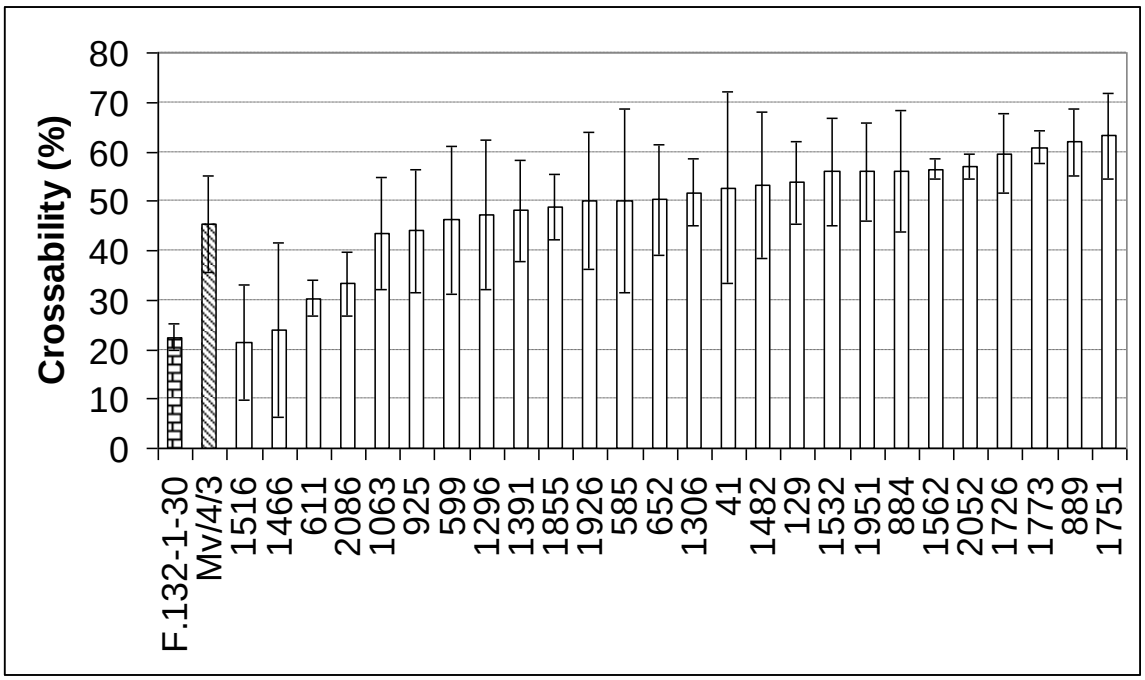

Fig.3: Distribution of the crossability (%) in the group B of DH lines. Standard deviation and confidence intervals calculated for each DH line.

Conclusions

Development of crossable winter wheat DH lines with superior agronomic traits, well adapted to local conditions represent a new challenge to extend donors spectra of related species for alien gene introgression into modern cultivated gene pool.

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Association Analysis of Microsatellite Markers with Morphological Traits of Grain in Bread Wheat Varieties of PBGI

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Introduction

Determining of specific genomic regions which control important agronomical and morphological traits is considered to be one of the main goals of applied genetics of agricultural plants. Grain shape, size, density and uniformity are important attributes which influence wheat milling performance and flour quality. Theoretical models predict that milling yield could be increased by optimizing grain shape and size with large and spherical grains being the optimum grain morphology (Gegas et al., 2010). Grain weight, which affects on several other wheat quality criteria, is thought to be related to the grain shape and size since these parameters determine the way the individual grain packs. Moreover, grain size was also found to be associated with various characteristics of flour, such as protein content and hydrolytic enzymes activity, which in turn determine baking quality and end-use suitability (Evers, 2000). In wheat association mapping was employed for the detection of quantitative trait loci (QTLs) for grain size and milling quality (Breseghello and Sorrells, 2006) where the association test was conducted between kernel traits and SSR markers in 95 elite wheat germplasms.

In the present study we have applied microsatellite markers (Röder et al., 1998, 2002) for the association with major loci connected with controlling of wheat grain morphology in a core collection from modern Ukrainian bread wheat varieties (Kolesnyk et al., 2013). Furthermore we report the distribution of alleles at microsatellite loci associated with increasing and decreasing values of wheat grain parameters obtained with the help of digital image analysis (DIA) (Williams et al., 2013). DIA methods that convert photographs into quantitative data based on measures of axes or pixel counts have been used by numerous research groups (Campbell et al., 1999; Breseghello and Sorrells, 2006, 2007). The main task of this research is focused on detection of significant associations between microsatellite markers and morphological traits, which can be used for applying in plant molecular breeding for a more effective screening of wheat genotypes with improved traits sufficient for increasing of wheat yield.

Materials and methods

Plant materials

The analyzed material consists of 48 bread winter wheat varieties (*Triticum aestivum* L.) originated in Plant Breeding and Genetic Institute (PBGI) and registered in State Register of plant varieties suitable for dissemination in Ukraine during different years: Hospodynia (2007), Scarbnytsia (2007), Kosovytsia (2008), Antonivka (2008), Zamozhnist' (2008), Blahodarka odes'ka (2009), Misiia odes'ka (2009), Dal'nyts'ka (2005), Yednist' (2008), Kiriia (2004), Liona (2005), Kuial'nyk (2003), Poshana (2004), Zaporuka (2008), Bunchuk (2009), Podiaka (2008), Oksana (2007), Zahrava odes'ka (2010), Epokha odes'ka (2010), Lytanivka (2008), Sluzhnytsia odes'ka (2009), Hoduval'nutsia odes'ka (2009), Istyna odes'ka (2010), Zmina (2007), Dovira (2009), Krasen' (2009), Otaman (2008), Borvii (2010), Turunchuk (2008), Diuk (2008), Nebokrai (2011), Khyst (2013), Pylypivka (2011), Zorepad (2011), Zhaivir (2010), Uzhynok

(2010), Hurt (2013), Dobrochyn (2013), Vatazhok (2011), Pol'ovyk (2009), Holubka odes'ka (2011), Kniahynia O'lha (2011), Lebidka odes'ka (2 samples, 2011), Zhuravka odes'ka (2011), Bezmezhna (2008), Lastivka odes'ka (2011). Varieties Albatros odes'kyi (1990) and two collection samples of variety Bezosta 1 (1955) were taken into research as standard (etalon) samples according to recommendation of Ukrainian Institute of Examination of Plant Varieties of Ministry of Agriculture and Food of Ukraine as a national standards provided for distinctness, uniformity and stability (DUS) of new varieties of bread wheat (*Triticum aestivum* L.) for the purpose of granting the Breeders' Right.

Genotypic data

Genomic DNA was extracted from seedlings using modified CTAB method (1998). Polymerase chain reactions (PCR) was performed on a Tertsyk thermocycler (DNA Technology, Russia) according to Röder et al. (1998). All data were evaluated by means of descriptive statistic instruments of EXCEL package. Averaged means and their errors were calculated for every group (if provided variety number in it was 4 and more) carrying different alleles of SSR markers, for each season separately. Significance of between-group differences was estimated by t-criterion (Rokitskii, 1973).

Phenotypic analyses

The seeds have been sown by plots in field experiment conditions in 2010/2011, 2011/2012, 2012/2013 growing seasons by the laboratory of Variety Investigation and Breeding Process Modeling of PBGI, located in Odessa, Ukraine (46° 27' 3", 30° 39' 18"). From each variety in a randomized way 5 seedlings were taken for microsatellite analysis (MS-analysis), of which 1 was used for the further growing under spike-row scheme. The obtained grain material was analyzed by extracting of 5 morphological traits from 2D digital images: area of grain image (Ag), perimeter (Pg), length (Lg), width (Wg) and grain circularity (Cg). On the studied material there was shown the previously determined hereditary nature of statistically significant associations of allelic state revealed by microsatellite markers with the expressiveness of a number of parameters of these morphological traits.

Results and discussion

Genetic diversity

Genetic diversity in studied varieties of interest was evaluated based on allelic richness (range of allele size and number of alleles per locus) as a measure of genetic variation; presence of specific alleles found in one variety but absent in others as a measure of genetic distinctiveness and uniformity; PIC of each SSR marker as a measure of genetic diversity. A total of 114 alleles were detected at 17 SSR markers among the 47 winter bread wheat varieties, and the number of alleles per locus ranged from 4 to 10 with an average of 6.7. The largest number of alleles (10) was found at *Xgwm155*-3A, *Xgwm325*-6D and *Xbarc126*-7D loci, whereas only four alleles were detected at *Xgwm190*-5D locus in the investigated varieties. The average PIC value was 0.67 with a range of 0.48 (*Xgwm190*-5D) – 0.84 (*Xgwm155*-3A) (Table 1).

Table 1: Microsatellite allelic polymorphism in 48 modern bread wheat varieties of PBGI

№	SSR markers	Allele size (bp)	Allelic No. / locus	PIC	Relative frequency of the most frequent allele
1	*Xgwm186-5A*	102 – 142	9	0.67	0.548 (102 bp)
2	*Xgwm095-2A*	110 – 130	6	0.65	0.490 (122 bp)
3	*Xgwm357-1A*	116 – 134	7	0.73	0.416 (125 bp)

4	*Xgwm18-1B*	186 – 196	5	0.72	0.396 (192 bp)
5	*Xgwm3-3D*	75 – 88	7	0.83	0.244 (86 bp)
6	*Xgwm165/I-4A*	185 – 195	5	0.70	0.360 (183 bp)
7	*Xgwm155-3A*	129 – 152	10	0.84	0.264 (149 bp)
8	*Xgwm389-3B*	117 – 145	7	0.82	0.248 (117 bp)
9	*Xgwm190-5D*	204 – 212	4	0.48	0.616 (208 bp)
10	*Xgwm325-6D*	115 – 150	10	0.83	0.280 (144 bp)
11	*Xgwm437-7D*	105 – 113	5	0.55	0.524 (107 bp)
12	*Xgwm44-7D*	176 – 187	6	0.59	0.576 (185 bp)
13	*Xbarc126-7D*	138 – 166	10	0.73	0.376 (164 bp)
14	*Xwmc405-7D*	210 – 222	6	0.62	0.464 (220 bp)
15	*Xgwm408-5B*	148 – 192	6	0.51	0.652 (188 bp)
16	*Taglgap-1B*	207 – 265	6	0.53	0.660 (218 bp)
17	*Xgwm577-7B*	137 – 175	5	0.65	0.532 (173 bp)

Trait analysis

Mean values of the evaluated traits in all seasons analyzed showed inconsiderable levels of inter-variety diversity: 4,8% for Ag, 2.9 % for Pg, 3.6 % for Lg, 2.7 % for Wg and 2.4 % for Cg (Table 2). The largest value of Ag was evaluated in varieties Dovira and Blahodarka odes'ka, while the smallest value of Ag had variety Zahrava odes'ka. Variety Dovira had also the largest value of Pg, while the smallest value of Pg was evaluated in variety Yednist'. This variety was characterized also by smallest value of Lg, while varieties Zaporuka, Dovira and Kosovytsia had the largest value of Lg. The shortest value of Wg was detected in variety Borvii, while varieties Antonivka, Lastivka odes'ka (2011) and two collection samples of variety Bezosta 1 had the largest value of Wg. Variety Yednist', which was characterized by the smallest values of Pg and Lg, had the biggest value of Cg, while the smallest value of Cg was evaluated in variety Nebokrai.

Table 2: Levels of inter-variety diversity for area of grain image (Ag), perimeter (Pg), length (Lg), width (Wg), circularity (Cg)*

DATA	Ag	Lg	Wg	Pg	Cg
Mean	17,07	6,70	3,23	17,07	0,73
SD	0,83	0,24	0,09	0,50	0,02
CV	4,84	3,59	2,75	2,94	2,39
min	15,43	6,14	3,03	15,95	0,69
max	18,72	7,20	3,43	18,07	0,77

* Ag, Pg, Lg, Wg are given in cm^2 and cm, accordingly; Cg was estimated as $Cg = 4\pi * Ag / Pg^2$; Mean – average values; SD – standard deviation; CV – coefficient of variation, %

Association analysis

Significant marker-trait associations (MTAs) for Ag, Pg, Lg, Wg and Cg in two – 2-3 analyzed years are given in table 3. Overall 9 MTAs for Ag, 16 for Pg, 18 for Lg, 3 for Wg and 21 for Cg were found to be stable and significant in 2-3 growing seasons. The SSR marker *Xgwm186*-5A was significantly associated with all analyzed traits and showed stability in 3 years for Ag, Pg and Lg. Markers *Xtaglgap*-1B, *Xgwm325*-6D and *Xgwm437*-7D showed stable associations with Pg, Lg and Cg in 2-3 years analyzed, while *Xgwm357*-1A was significantly associated with Ag,

Pg, Lg and Cg. 4 SSR alleles for Ag, 8 for Pg, 8 for Lg, and 1 for Wg were found to significantly associated with the increase of these values, respectively, while 5 SSR alleles for Ag, 8 for Pg, 9 for Lg, and 2 for Wg were associated with their reduction. As for Cg, 10 SSR alleles were found to be significantly associated with the larger value of Cg and 11 SSR alleles with the smaller value of Cg; 3 SSR alleles showed alternative effect on the value of Cg.

Table 3: The number of alleles according to their associations with area of grain image (Ag), perimeter (Pg), length (Lg), width (Wg) and grain circularity (Cg)

Characteristics	Ag	Pg	Lg	Wg	Cg
Number of alleles increasing the value of studied trait	4	8	8	1	10
Number of alleles decreasing the value of studied trait	5	8	9	2	11
Alternate	1	2	1	1	3
Rest	99	91	91	105	85

For Ag 9 marker trait associations (MTAs) were shown to be stable in 2-3 growing seasons, namely alleles *Xgwm357*116, *Xgwm389*136, *Xgwm165/1*191, *Xgwm186*135 were found to be significantly associated with the larger value of Ag while alleles *Xgwm357*125, *Xgwm389*119, *Xgwm165/1*195, *Xgwm186*102, *Xgwm186*115 showed to be significantly associated with the smaller value of Ag. Additionally during 2 growing seasons allele *Xgwm186*142 was alternatively associated both with smaller and larger values of Ag. For the value of Pg 4 MTAs were found to be stable in 3 growing seasons and 12 MTAs were proved to be significant in 2 growing seasons. Overall 8 SSR markers were associated with the larger value of Pg, 8 alleles were associated with the smaller value of PH and alleles *Xgwm186*142 and *Xgwm165/1*191 were alternatively associated both with smaller and larger values of Pg in 2 growing seasons. 21 MTAs for Cg were found to be stable and significant during 2-3 growing seasons. The smaller value of Cg was significantly associated with alleles *Xgwm357*116, *Xgwm357*125, *Xtaglgap*218, *Xgwm095*130, *Xgwm186*113, *Xgwm186*115, *Xgwm325*115, *Xgwm325*144, *Xgwm325*146, *Xgwm437*107, *Xgwm437*113 while the larger value of Cg showed associations with alleles *Xgwm357*123, *Xgwm357*128, *Xtaglgap*238, *Xgwm095*122, *Xgwm095*128, *Xgwm3*77, *Xgwm155*141, *Xgwm186*102, *Xgwm325*148, *Xgwm437*109. Alleles *Xgwm3*86, *Xgwm3*88, *Xgwm155*149 was detected to be alternatively associated both with smaller and larger values of Cg in 2 growing seasons. 18 MTAs for Lg and 3 MTAs for Wg were proved to be significant in 2-3 growing seasons. The analysis revealed that 8 alleles were associated with the larger value of Lg and 9 alleles with the smaller value of Lg; allele *Xgwm357*125 was detected to be alternatively associated both with smaller and larger values of Lg in 2 growing seasons. As for Wg alleles *Xgwm3*81, *Xgwm3*83 were associated with the smaller value of Wg, allele *Xgwm3*79 showed to be significantly associated with the larger value of Wg and allele *Xgwm186*102 was alternatively associated both with smaller and larger values of Wg in 2 growing seasons.

Thus, the association analysis with microsatellite markers was performed to map the regions associated with expression of morphological traits. The presence of contrast allele pairs in different microsatellite loci associated with either increasing or decreasing values of grain morphology during several seasons can be explained by the inheritance of those contrast alleles, which can be situated near major morphology QTLs. Grain morphology represents a number of polygenic traits that are controlled by the different components of the genetic systems in their interaction with each other and with the changing environmental conditions. Such changing environmental factors can cause the alternative way of association of the same microsatellite

allele with either increasing or decreasing values of grain morphology in different seasons of study.

Stable marker-trait associations can be used in breeding programs aiming at combining the best alleles in advanced wheat genotypes with increased grain size and/or improved grain weight.

Conclusions

This study demonstrated the presence of diversity among modern varieties originated from PBGI (Odessa, Ukraine). In order to facilitate and improve breeding process, extensive studies focused on finding traits of interest associated with microsatellite markers and different genomic regions of wheat are required and could be assisted by molecular marker technologies. In conclusion, 4 SSR alleles for Ag, 8 for Pg, 8 for Lg, 1 for Wg and 10 for Cg were found to significantly associated with the increase of these values, respectively, while 5 SSR alleles for Ag, 8 for Pg, 9 for Lg, 2 for Wg and 11 for Cg were associated with their reduction. 8 SSR alleles showed alternative effect on different values of wheat grain morphology.

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120 years of winter wheat breeding in Germany – a case study

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Introduction

Wheat is the oldest major food in the human diet. Beside rice it is the most common food in the world, since it is grown on large areas in comparison with the rest of the crops. In 2013/2014 the global wheat growing area was 218 million hectares; 713 million tons of grains were harvested. In Germany the wheat growing area was 3.1 million hectares yielding in 25 million tons (FAOSTAT 2013). The development of the area and yield of winter wheat in Germany from 1891 till 2013 is shown in figure 1 (DFG 1891 to 2013).

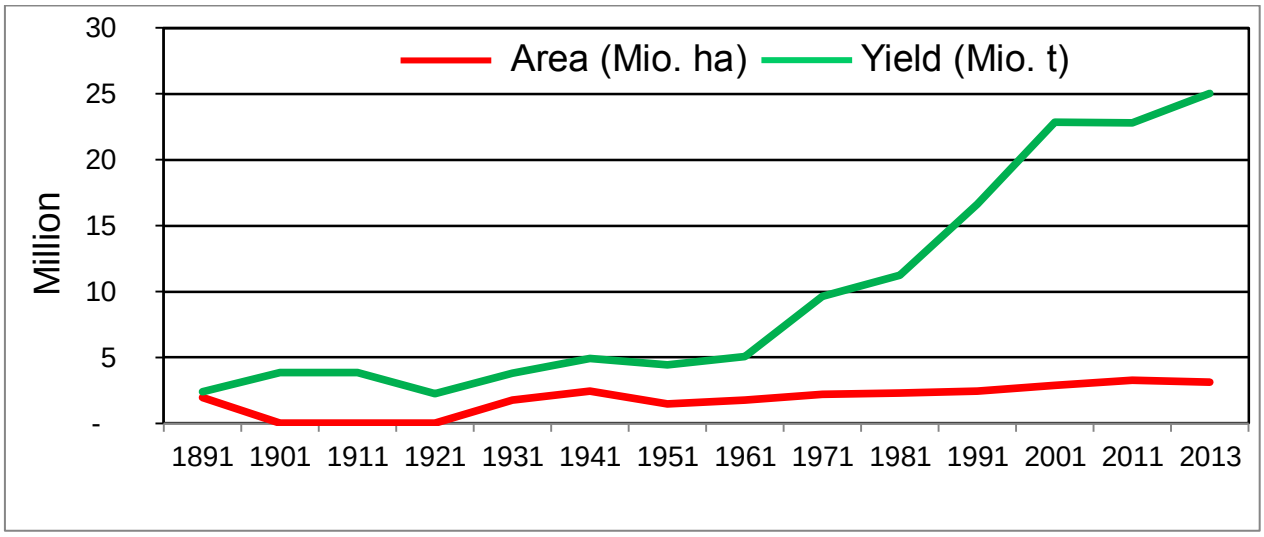

Fig.1: Area and yield of winter wheat in Germany 1891-2013

The aim of the present study was to identify the development of breeding progress for winter wheat in Germany during the last 120 years. Grain yield and other agronomic characters were considered.

Materials and methods

In total 20 winter wheat cultivars were investigated originated from Germany and grown by the farmers in the periods between 1891 and 1910 as well as 1991-2010, respectively (table 1).

Table 1: Cultivars from different growing periods used in the present study

Growing period	Cultivar name
1891 – 1910	Rimpaus Früher Bastard Rimpaus Dickkopf Strubes Dickkopf Cimbals Großherzog von Sachsen Steigers Leutewitzer Dickkopf Breustedts Extra Dickkopf Janetzkis Frühe Kreuzung Kraffts Siegerländer Ruppiner Brauner Landweizen Ackermanns Brauner Dickkopf
1991 – 2010	Ritmo Zentos Astron Borenos Orestis Akteur Cubus Dekan Drifter Tommi

The material was grown at the experimental fields of IPK in Gatersleben during the season 2013/2014 in plots having a size of 1.5 x 1 m. The sowing date was 9 October, 2013.

The following agronomic traits were considered:

Days to flowering after sowing
Plant height (cm)
Lodging (scale 1-3, with 3 = strong lodging, 1 = no lodging)
Grain yield (g; 20 spikes)
Straw yield (g; 20 tillers)
Harvest index (20 spikes)
Grain number per spike (average of 20 spikes)
Thousand grain weight (g; 20 spikes)
Grain yield per plot (g)

Results and discussion

The results for the traits investigated are given in the figures 2 – 7. With respect to flowering time there is a tendency that the modern cultivars are earlier, although the cultivar ‘Rimpaus Früher Bastard’ (Rimpau’s Early Crossbreed) was as early as the earliest modern cultivars ‘Borenos’ and ‘Cubus’ (Fig. 2). For plant height a clear reduction in the modern cultivars was observed ranging from 90 to 120 cm. In contrast, the old cultivars reached 150 to 180 cm (Fig. 3). In a study comparing German winter wheat cultivars released between 1966 and 2007 Ahlemeyer and Friedt (2012) observed an early flowering time and a reduced plant height in the modern cultivars. The latter was mainly due to the introduction of the *Rht* genes. Also Austin et al. (1980) found that already in the late 1970s the newer, high yielding, varieties were shorter and reached anthesis earlier than the older ones.

The reduction in plant height has an evident effect on lodging resistance having a score of 1 for all modern cultivars. However, three of the tall cultivars in the range between 140 and 160 cm did also have a score of 1 (Fig. 4). The reason may be a special elasticity or cell wall stability of the tillers. The reduced plant height is also the reason for a reduced straw yield (20 tillers) of the modern cultivars as shown in figure 5. With respect to the yield of 20 single spikes no obvious tendency over the years was found, however, the harvest index was increased (Fig. 6 and 7). For the yield components grain number per spike and thousand grain weight again no clear tendency was observed (Fig. 8 and 9). There was a slight tendency for an increased grain weight. Finally, the grain yield of the plots did show an increase in the modern cultivars (Fig. 10) which may be due to a higher number of spikes per square meter (not scored). This would be in agreement with results of Canevara et al. (1994) indicating that the yield increase in winter wheat is due to a higher number of fertile tillers. In contrast, Ahlemeyer and Friedt (2012) detected an increase in the grain number in German winter wheat cultivars released between 1966 and 2007 whereas the thousand grain weight and number of fertile tillers was not participating in yield increase. The reason for this disagreement may be due to the different time periods under investigation. In addition, many other factors as adaptability or disease resistance may have contributed to yield increase.

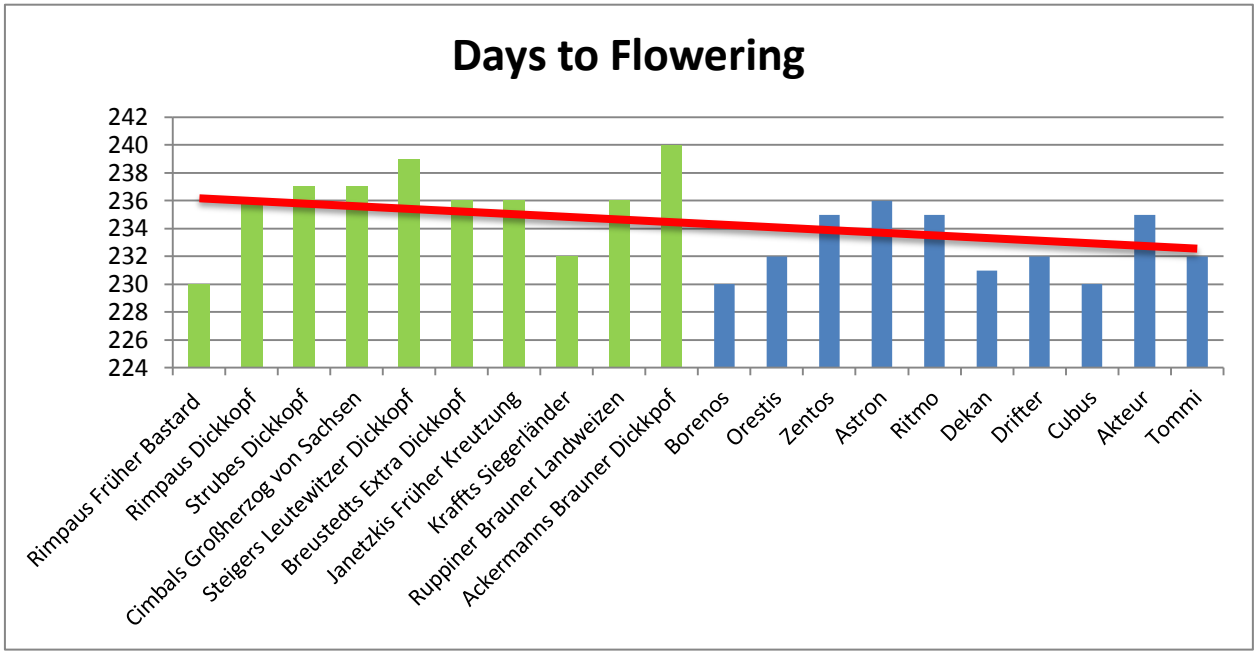

Fig. 2: Days to flowering after sowing. Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

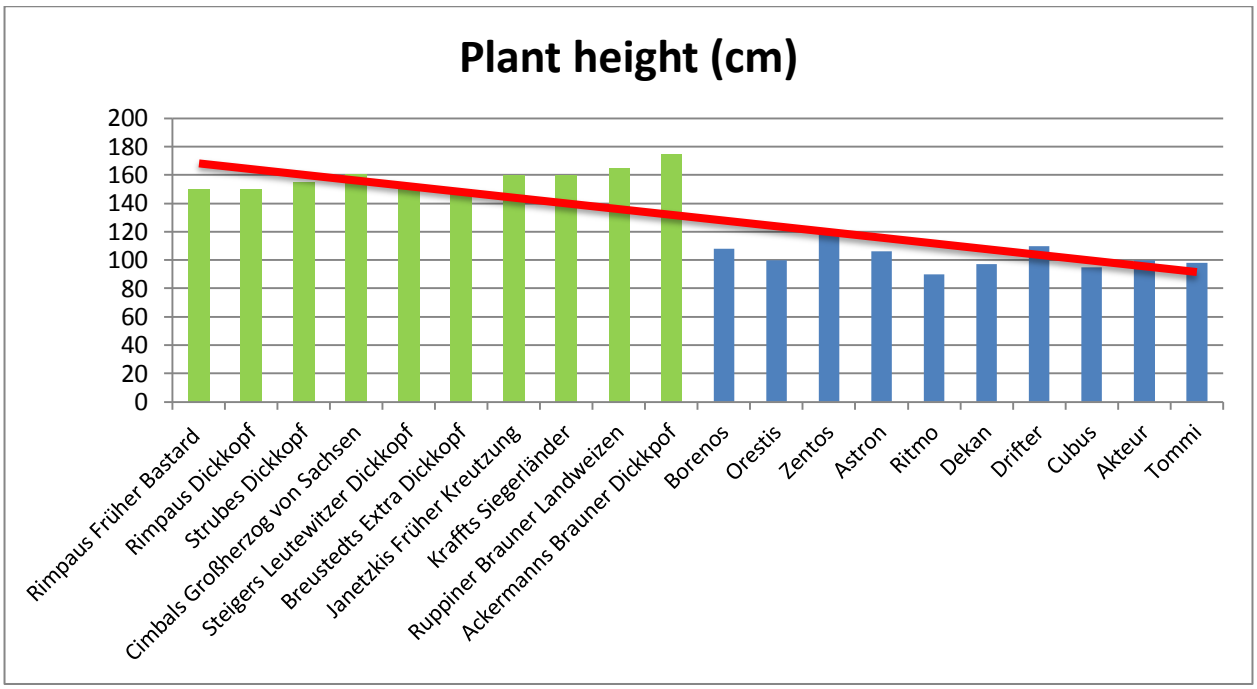

Fig. 3: Plant height. Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

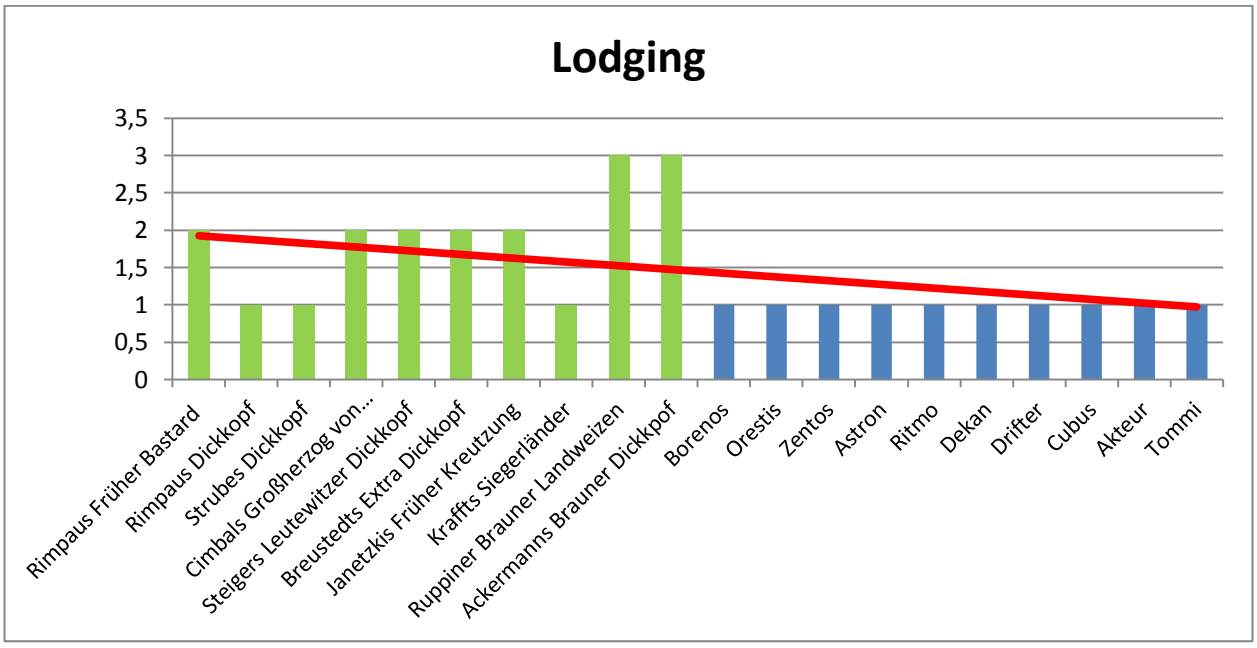

Fig. 4: Lodging (3 = strong lodging, 2 = moderate lodging, 1 = no lodging). Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

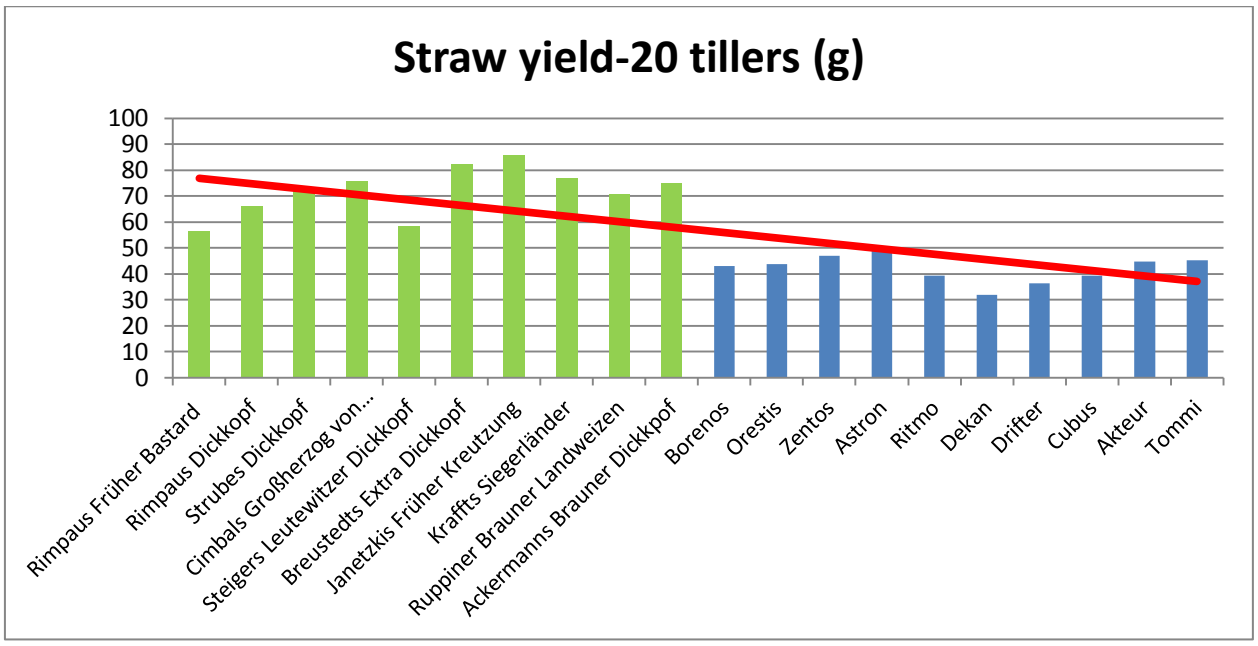

Fig. 5: Straw yield of 20 tillers. Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

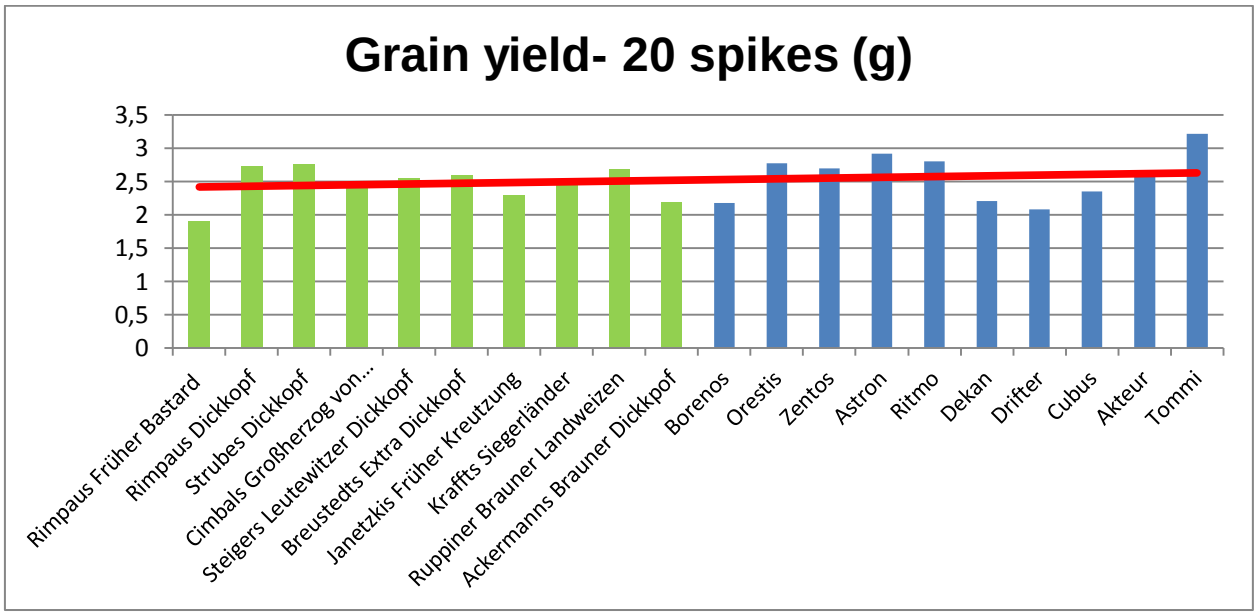

Fig. 6: Grain yield of 20 spikes. Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

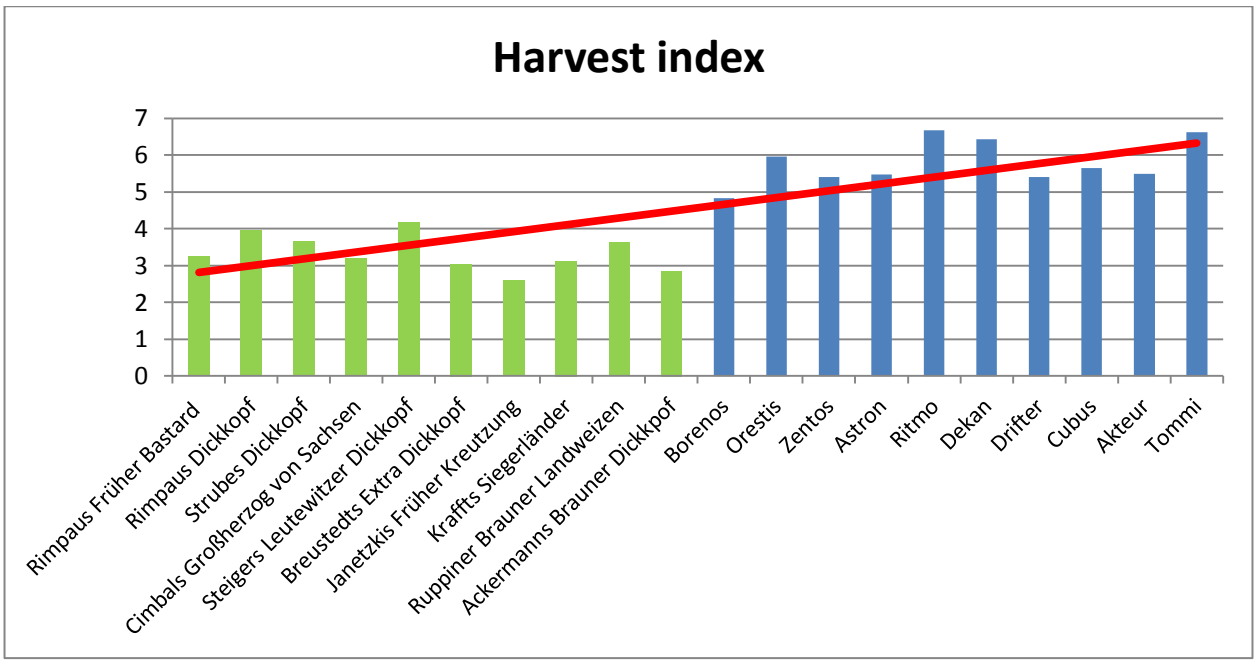

Fig. 7: Harvest index. Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

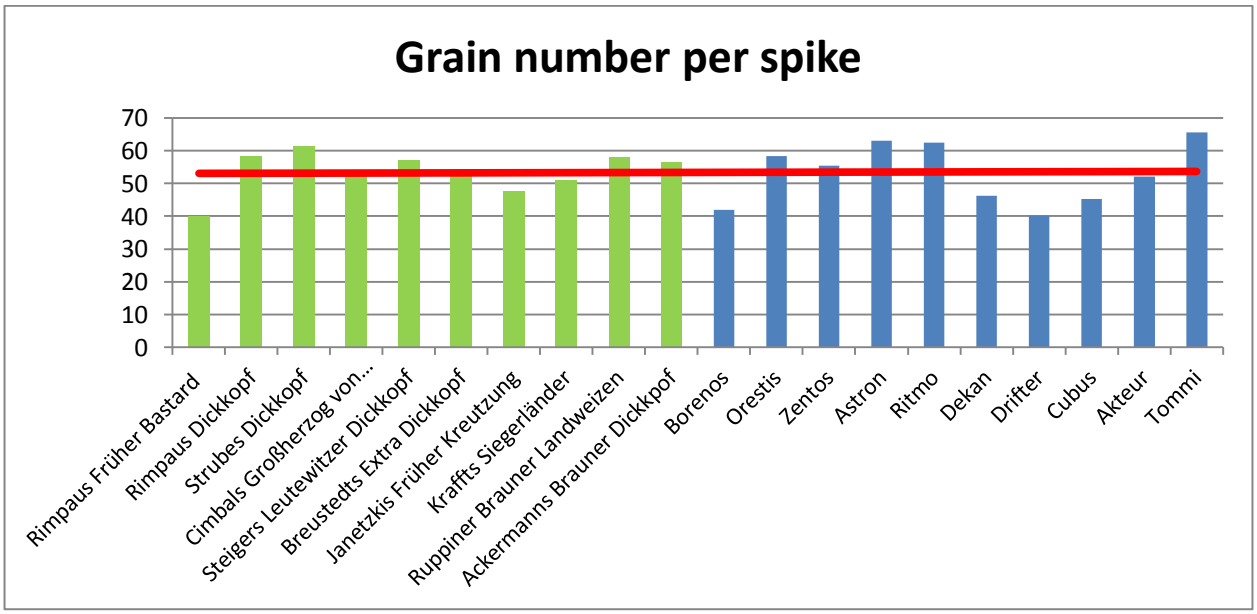

Fig. 8: Grain number per spike. Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

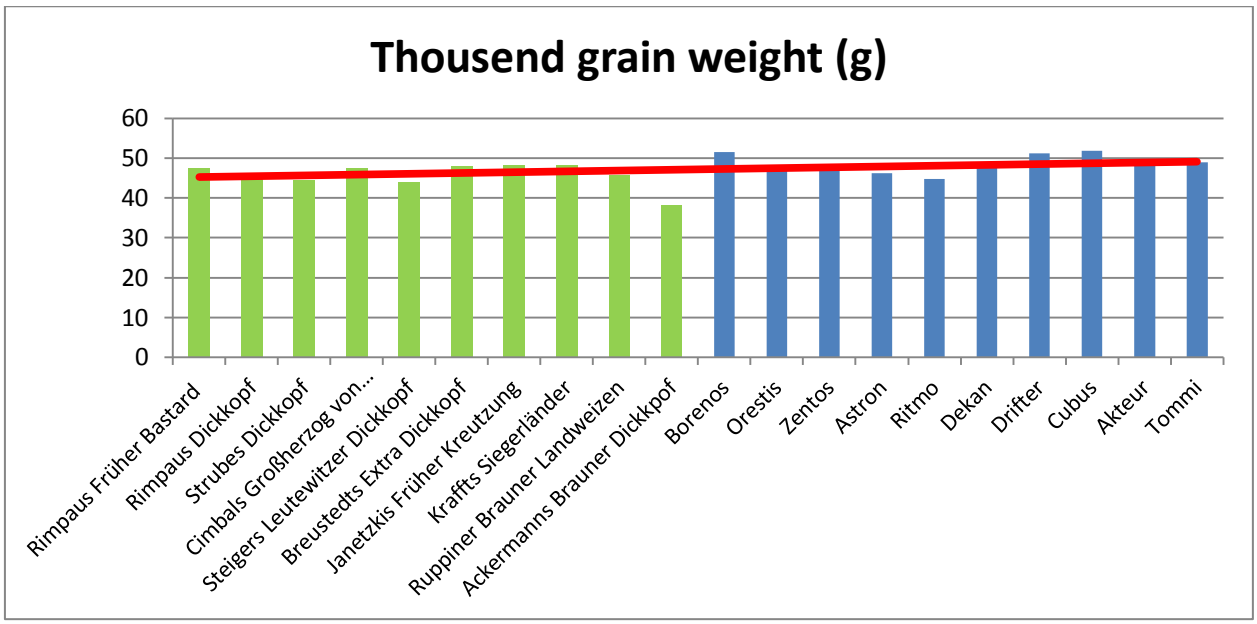

Fig. 9: Thousand grain weight. Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

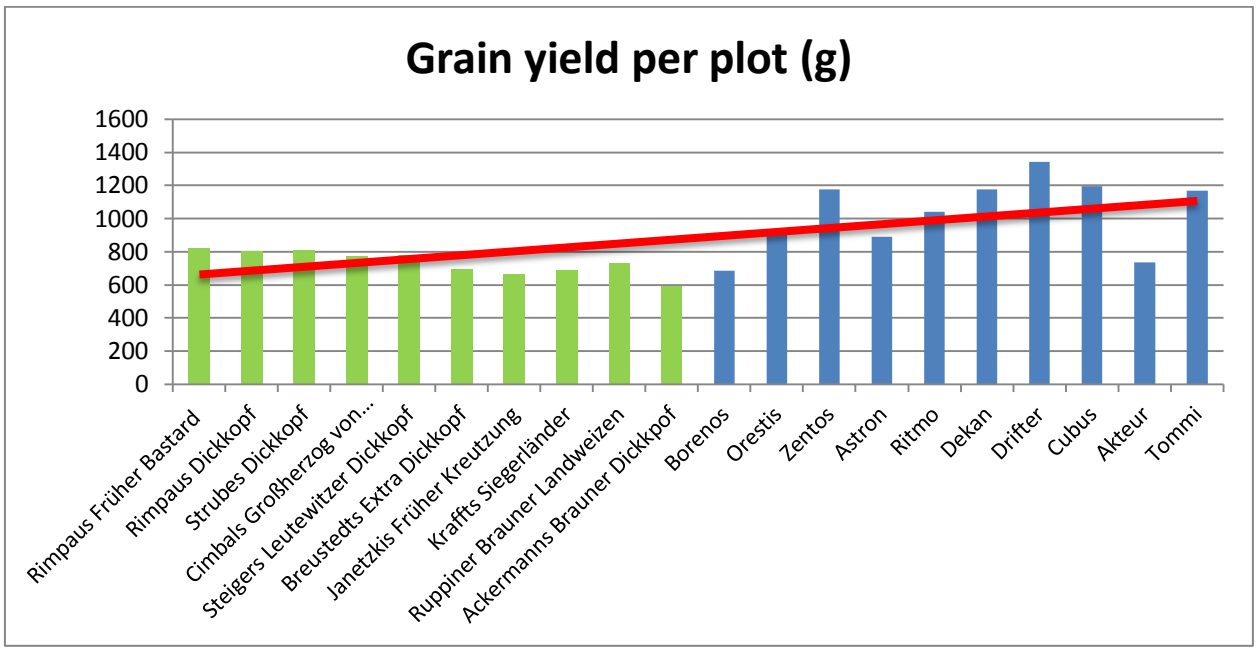

Fig. 10: Grain yield per plot. Green columns: Old cultivars (1891 - 1910); Blue columns: Modern cultivars (1991 – 2010).

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Research on improvement of resistance to powdery mildew in oat

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Powdery mildew caused by *Blumeria graminis* DC. f. sp. *avenae* Em. Marchal. is one of the most important foliar disease of common oat in the cooler and humid regions of north-west Europe (Schwarzbach and Smith 1988) and south-east United States of America (Leath et al. 1991). In Germany and Great Britain is the most serious fungal disease of oat (Jones 1977, Roderick et al. 2000, Herrmann and Roderick 1996). In recent years powdery mildew was reported in Central and Eastern Europe, also in Poland intensification of powdery mildew symptoms were observed (Sebesta et al. 1991, own observation). The annual crop losses from mildew infections are estimated to range from 5-10% up to 40% in years of low and high disease pressure (Jones 1977). Weather conditions have a significant effect on the development of the fungus. It was observed that the disease becomes more intense after mild winters and warm springs (Priestley and Bayles 1979). The result of the effect of powdery mildew is a reduction of photosynthesis efficiency, a drop in the number of grains and grain weight as well as a decrease in the content of carbohydrates in grain (Roderick and Jones, 1988; Roderick et al. 2000). The ability to infect plants within a wide range of temperatures and humidity constitutes an important epidemiological property of powdery mildew. High genetic changeability and the ability to generate new forms by mutations and DNA recombinations make this pathogen easily adaptable to new conditions (Bennet, 1984; Bayles, 1997).

Till date 8 resistant gene to powdery mildew has been identified (*Pm1-Pm8*). In breeding programmes only three of them were commonly used. Hsam et al. (1997) tested 256 oat cultivars and breeding lines collected from Western Europe and North America. Among this cultivars and breeding lines eight had resistance patterns of *Pm6* , six cultivars of *Pm1,* and eleven cultivars *Pm3*. Among cultivars and breeding lines from Northern and Eastern Europe Hsam et al. (1998) find that only 5% tested genotypes possess resistant genes. Five genotypes showed the resistance of *Pm6*, one cultivar possess *Pm3* gene. Okoń (2012) screened Polish common oat cultivars and showed that only four posses resistant gene against powdery mildew. Cultivar Dragon possess *Pm6* gene, cultivar Skrzat *Pm1*. In cultivars Deresz and Hetman *Pm3* resistant gene was identified. Resistance corresponding to OMR4 were not found in the tested Polish common oat cultivars. Cultivar Canyon shoved very interesting results. This cultivar was resistant to all isolates used in host-pathogen tests; this pattern of resistance was different than obtained to standard differential cultivars and lines (Table 1). This research showed that in polish breeding programmes only three resistant gene were used, but there is no information about effectiveness of this genes.

Table 1: Reaction of 30 oat cultivars grown in Poland after inoculation with 6 differential isolates of powdery mildew (Okoń 2012).

Cultivar/line	***Blumeriagraminis* DC. f.sp. *avenae*Em.**						**Oat Mildew Resistance (OMR)**	**Pm gene**
	M1	**M**	**M1**	**M1**	**M1**	**M2**		
Fuchs	s[1]	s	s	s	s	s	OMR0	-
Bruno	s	i[2]	r[3]	r	i	r	OMR1	*Pm6*
Jumbo	r	r	r	i	s	i	OMR2	*Pm1*
Mostyn	s	r	s	s	r	r	OMR3	*Pm3*
AV1860	r	r	r	r	s	s	OMR4	*Pm4*

Akt	s	s	s	s	i	i	-	-
Arab	s	s	s	s	s	i	-	-
Bachmat	s	r	i	s	s	s	-	-
Bajka	s	s	s	s	s	s	-	-
Borowiak	s	i	s	s	s	s	-	-
Boryna	s	s	s	s	s	s	-	-
Borys	s	s	s	s	s	S	-	-
Cacko	s	i	i	i	s	s	-	-
Cekin	s	s	s	s	s	s	-	-
Chwat	i	i	i	s	s	i	-	-
Deresz	s	r	s	s	r	r	OMR3	*Pm3*
Dragon	s	i	r	r	i	r	OMR1	*Pm6*
Dukat	s	s	s	s	s	s	-	-
Farys	s	s	s	s	s	i	-	-
German	s	s	s	s	s	s	-	-
Góral	s	i	i	i	i	i	-	-
Grajcar	s	s	s	i	i	s	-	-
Hetman	s	r	s	s	r	r	OMR3	*Pm3*
Jawor	s	s	s	s	s	s	-	-
Karol	s	s	s	s	s	s	-	-
Kasztan	i	i	i	s	i	s	-	-
Komes	s	s	s	s	s	s	-	-
Kwant	s	s	s	s	s	s	-	-
Santor	s	s	s	s	s	s	-	-
Sławko	s	s	s	s	s	s	-	-
Sam	s	s	s	s	s	s	-	-
Skrzat	r	r	r	i	s	i	OMR2	*Pm1*
Canyon	r	r	r	r	r	r	U[4]	U
Sprinter	s	s	s	s	s	s	-	-
Polar	s	s	s	s	s	s	-	-

[1]s = susceptible,[2] i – intermediate, [3]r – resistance, [4]u - unknown

Okoń (2015) estimate effectiveness of resistance genes to powdery mildew in Polish condition using host-pathogen tests. 55 powdery mildew single spore isolates from different part of Poland were used in this experiment. This research showed that the most effectives genes in Polish condition were *Pm4* and *Pm7*, this genes can be used in breading programmes to increase the level of resistance in cultivated oat. Host-Pathogen tests showed that resistance conditioned by *Pm1, Pm3* and *Pm6* was breaking down in Polish condition (Table 2).

Table 2: Reaction of control oat lines and cultivars to different isolates of powdery mildew (Okoń 2015).

s- susceptible, I – intermediate response, r- resistant

Origin of powdery mildew isolates	izolat number	Bruno *Pm6*	Jumbo *Pm1*	Mostyn *Pm3*	Av1860 *Pm4*	APR122 *Pm7*	Canyon	Fuchs
Choryń	1	s	s	s	r	r	r	s
	2	i	i	s	r	r	r	s
	3	s	i	s	r	r	i	s
	4	s	s	s	r	r	r	s
	5	s	s	s	r	r	i	s
	6	s	s	s	r	r	i	s
	7	s	s	s	r	r	r	s
	8	s	i	s	r	r	i	s
	9	s	s	s	r	r	i	s
	10	s	s	s	r	r	i	s
	11	s	s	s	r	r	i	s

	12	s	s	s	r	r	r	s
	13	s	i	s	r	r	r	s
	14	s	s	s	r	r	i	s
	15	s	s	i	r	r	r	s
	16	s	s	s	r	r	r	s
	17	i	s	s	r	r	r	s
	18	i	s	s	r	r	r	s
	19	i	i	s	r	r	i	s
	20	i	i	s	r	r	i	s
	21	i	s	s	r	r	i	s
	22	i	s	s	r	r	i	s
Strzelce	1	s	s	s	r	r	i	s
	2	i	s	s	r	r	i	s
	3	s	s	s	r	i	r	s
	4	i	s	s	r	i	r	s
	5	i	s	s	r	r	r	s
	6	s	i	s	r	r	r	s
	7	s	s	s	r	r	r	s
	8	s	i	s	r	r	r	s
	9	s	s	s	r	r	r	s
	10	s	s	s	r	r	i	s
	11	s	s	s	r	r	i	s
	12	s	s	s	r	r	r	s
	13	s	s	s	r	r	r	s
	14	s	i	s	r	r	r	s
	15	s	i	s	r	r	r	s
	16	s	i	s	r	r	r	s
	17	s	s	s	r	i	r	s
	18	s	s	s	r	r	r	s
	19	s	s	s	r	r	i	s
	20	s	s	s	r	r	i	s
	21	s	s	s	r	r	i	s
	22	s	s	s	r	r	r	s
Czesławice	1	s	s	s	r	r	i	s
	2	s	s	s	r	r	r	s
	3	i	s	s	r	r	r	s
	4	s	s	s	r	r	i	s
	5	s	s	s	r	r	i	s
	6	s	s	s	r	r	i	s
	7	s	s	s	r	r	i	s
	8	s	s	s	r	r	i	s
	9	s	s	s	r	r	r	s
	10	s	s	s	r	r	r	s
	11	s	s	s	r	r	i	s

Our own observations carried out in 2010-2014 both in laboratory and field conditions showed that effectiveness of *Pm1, Pm3* and *Pm6* genes has been change. In 2010 *Pm1* and *Pm3* genes showed resistant or intermediate response to powdery mildew isolates collected in Poland. In 2014 effectiveness of *Pm1* gene was breaking down by all tested isolates of powdery mildew, also in field conditions this gene was susceptible. *Pm3* gene in field condition showed intermediate response, but in laboratory condition resistance of this gene was breaking down by more than 50% of tested isolates. In polish condition only *Pm4* and *Pm7* genes are still effective against powdery mildew, but the level of resistance of *Pm7* gene begins to be breaking down. There was observed that more and more isolates infects the line with *Pm7* gene.

Therefore there is a need to identify new and effective sources of resistance. In genus *Avena* exist many wild species which could be used as a potential sources of resistance. Our

investigation showed that tertaploid species characterized very high level of resistance to powdery mildew. Because of strong sterility barriers which isolate species of lower ploidy levels from the cultivated forms the transfer of resistant genes cannot be achieved by means of a similar backcrossing programme. More effective transfer of resistant genes could be achieved using species which share all genomes with *A. sativa*. Our experiments based on host-pathogen tests showed that among *A sterilis* genotypes exist potential donors of resistant genes (Fig. 1).

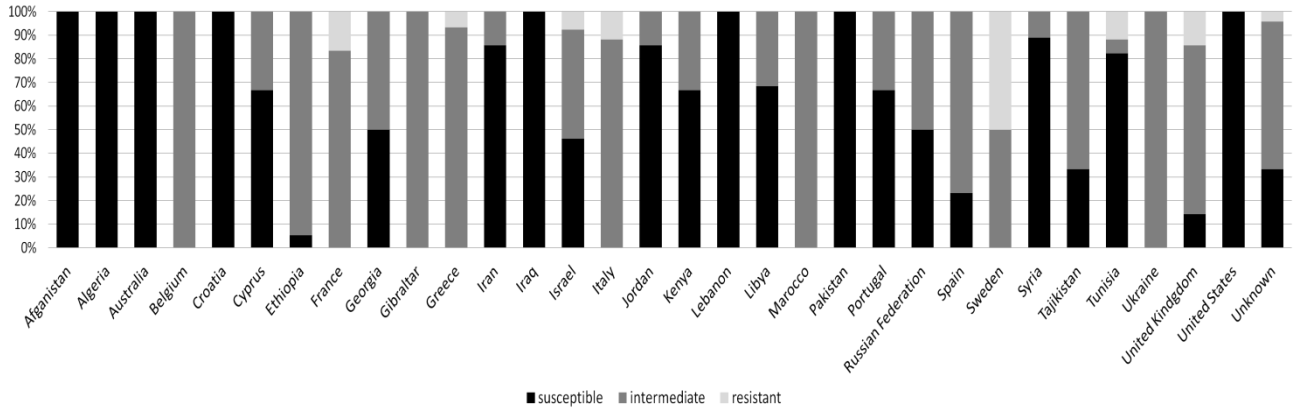

Fig. 1: Reaction of *A. sterilis* genotypes from different countries to powdery mildew isolates.

In future work we are going to characterize the new sources of resistance to powdery mildew in *A. sterilis* genotypes and transfer this genes into cultivated oat.

Part of this work was carried out in the framework of the Programme LEADER V project number: LIDER V/21/p325/L-5/13/NCBR/2014 "Identification of new and effective resistance genes to fungal diseases in oats and development of DNA markers for their identification", supported by the National Research and Development Centre.

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Putative markers towards *Dw6* dwarfing gene in oats

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Abstract

Lodging, one of the main factors causing quantitative and qualitative losses in the yield of grain, could be prevented by use of varieties with increased tolerance supported by dwarfing genes In Polish breeding programs of oats dominant *Dw6* gene derived from OT207 line is used to shorten the straw. This gene allows to obtain the specimens characterized by two most desirable in the cultivation of cereals features: increased resistance to lodging and high yielding.

The primary aim of the study was the identification of putative *Dw6* gene markers by means of RAPD and BSA approach. A total of 600 RAPD primers were tested for polymorphisms in the bulk of short and tall F_2 individuals. Only 1 marker ($G12^{1200}$) was associated with plant height.

Introduction

New forms characterized by high quality and increased yield of seeds as well as tolerant to biotic and abiotic stresses are the principal purpose of cereal breeding programs. Cultivation of intensive forms that positively react towards plant protection and increased fertilization enables achieving high yield. However, in the case of lodging efficient plant vegetation is diminished. This phenomenon is in fact one of the main factors causing quantitative and qualitative losses in the yield of grain. The extent of the damage depends on the lodging expanse and timing of its occurrence. Lodging exhibits detrimental effects on seed and straw quality (Börner et al. 1999). Moreover, efficient conduct of photosynthesis and proper uptake of water and nutrients are disrupted, which inevitably leads to inhibition of normal growth and development of plants.

Lodging could be prevented via application of retardants. However, the use of chemicals seriously affect not only the environment but also the human and animal health, especially when the residues of preparations are collected in seeds. What is more, due to the lack of sufficient studies and distinct reaction of oat varieties, retardants are usually not recommended.

The alternative way that could be considered for lodging prevention is the use of varieties with increased tolerance supported by dwarfing genes. In Polish breeding programs of oats dominant dwarfing *Dw6* gene derived from OT207 line is used to shorten the straw (Milach and Federizzi 2001). *Dw6* reduces the height of individuals belonging to the *Avena* genus up to 60% of basal by truncation of two or three upper internodes. This gene, sensitive to exogenous application of gibberellic acid, allows to obtain the specimens characterized by two most desirable in the cultivation of cereals features: increased resistance to lodging and high yielding (Burrows 1978; Marshall and Murphy 1981; Milach et al. 1998).

The primary aim of the study was the identification of putative *Dw6* gene markers by means of RAPD and BSA approach.

Materials and methods

The F_2 biparental oat mapping population ('Celer' x STH 9210) was the object of the study. The STH 9210 is a line with *Dw6* gene determining short stature whereas 'Celer' is the Polish breeding cultivar growing up to 120 cm high.

The heights of 110 F_2 individuals were measured from the soil surface to the top of the panicle on the main tiller of each plant at maturity. To discriminate between homozygotes and heterozygotes for short straw, 50 F_3 seeds from each self-pollinated F_2 individual were sown and the heights of the plants measured. On the basis of F_3 generation observations homozygous F_2 plants were selected and used to look for markers. The BSA method was used to examine molecular markers for plant height. For this purpose, DNA from the 15 shortest and 15 tallest F_2 individuals were pooled.

Random amplification of DNA fragments was carried out using 600 arbitral RAPD primers. PCR was performed in a volume of 10 µl with an optimized composition comprising: deionized water, a PCR buffer, $MgCl_2$, dNTPs, arbitral primer, DNA Taq polymerase and proper DNA sample. To verify the putative markers reactions of the selected primers with DNA of random F_2 homozygous plants were performed.

Results

The average height of 'Celer' specimens was 107 cm and that of 'STH 9210' was 75 cm. Plant heights in the F_2 population varied from 58 to 133 cm, with a mean of 86.5 cm. The distribution of heights indicates a monogenic dominant inheritance of the dwarfing gene. We classified the F_2 individuals into homozygotes (non-segregating progenies) and heterozygotes (segregating progenies) by measuring the heights in F_3 generation. The segregation of the different genotype classes (26:55:29) fits the 1:2:1 ratio expected for a monogenic trait.

A total of 600 RAPD primers were tested for polymorphisms in the bulk of short and tall F_2 individuals. Twelve primers (2%) produced polymorphic markers. These markers were analyzed in the random individual plants of the bulks. Only 1 marker ($G12^{1200}$) was associated with plant height and the total F_2 population was analyzed with it (Fig. 1). The 84 F_2 individuals carrying the $G12^{1200}$ marker had a height of 74 ± 15 cm and the 25 F_2 individuals without it had a height of 121 ± 12 cm. The association of the marker with height was statistically significant ($P< 0.05$, $\chi^2 = 0.012$). The RAPD phenotype of 1 individuals tested (0,9%) did not correlate to height.

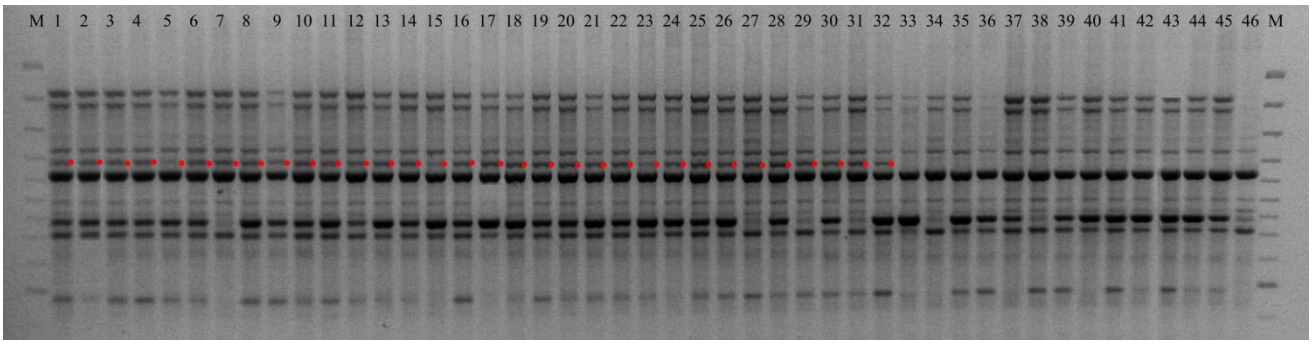

Fig. 1: RAPD profile of amplification obtained with G12 primer in homozygous F_2 individuals of 'Celer' x STH 9210 population.

Summary

Promising results arising from the application of dwarfing genes in breeding programs suggest the need for intense search for molecular markers coupled with them (Paczos-Grzęda and Grądzielewska 2012). One of the first attempts was made by Milach et al. (1997). The use of RFLP method combined with Bulk Segregant Analysis allowed them to determine precisely the position of target gene on the basis of identified Xumn145B marker located 3.3 cM away. Codominant SNP markers were obtained from the conversion of polymorphic RAPD products and non-specific REMAP markers (Tanhuanpaa et al. 2006). It was also found that the *Dw6* dwarfing gene encodes probably one of the subunits of vacuolar V-ATPase (Molnar et al. 2012).

The above-mentioned putative markers for *Dw6* dwarfing gene do not allow to identify differences between short and high Polish line forms or still require detailed verification. Identification of $G12^{1200}$ marker gives the ability to convert the resulting fragment in the specific SCAR marker. This procedure would allow for an assessment of the gene introduction effectiveness. It is necessary to carry out the sequencing of differentiating fragments and design specific primers enabling further analysis. The conversion eliminates the main disadvantages of RAPD method: increases the reproducibility of the results obtained and provides greater specificity of identification.

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Chromosomal regions in genome D of *Triticum aestivum* L. associated with the activity of different lipoxygenase forms under drought

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Introduction

Adaptation of higher plants to biotic and abiotic stress is often accompanied by the occurrence of lipid peroxidation and the derived metabolites therefrom are called oxylipins. The synthesis of oxylipins is first catalyzed by different forms of lipoxygenases (LOXs), which add molecular oxygen to polyunsaturated fatty acids containing a *cis,cis*-1,4 pentadiene moiety for the formation of fatty acid hydroperoxides. These hydroperoxides are substrates for other enzymes and can be converted through different reactions of the LOX pathway resulting in synthesis of phytohormones, like jasmonic acid (JA) or traumatin, C6 volatiles, divinyl ethers and others. LOX-derived compounds are potent signaling molecules in the plants defense response (Liavonchanka and Feussner 2006).

Although the multiple isozymes of expressed LOXs have been identified in cereal species, the physiological role of LOXs in the certain cellular compartments of different plant organs has yet to be established. Little is known about the genetic variability of their activity. Quantitative trait loci (QTL) mapping of the activities of both soluble and membrane-bound LOXs can provide the information on chromosomal location of unknown genes that influence the quantitative variation for complex traits associated with plants resistance to stress including drought.

The aim of the work was to identify the QTLs associated with the activity of different LOX isoforms in D-genome of bread wheat using Chinese Spring/Synthetic 6x introgression lines and to search the correlations of these activities with a range of physiological traits of wheat plant.

Materials and methods

The genetic material used for the analysis included the parents Chinese Spring (CS) and Synthetic 6x (Syn) and the mapping population of *Triticum aestivum–Aegilops taushii* D-genome introgression lines (DILs) CS/Syn, each line harboring a single *Ae. tauschii* segment in the homozygous state (Pestsova et al. 2006). The investigated set consisted of 49 out of the possible 80 lines. Twenty of the DILs involve chromosome 2D, seven chromosome 4D, eight chromosome 5D and 13 chromosome 7D.

Plants were grown under controlled conditions in greenhouse (environment 1) and climatic chamber CLF PlantMaster (CLF Plant Climatic GMBH, Wertingen, Germany) (environment 2) mounted on the phytotron SIFIBR SB RAS under two contrasting moisture regimes: normal (60% of the full moisture capacity of the soil) and insufficient (30% water saturation) as described in detail by Osipova et al. (2013). Relative water content (RWC), shoot biomass, gas exchange and chlorophyll fluorescence of the flag leaf and yield components and tolerance index

(TI) were determined as described earlier (Osipova et al. 2011, 2013). The three day-old seedlings were germinated on the water (control) and 12% polyethylene glycol solution that simulates the osmotic stress under water deficit. LOX extraction and determination of enzyme activity methods were described earlier (Permyakova et al., 2012). The last was modified for the Infinite M200 PRO microplate reader (Tecan Group Ltd., Männedorf, Switzerland). 200 μL reaction mixture contained 5 μL of enzyme extract in all experiments. The response of each trait to moisture stress was assessed in the form of a tolerance index (IT). IT=Td/Tc·100, where Td - mean of trait of the droughted plants of a given line, Tc - mean of trait of the well-watered plants of a given line. Associations between phenotype and genotype were sought using the single marker regression routine implemented in QGENE software (Nelson 1997).

Results and discussion

Figure1 shows the revealed chromosomal regions associated with LOX activity in different organs of wheat. Activity of soluble seed LOX was linked to the distal region of the short arm of chromosome 4D regardless of the water supply. This chromosomal region is known for the presence of structural gene of LOX isozyme (Feng et al. 2010).

The variability of soluble and membrane-bound LOX activities of seedlings germinated under PEG were mapped to the regions located on different arms of chromosome 5D. One of the known loci responsible for polar lipid levels- *Fpl-1*, was found on the short arm, while another one, *Fpl-2* - on the long arm of chromosome 5D. The LOX loci were detected in our study in a similar position. This suggests the involvement of the enzyme in the metabolism of this type of lipids. The revealed locus associated with the activity of soluble LOX on the long arm may coincide with the known structural LOX gene (Feng et al. 2010) and also with the vernalization response gene *Vrn-D1* (Nelson et al. 1995) and QTLs of wheat-development parameters (Pestsova et al. 2006, Landjeva et al. 2010). In our work the parameters biomass, carotenoid content (Car) and electron transport rate at 160 μmol photons/m^2·s (ETR160) in one of the two locations have been mapped in the given chromosomal region (Table 1). The QTL associated with the activity of membrane-bonded LOX was located on the short arm of chromosome 5D. It was co-located with transpiration rate (TR), stomatal conductance (SC), water use efficiency (WUE) and effective photochemical quantum yield of PS II (Y(II)) in well-watered conditions (WW), stem length (StL) under water deficient (WD) and tolerance indices (IT) of several parameters: biomass, ETR160, initial slope of light curve, related to maximum yield of photosynthesis electrons/photons (α), StL, spikelet number (SpN) and grain weight (GW). Likely, this LOX locus may play a role in regulating the grain texture (softness or hardness). This assumption is based on the physiological and functional relationships between the puroindolines (PINs), - the lipid-transfer proteins associated with grain hardness (Pauly et al. 2013), the lipid-degrading enzyme LOX, and the polar lipids of starch grains that serve as a preferable substrate for LOX. The known results of the genomic studies show the co-localization of the genes *Pina-D1*, *Pinb-D1* and *Fpl-2* in the main locus controlling the grain hardness (*Ha*) in the distal end of the 5D chromosome (Turnbull and Rahman 2002). We found a minor QTL associated with membrane-bound LOX activities of seedlings in a close position.

On chromosome 7D, the LOX activities in leaves of wheat grown under drought both soluble and membrane-bound were linked with the centromeric region (Fig. 1). In the same region the photosynthetic rate and it's IT, as well as WUE were mapped (Table 1). This LOX QTL may be relevant for tolerance to a biotic stress because the known locus is associated with the resistance to Septoria tritici blotch in a similar position (Simon et al., 2007). Moreover, QTL associated with LOX activity on 7D chromosome, probably, also affect the endosperm texture. It was positioned near to the known locus of the variant form of puroindolin b which homoeoalleles were detected on all homoeologous group-7 chromosomes of wheat (Chen et al. 2011).

In addition, locus associated with the variability for the soluble LOX activity under drought was found in the centromeric region of chromosome 2D but with its IT in the distal region of the short arm of 2D. The activity of soluble LOX of chloroplasts under drought was associated with the marker *Xgwm261*. The first locus (SLOX) has been co-localized with two parameters of chlorophyll fluorescence, the second (IT SLOX) - with the photosynthetic electron transport. Furthermore, IT SLOX was co-located with the gas exchange parameters under water deficit, but a QTL of chloroplast LOX activity was co-located with the same parameters in well-watered condition (Table 1).

Correlation analysis revealed a positive relationship between the seed SLOX activity and grain productivity, more pronounced under drought. Both SLOX and MLOX activities in leaves under water deficit positively correlated with some parameters of grain productivity, gas exchange and photosynthesis and also with biomass, RWC and photosynthetic pigments content. In contrast, ChlSLOX activity had negative relationship with some above parameters except photosynthetic pigments content (data not shown). All of the leave LOXs showed a negative impact on the effectiveness of photochemical energy. However, SLOX had a positive impact on the electrons transport contrast to the chloroplast enzyme form.

Our results suggest that in wheat leaves the cytosolic enzyme form (SLOX) may have genetic regulation either in separate or in common with membrane-bonded LOX form (MLOX). Unlike, the chloroplast LOXs has an independent genetic regulation and the specific function under stress. ChlSLOXs are likely involved in the degradation of lipids and chlorophylls in thylakoid membranes and also may participate in non-photochemical quenching of chlorophyll fluorescence. It is possible that SLOX and MLOX are linked with the synthesis of jasmonic acid and other oxylipins, thus playing the roles in regulating of plant growth and defense status.

The QTL regions revealed in our work associated with the variability for parameters of photosynthesis, gas exchange and biomass and, ultimately, on grain productivity of wheat plant under drought. At the same time, they carry the genes for lipid metabolism pathways initiated by the individual LOXs. The genetic study of different LOX forms may shed some light on the processes regulating drought tolerance. Further research is required to refine the position of minor LOX loci.

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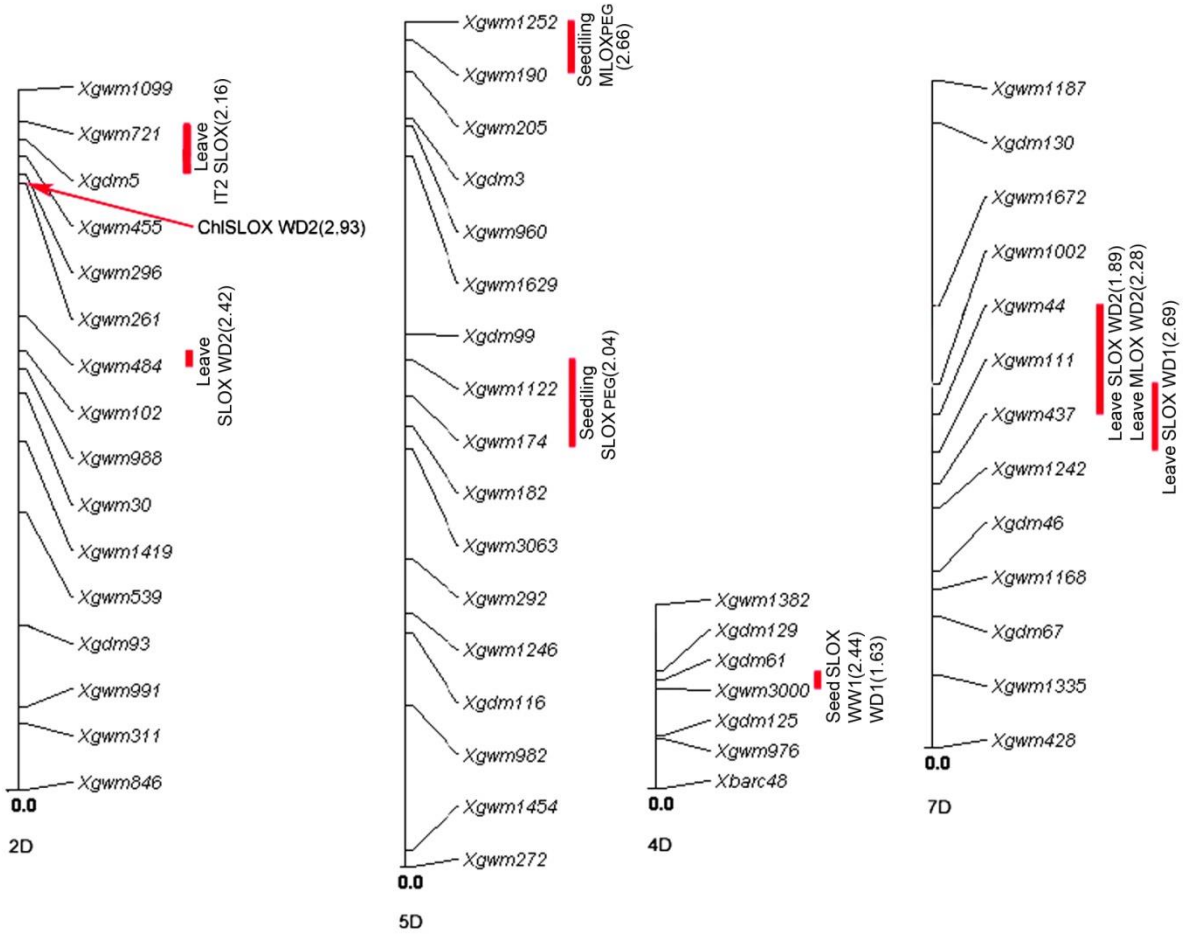

Fig. 1: Regions of bread wheat D-genome chromosomes harbored QTLs associated with the activity of different LOX forms under different conditions. SLOX- activity of soluble LOX; MLOX- activity of microsomal LOX; ChlSLOX - activity of soluble LOX of chloroplasts. WW –well-watered condition; WD –water deficit. 1 – plants were grown in a growing house; 2 - plants were grown in a climatic chamber; PEG - seedlings were germinated in 12% polyethylene glycol solution.

Table 1: Markers associated with the activity of different forms of wheat LOX and their association with the chromosome positions of various physiological traits

LOX loci	Marker	Chro-moso-me	Source of higher activity	Associated physiological traits (LOD>2,0)
Seed SLOX WW1; WD1	Xgdm129	4D	Syn	WUE WW2
Seedling SLOX PEG	Xgwm1122	5D	CS	Car WD1, Biomass WD2, ETR_{160} WD1
Seedling MLOX PEG	Xgwm190	5D	CS	TR WW1, Sc WW1, WUE W1, Y(II) WW2, IT1α, T1GW, IT1 SpN, IT1 StL, StL WD1
Leave SLOX WD1	Xgwm111	7D	CS	IT2 Phr, WUE WD2
Leave SLOX WD2	Xgwm102	2D	CS	LK WD1, α WD1
Leave MLOX WD2	Xgwm1002	7D	CS	Ph WD2
Leave SLOX WD2	Xgwm1002	7D	CS	Ph WD2
ChSLOX WD2	Xgwm261	2D	CS	TR WW1, Sc WW1, Ph WW1
Leave IT2 SLOX	Xgwm455	2D	CS	ETR_{max} WD1,2, Sc WD1, TR WD2

SLOX- activity of soluble LOX; MLOX- activity of microsomal LOX; ChlSLOX - activity of soluble LOX of chloroplasts; StL- stem length; SpN -spikelet number; GW - grain weight; TR - transpiration rate; SC - stomatal conductance; Phr - photosynthetic rate; WUE – water use efficiency; Y(II) - effective photochemical quantum yield of PS II; ETR160 - electron transport rate at 160 μmol photons/(m^2·s); ETRmax - maximum electron transport rate; α - initial slope of light curve, related to maximum yield of photosynthesis electrons/photons; LK - the light intensity at which the α and ETR lines intersect (unit: μmol photons/(m2·s)); Car - carotenoid content; IT - tolerance indice.

WW –well-watered conlition; WD –water deficit. 1 - plants were grown in the growing house; 2 - plants were grown in the climatic chamber; PEG - seedlings was germinated in 12% polyethylene glycol solution.

Association of introgressions in 2A, 2B and 5A chromosomes of bread wheat from *Triticum timofeevii* Zhuk. with parameters of gas exchange, chlorophyll fluorescence and activity of antioxidant enzymes under normal and water deficit conditions

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Alien hybridization in cereals is used for comparative investigations of genome structure and evolution as well as for extracting the useful genes from a wild gene pool. The tetraploid species *Triticum timopheevii* Zhuk. has long been used as a source of genes for resistance to fungi diseases. The line 821 (Fi. 1) was developed on the genetic background of drought resistant but very susceptible for diseases cultivar Saratovskaya 29 (S29), and resistant to leaf rust (Budashkina, Kalinina, 2001). According the genotyping data, the line carries big introgressions in 2A and 2B chromosomes and a small introgression in subtelomeric region of 5A chromosome (Leonova et al. 2001). The line was studied for a number of physiological and biochemical parameters under conditions of normal and restricted water supply in order to analyze the impact of introgressions on reaction to water deficit.

Materials and methods

Plants were grown under controlled conditions in the climatic chamber CLF PlantMaster (CLF Plant Climatic GMBH, Wertingen, Germany) mounted on the phytotron of SIFIBR SB RAS in a 1:1:1 humus:sand:peat mixture, and maintained under a 16 h photoperiod supplied at 600 μmol m -2s-1, a day/night temperature of 23/16 °C and 60 % relative humidity. One pot was maintained in a well-watered state (60% saturation), while water was withheld from the other pot, starting at the three leaf stage and continuing until the soil water content had fallen to 30 % of saturation, as determined by Osipova et al. (2011). Water stress was applied at 3-leaves stage. The following physiological and biochemical traits were studied: shoot biomass, (g); transpiration rate (E), stomatal conductance (SC), rate of CO2 assimilation (A), WUE (A/E) using using a portable gas exchange system (LCi Photosynthesis System, ADC BioScientific Ltd., Hoddesdon, England); potential (Fv/Fm) and real (Yield) efficiency of photosynthesis, rate of electron transport in photosystem II (ETR), non-photochemical quenching of fluorescence (NPQ) using a PAM-250 chlorophyll fluorometer (Heinz Walz GmbH, Effeltrich, Germany); leaf pigment content (chlorophyll A, chlorophyll B, carotenoids) was studied spectrophotometrically. Activity of antioxidant enzymes: dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), glutathione reductase (GR), superoxide dismutase (SOD) and catalase (CAT), and lipoxygenase (LOX) was studied using an Infinite M200 PRO microplate reader (Tecan Group Ltd., Männedorf, Switzerland); 2. All parameters were determined on the stage of heading. The flag leaf on the leading tiller of eight of the ten plants per line was used for all manipulations.

To asses the mean values of traits and significance of genotype and environment effects on physiological and biochemical parameters of plants two-way ANOVA was used. One-way ANOVA was used for comparison of means within one genotype. MS Excel prgram was used for calculations.

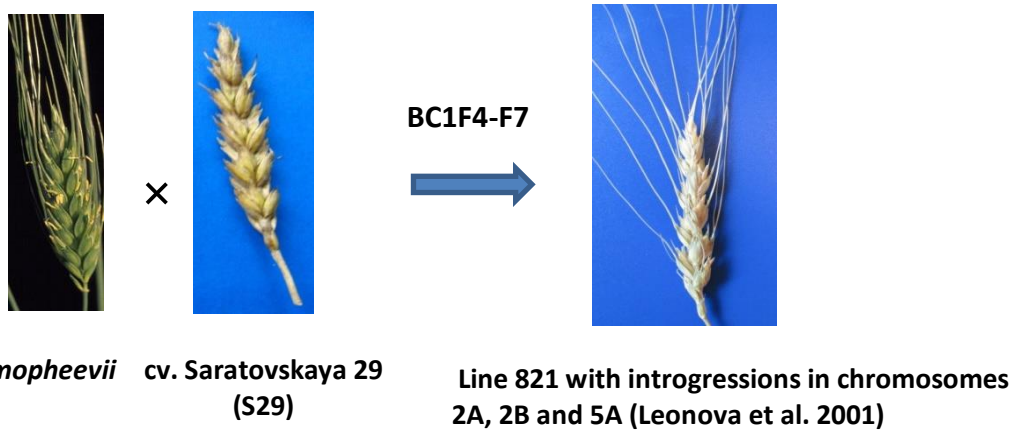

Fig. 1: Origin of the line 821 with introgression from *T. timopheevii*

Results

The line 821 substantially differed from the initial drought tolerant cultivar for gas exchange (Table 1), pigments content in leaves (Table 2), chlorophyll fluorescence (Table 3) and activity of antioxidant enzymes (Table 4) under both conditions. Under normal watering the parental cultivar differed by decreased parameters of gas exchange and photosynthesis comparing to the line 821 but had higher water use efficiency (WUE). Under drought, the line noticeably lowered these parameters in contrast to S29 in which these parameters increased and WUE was also substantially higher. S29 had a higher rate of electron transport and real effect of photosynthesis, possibly, because of a higher chlorophyll A and B content in leaves comparing to 821. Additionally, the carotenoids content was increased in S29 under drought. This pigment participates in a dissipation of excessive light energy and protects the photosynthetic apparatus from photooxidation. Under water stress, the total activity of antioxidant enzymes in leaves of S29 was 3 times higher than in 821 line, and LOX activity—2 times lower. Possibly, this also contributed to a higher resistance of photosynthetic apparatus and other cell systems, prone to influence of oxidative stress which accompanies water stress.

Conclusions

Under water deficit the drought tolerant cultivar S29 in 1,6 – 1,7 times raised all gas exchange parameters and in contrast to 821 line retained a high WUE and greatly increased the antioxidant enzymes activity. Introgressions in 2A, 2B and 5A chromosomes substantially change physiological and biochemical parameters of initial cultivar. It may be supposed that 2A, 2B and 5A chromosomes carry the important genetic complexes responsible for reaction of living systems of wheat plant on changeable environment.

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Table1: Average meanings of shoot biomass and photosynthetic parameters of parental cultivar Saratovskaya 29 (S29) and line 821 with introgressions from *T. timopheevii* under contrasting water regimes

Growing conditions	Shoot biomass, g		E* (mmol m^{-2} s^{-1})		SC (mmol $m^{-2\ s-1}$)		A (µmol $m^{-2\ s-1}$)		WUE (A/E)	
	S29	821	S29	821	S29	821	S29	821	S29	821
Normal watering	4,72	3,48	0,318	1,302	22,4	102,00	1,84	5,28	6,4	4,1
Drought	3,02	2,62	0,520	0,693	38,9	50,9	2,94	3,55	6,7	5,4
F_G	14,2***		130,8***		134,8***		165,0***		17,3***	
F_E	34,0***		16,2***		19,2***		4,0 ns		3,3 ns	

Abbreviations see in Material and methods

Table 2: Average meanings of pigment content in leaves (mg/g) of parental cultivar Saratovskaya 29 (S29) and line 821 with introgressions from *T. timopheevii* under contrasting water regimes

Growing conditions	Chlorophyll A		Chlorophyll B		Carotenoids	
	S29	821	S29	821	S29	821
Normal watering	2,4	2,1	0,91	0,85	0,48	0,47
Drought	2,1	1,8	1,0	0,78	0,56	0,44
F_G	6,03*		9,61**		8,57**	
F_E	7,60**		0,11 ns		0,98 ns	

Table 3: Average meanings of chlorophyll fluorescence параметров of parental cultivar Saratovskaya 29 (S29) and line 821 with introgressions from *T. timopheevii* under contrasting water regimes

Growing conditions	Fv/Fm		ETR (μmol·electrons $m^{-2} \cdot s^{-1}$)		NPQ		Yield	
	S29	821	S29	821	S29	821	S29	821
Normal watering	0,759	0,739	38,0	32,7	0,257	0,375	0,568	0,488
Drought	0,776	0,786	36,8	33,1	0,282	0,304	0,55	0,494
F_G	1,2 ns		73,0***		11,8**		74,4***	
F_E	46,4***		0,6 ns		1,4 ns		0,5 ns	

Abbreviations see in Material and methods

Table 4: Average meanings of dehydroascorbate reductase (DHAR), ascorbate peroxidase (APX), glutathione reductase (GR)), catalase (CAT), superoxide dismutase (SOD) and lipoxygenase (LOX) of parental cultivar Saratovskaya 29 (S29) and line 821 with introgressions from *T. timopheevii* under contrasting water regimes

Growing conditions	DHAR		APX		GR		CAT		SOD		LOX		∑ DHAR, APX, GR, CAT, SOD	
	S29	821	S29	821	S29	821	S29	821	S29	821	S29	821	C29	821
Normal watering	74,3	73,0	136,3	80,1	20,1	17,0	10,63	9,44	2,70	2,49	3,49	1,48	244,0	181,9
Drought	113,8	90,3	313,8	105,9	22,8	17,6	6,54	8,99	2,51	2,50	2,72	5,88	665,0	225,3
F_G	4,9*		24,9***		12,9**		1,9 ns		25,4***		0,9 ns		-	-
F_E	25,7***		23,1***		2,0 ns		25,4***		16,9***		8,6**		-	-

Abbreviations see in Material and methods

Isolation and characterization of regulatory genes *TaMyc1* (*Pp3*) and *HvMpc1* (*Ant1*) for anthocyanin biosynthesis in wheat and barley

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Functional food reach in anthocyanins has an important role in human diets, reducing diseases risk (Lila et al. 2004). Cereals with anthocyanin-colored grains are considered as a source of these healthy molecules. Furthermore, anthocyanin production in in various parts of plant is related with protection from unfavorable environment conditions, because anthocyanins are capable of absorbing high-energy quanta, preventing the development of photooxidative stress in excess of UV-light or can scavenge free radicals generated during oxidative stress under extreme temperatures, drought, salinity, infection, or wounding (Chalker-Scott 1999, Gould 2004, Khlestkina 2013). In the current study, the regulatory genes *TaMyc1* (*Pp3*) and *HvMpc1* activating anthocyanin synthesis in wheat pericarp and barley leaf sheath were isolated and characterized for the first time.

Materials and methods

Wheat and barley near-isogenic lines (NILs) were used. Bread wheat NILs carrying different combinations of dominant and recessive *Pp-A1*, *Pp-D1* and Pp3 alleles (Table 1) were developed in cv. Saratovskaya 29 (Arbuzova e t al. 1998; Gordeeva et al. 2015). The set of *Ant1* NILs, developed in cv. Bowman (Druka et al. 2011), comprised three *Ant1*-carrying, purple leaf sheath selections (NGB20651, 22213 and 22272), along with the green leaf sheath cv. Bowman (NGB22812) of genotype *ant1ant1*. Plants were grown under a 12h photoperiod, with the temperature maintained in the range 20-25°C. Tissue used for RNA extraction were collected (wheat pericarp and barley leaf sheath), and 0.7 µg aliquot of total RNA was converted to single-stranded cDNA via reverse transcription using a RevertAid™ kit (Thermo Fisher Scientific Inc., Waltham, MA, USA) primed with $(dT)_{15}$. The maize genes *C1* and *Lc* sequences were used to find identical sequences in wheat and barley databases. Pairs of primers targeting putative *C1* and *Lc* homologs (*HvMpc1* and *TaMyc*) were designed using OLIGO software (Offerman and Rychlik 2003). The amplicons were gel-purified using a DNA Clean kit (Cytokine, St. Petersburg, Russia) and sequenced in both directions. RT-PCRs were performed using primer pairs specific to *HvMpc1* and *TaMyc1* as well as a set of anthocyanin biosynthesis genes and *Ubc* as control.

Table 1: The set of NILs carrying various combinations of *Pp* alleles. The lines recently obtained by marker-assisted backcrossing are bold.

Designation	*Pp-A1* (7A)	*Pp-D1* (7D)	*Pp3* (2A)	Pericarp	References
i:S29*Pp-A1Pp-D1Pp3*^{PF}	dominant	dominant	dominant	dark purple	Arbuzova et al. 1998; Tereshchenko et al. 2012; Gordeeva et al. 2015
i:S29*Pp-A1Pp-D1pp3*^{PF}	**dominant**	**dominant**	**recessive**	**uncolored**	Gordeeva et al. 2015
i:S29*Pp-A1pp-D1Pp3*^{PF}	**dominant**	**recessive**	**dominant**	**light purple**	Gordeeva et al. 2015
i:S29*pp-A1pp-D1Pp3*^{PF}	**recessive**	**recessive**	**dominant**	**uncolored**	Gordeeva et al. 2015
i:S29*Pp-A1pp-D1pp3*	dominant	recessive	recessive	uncolored	*Pp-A1* was identified in the current study
i:S29*pp-A1pp-D1pp3*	recessive	recessive	recessive	uncolored	Khlestkina et al. 2010b; Gordeeva et al. 2015
i:S29*Pp-A1Pp-D1Pp3*^{P}	dominant	dominant	dominant	dark purple	Arbuzova et al. 1998; Tereshchenko et al. 2012; Gordeeva et al. 2015
i:S29*Pp-A1Pp-D1pp3*^{P}	**dominant**	**dominant**	**recessive**	**uncolored**	Gordeeva et al. 2015
i:S29*Pp-A1pp-D1Pp3*^{P}	**dominant**	**recessive**	**dominant**	**light purple**	Gordeeva et al. 2015
i:S29*pp-A1pp-D1Pp3*^{P}	**recessive**	**recessive**	**dominant**	**uncolored**	Gordeeva et al. 2015

Results and discussion

Barley Ant 1. The characteristic feature of *ant1* mutants in barley (*Hordeum vulgare* L., *2n = 2x* = 14; genome HH) is the absence of any anthocyanin in the stem, auricle, awn, lemma (Jende-Strid and Lundqvist 1978) or basal leaf sheath (Franckowiak and Lundqvist 2012). The *Ant1* gene was mapped within a centromeric region of the short arm of chromosome 7H (Lundqvist et al. 1996). Here, the maize *C1* sequence was exploited to allow the homology-based cloning and sequencing of *Ant1* (Shoeva et al. 2015). *C1* is known to encode a member of the R2R3 MYB-like family of transcription factors (Paz-Ares et al. 1987), which have been shown to represent an important component of the anthocyanin synthesis regulatory network. Three NILs having dominant *Ant1* alleles were compared with Bowman (recessive *ant1*). Microsatellite genotyping revealed donor segments in chromosomes 7H of the NILs with dominant *Ant1*, overlapping in the region between the markers *Xgbms0226* and *Xgbms0240* (Fig. 1a). The isolated sequence was designated *HvMpc1* (*Hordeum vulgare* MYB-like protein C1). *HvMpc1* differed in the lines with dominant and recessive *Ant1* alleles by putative functional mutations in the promoter region. In particular, the *ant1* allele's promoter sequence includes fewer of the *cis*-regulatory elements important for light responsiveness, GT-1 sites and I boxes (potentially functional cis elements associated with tissue pigmentation), than does that of the *Ant1* allele. The structural divergence between *HvMpc1* alleles at the promoter region may underlie their different expression: Bowman's *HvMpc1* is not transcribed, whereas it is active in leaf sheaths of the lines carrying dominant *Ant1* (Fig. 1b). Expression of the anthocyanin biosynthesis structural genes (*Chi*, *F3h*, *Dfr*, and *Ans*) was increased significantly in the presence of the dominant *Ant1* alleles (Fig. 1b). Overall, we conclude that the *Ant1* gene matches *HvMpc1* and encodes the R2R3 MYB regulatory factor.

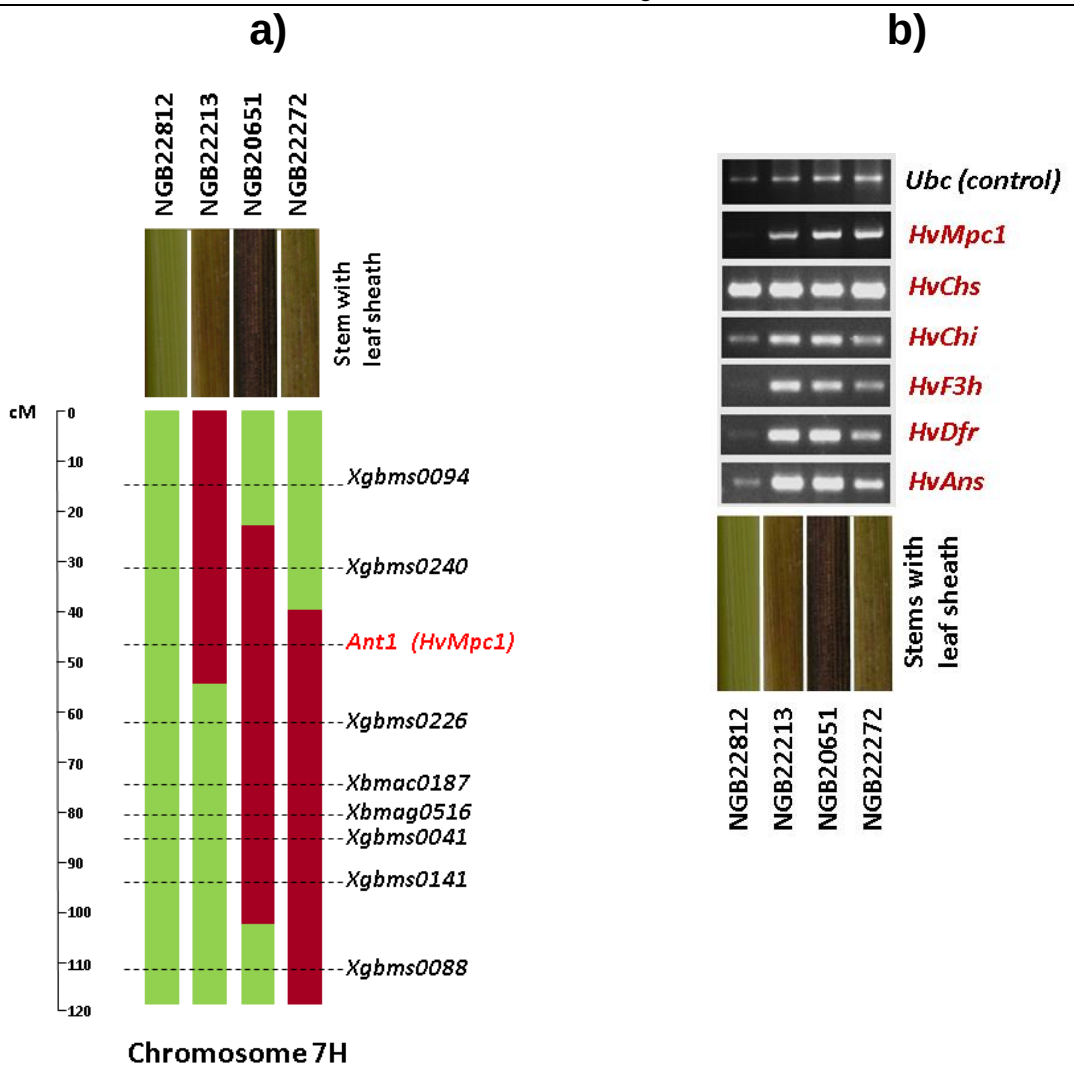

Fig. 1: Phenotype and genotype of cv. Bowman (NGB22812; *ant1*) and the three *Ant1* NILs NGB20651, NGB22213 and NGB22272 (**a**): *Ant1* donor segments on chromosome 7H remaining in the NILs (in red), as revealed by microsatellite genotyping. Transcription profiling of the anthocyanin synthesis genes (*Mpc1* – MYB-like protein C1; *Chs* – chalcone synthase, *Chi* – chalcone flavanone isomerase, *F3h* – flavanone 3-hydroxylase, *Dfr* – dihydroflavonol 4-reductase, *Ans* – anthocyanidin synthase) in the leaf sheath of cv. Bowman and the *Ant1* NILs (**b**).

Wheat Pp3. In bread wheat (*Triticum aestivum* L., *2n = 6x* = 42; genome BBAADD) grain the anthocyanins reside either in the pericarp or in aleurone layer; the grain of some accessions has a purple or blue appearance as a result of the anthocyanin content of one or other of these tissues (Zeven et al. 1991). For purple grain pigmentation interaction of the factors encoded by dominant *Pp-1* and *Pp3* genes is needed. The homoeoallelic *Pp-1* genes map to the short arms of the homeologous group 7 chromosomes, and the latter to chromosome arm 2AL (Arbuzova et al. 1998, Dobrovolskaya et al. 2006, Khlestkina et al. 2010, Tereshchenko et al. 2012, Gordeeva et al. 2015). Based on a similar function and chromosomal localization, genes that encode Myc-like regulatory genes of the anthocyanin biosynthesis in rice (*Pb*/*Ra*) and maize (*Lc*/*R*) can be considered orthologs of the *Pp3* gene (Dooner, Kermicle, 1976; Ludwig et al., 1989; Hu et al., 1996; Wang, Shu, 2007).Using homology-based cloning approach 5 copies of the *TaMyc* gene were isolated from bread wheat. The *TaMyc* genes were mapped to chromosomes 2AL (2 genes), 2BL (1 gene) and 2DL (2 genes) of bread wheat (Fig. 2). *TaMyc1* and *TaMyc2* colocate with the *Pp3* gene determining purple pericarp color (Fig. 2).

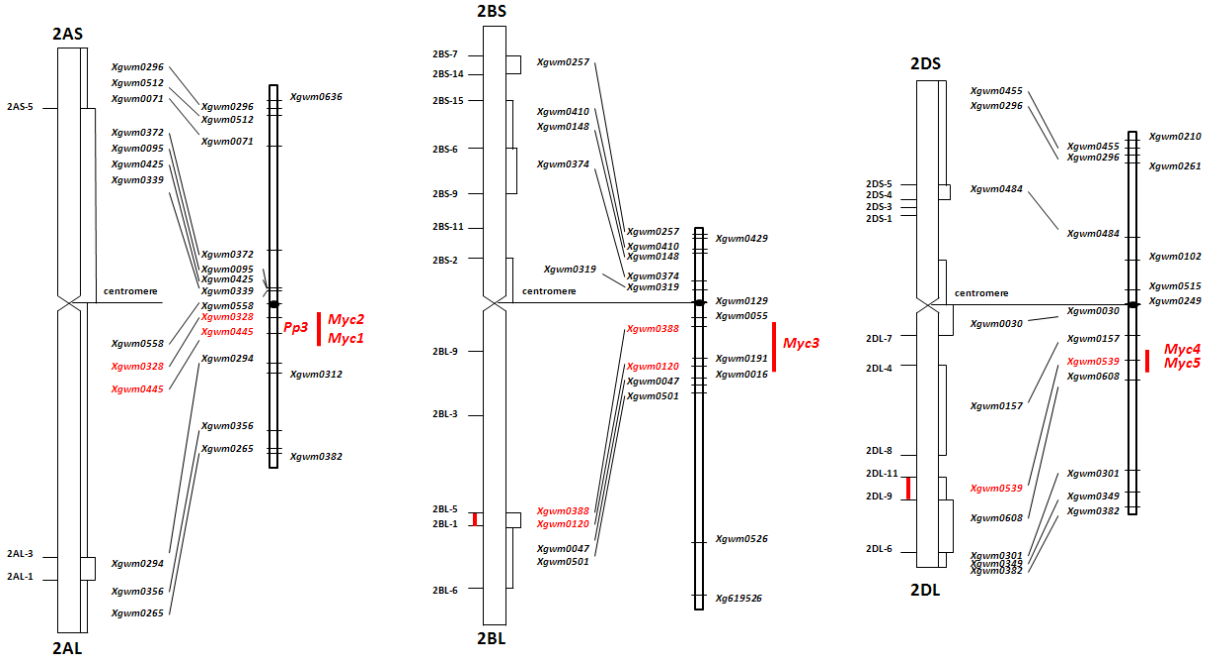

Fig. 2. Physical mapping of the cloned *TaMyc* sequences, using wheat cv. Chinese Spring deletion lines. The corresponding genetic maps are shown to the right.

The *TaMyc1* gene was expressed specifically in the bread wheat pericarp colored by anthocyanins, whereas the *TaMyc2-5* genes were transcribed neither in colored nor in non-colored pericarps. Full-length nucleotide sequence of *TaMyc1* was isolated. The open reading frame encode a protein harboring a conserved basic helix-loop-helix (bHLH) domain. Overall, the data on chromosome mapping, structural organization and functional activity of the *TaMyc1* sequence allow concluding that the *TaMyc1* gene is *Pp3* and encodes for bHLH regulatory factor (Shoeva et al. 2014). The *Pp-1* gene is suggested to encode a MYB-like transcriptional activator. To investigate the regulatory mechanisms in the AB gene network in wheat pericarp, precise genetic lines with different combinations of the *Pp* alleles were created (Table 1; Gordeeva et al. 2015) and used in transcriptional assay of the *TaMyc1* gene (Fig. 3). It was shown that in genotypes harbouring the dominant allele at *Pp-D1* the transcriptional abundance of *TaMyc1* was

lower than in those carrying the recessive allele (Fig. 3). These data indicated that there is an influence exerted by the *Pp-D1* genes on *TaMyc1* expression, but the nature of the underlying mechanism is obscure. A possible model might involve negative feedback, in which the presence of an active MYB-bHLH complex represses the transcription of *TaMyc1* and leads to optimal proportion of the regulatory partners in functional MYB-bHLH complex (Shoeva et al. 2014).

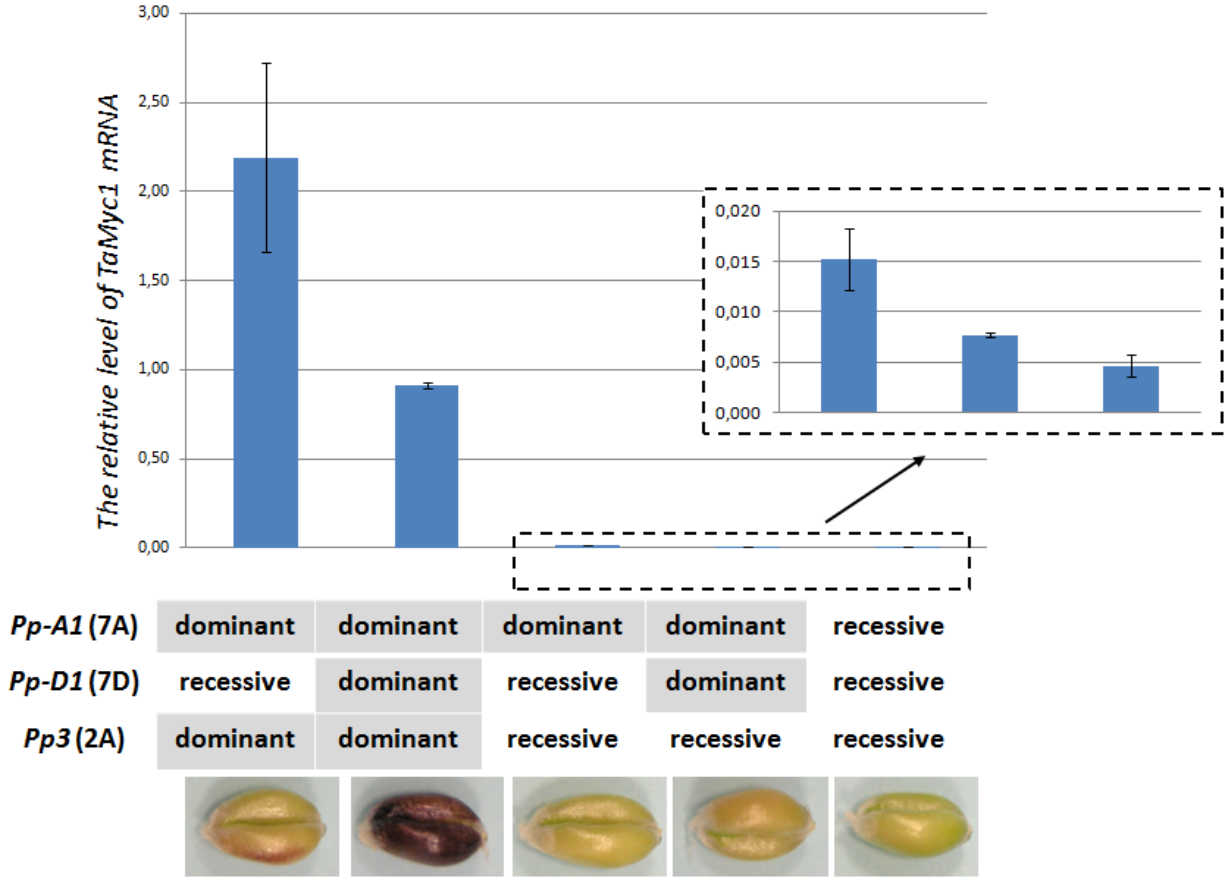

Fig. 3: *TaMyc1* transcription in the pericarp of near isogenic lines carrying various combinations of *Pp* alleles.

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Genotyping and linkage analysis of wheat experimental populations using KASP platform

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Introduction

SNP (single nucleotide polymorphism) markers are single-base differences between individuals and represent the most common type of sequence differences between alleles in plant genomes (Batley and Edwards, 2007). They are direct markers, providing the exact nature of the allelic variants. A genome-wide analysis of SNP diversity in the world's major cereal crops revealed that the frequency of sequence polymorphism was the highest in polyploid taxa such is allohexaploid wheat. Even so, large-scale genotyping in wheat is still a great challenge because of the presence of the homeologous and paralogous genes as a consequence of complex evolution of wheat genome (Barker and Edwards, 2009).

In the past few years the development of high-throughput genomic technologies has resulted in numerous SNP genotyping platforms that differ in allele discrimination techniques, detection methods and reaction formats. Within these technologies kompetitive allele specific polymerase chain reaction (KASP) genotyping has emerged as one of the most cost-effective and still highly informative and reliable methodologies involving SNP markers. KASP is homogeneous, fluorescence-based genotyping technology developed by KBioscience. It is based on FRET (Fluorescent Resonance Energy Transfer) that allows for the detection of SNP's right after PCR, without prior separation step and makes this genotyping technology high-throughput (http://www.ksre.ksu.edu/igenomics/doc1363.ashx). Besides, KASP offers flexibility in applications that require small to moderate numbers of markers, such as quantitative trait loci (QTL) mapping in biparental populations, marker-assisted recurrent selection, marker-assisted backcrossing, and QTL fine mapping (Semagn et al., 2014).

Usefulness of KASP markers for wheat genotyping was first reported by Allen et al. (2011). After that, a number of papers followed where KASP assays were used for scoring SNPs variation in the proximity of candidate genes for some agronomical important traits (Diaz et al., 2012; Zikhali et al., 2014) along with studies where examples of succesfull conversion of SNP markers to KASP assays further used for marker assisted selection were demostrated (Cabral et al., 2014; Liu et al., 2014). The objective of this paper was to validate usefulness of kompetitive allele specific polymerase chain reaction (KASP) assays for evaluation of genetic variation and for construction of linkage maps in wheat.

Material and methods

Two sets of KASP markers belonging to the core set of 960 KASP assays available from CerealsDB (Wilkinson et al., 2012), were used for molecular evaluation of two F7 SSD mapping populations, NS36-98/Paragon and Magnif41/Paragon, consisted of 83 and 112 lines, respectively. These populations were developed in collaboration between IFVC and JIC to identify QTL controlling canopy architecture and studied as part of the EU FP7 project ADAPTAWHEAT.

DNA was extracted from all samples using modified protocol of Pallota et al. (2003), quantified using a NanoDrop 8000 spectrophotometer (Thermo Scientific) and diluted to 5-10 ng/µl in sterile distilled water for use in KASP–SNP PCR reactions. Genotyping reactions were performed in a Hydrocycler (KBioscience) in a final volume of 1 µL containing 1 x KASP Reaction Mix (KBioscience), 0.07 µL assay mix (containing 12 µM each allele-specific forward primer and 30 µM reverse primer) and 10–20 ng genomic DNA. The following cycling conditions were used: 15 min at 94 °C; 10 touchdown cycles of 20 s at 94 °C, 60 s at 61–55 °C (dropping 0.6 °C per cycle); and 29 cycles of 20 s at 94 °C, 60 s at 55 ° C. Fluorescence detection of the reactions was performed using a Pherastar plate reader (BMG LABTECH). Obtained data were analysed using the KlusterCaller software (KBioscience).

Linkage maps were constructed using MapDisto 1.7.6.5 software and Kosambi mapping function.

Results

A total of 154 KASP markers, polymorphic between parents were applied for evaluation of NS36-98/Paragon population. 143 markers were distributed in 30 linkage groups, while 11 markers were unassigned. Total map size was 947 cM. The largest linkage group was 2B2 (104 cM) where 12 markers were assigned.

Genetic variation of the second population was accessed by 75 markers common with the first set and with additional 54 markers polymorphic between Magnif41 and Paragon. Total map size was 960 cM, with 118 markers assigned in 28 linkage groups, while 11 markers remained unassigned (Table 1.). Here, the largest linkage group was 5B (193 cM) were 16 markers were positioned.

Table 1. Number of KASP markers, detected linkage groups and unassigned markers

	NS36-98/Paragon			Magnif/Paragon		
Chromosome	Number of KASP markers	Number of Linkage Groups	Number of unassigned markers	Number of KASP markers	Number of Linkage Groups	Number of unassigned markers
1A	8	2	-	6	2	-
1B	9	2	-	7	2	1
1D	3	1	1	2	-	2
2A	9	1	1	8	2	-
2B	14	2	-	12	2	-
2D	5	1	2	6	1	-
3A	12	2	-	9	2	1
3B	12	2	1	7	2	1
4A	6	2	-	9	2	-
4B	7	1	1	1	-	1
4D	1	-	1	3	1	1
5A	13	2	-	9	2	-
5B	15	2	1	17	1	1
5D	1	-	1	5	1	1
6A	9	2	-	5	2	-
6B	9	2	-	9	2	-

	NS36-98/Paragon			Magnif/Paragon		
Chromosome	Number of KASP markers	Number of Linkage Groups	Number of unassigned markers	Number of KASP markers	Number of Linkage Groups	Number of unassigned markers
6D	4	2	-	2	1	-
7A	8	2	-	8	2	1
7B	4	1	1	1	-	-
7D	5	1	1	3	1	1
Total	154	30	11	129	28	11

Conclusions

According to obtained results, KASP markers are a good choice for further QTL analysis and marker assisted selection in these wheat populations. The common parent Paragon, is shared in a number of populations within the UKs Wheat Improvement Strategic Programme (WISP) and taken together these linked materials can be used to conduct a joint analysis of key traits.

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The effect of alien introgressions in wheat genome on drougth and salinity tolerance and total *Chi* expression

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Alien genetic material is widely used for improvement of bread wheat (*Triticum aestivum* L., 2n = 6x = 42, BBAADD). The species *T. timopheevii* (2n = 4x = 28, GGAA) and *Aegilops speltoides* (2n = 2x = 14, SS) can be donors of alleles for specific resistance to various phytopathogenes and a potential source of genes conferring tolerance to abiotic stress factors. The aim of this study was estimation of *Ae. speltoides* and *T. timopheevii* genetic material effect on drought and salinity stress tolerance and on total expression of the *Chi* gene in wheat seedlings. The *Chi* (chalcone-flavanone isomerase) gene belongs to a large group of the plant defense genes.

Materials and methods

Spring bread wheat Novosibirskaya 29, Rodina-1, and Saratovskaya 29, as well as the lines derived from them were investigated (Table 1).

Artificial drought was created using the neutral osmolyte polyethylene glycol (PEG 6000) according to the modified method of Balint et al. (2008). For salinity tolerance test 150 mM NaCl solution was used. The seeds were placed in UV-treated Petri dishes on filter paper moistened with distilled water, kept for 24 h at 4°C in the dark for the synchronization of germination and after that for 24 h at 20°C and a photoperiod of 12 h light/12 h darkness. The germinated seeds were transferred to Petri dishes containing 15% PEG 6000 or 150 mM NaCl or distilled water and kept for 72 h at 20°C and a photoperiod of 12 h light/12 h darkness; on the seventh day after germination the shoot weight was measured. For each line/test the experiment was performed in triplicate. For each line 96 plants were analyzed. The tolerance index of each line was calculated using the following formula: the ratio of the shoot weight under stress to the corresponding value obtained in control experiments (in distilled water).

The lines treated with 100 mM NaCl were used to perform *Chi* gene expression analysis. A Zymo Research Plant RNA MiniPrep™ kit (www.zymoresearch.com), followed by DNAse treatment, was employed to extract RNA from roots and shoots of four day old seedlings germinated at 20ºC under a 12h photoperiod (in three replicates) or from leaves, culm, caryopsis pericarp in greenhouse-grown plants. Single-stranded cDNA was synthesized from 3μg total RNA using a $(dT)_{15}$ primer and the Fermentas RevertAid™ first strand cDNA synthesis kit (www.thermoscientificbio.com) and used in RT-PCR. qRT-PCR was performed applying a SYNTOL SYBR Green I kit (http://www.syntol.ru). The amplifications were performed in an ABI Prism 7000 Sequence Detection System.

The significance of the differences was evaluated using the nonparametric Mann-Whitney test (U-test).

Table 1: Description of the plant material used in this study.

Designation	Description	Alien material	Reference
N29	*T. aestivum* Novosibirskaya 29	-	-
N29–*Ae. speltoides* 5SL*	Introgression line Novosibirskaya 29– *Ae. speltoides* 21-4/933-1-5SL	5BS · 5BL-5SL translocation (donor - *Ae. speltoides* k-389)	Salina et al. 2013
Rod.	*T. aestivum* Rodina-1 (differs from cv. Rodina by the absence of 1RS · 1BL translocation)	-	Adonina et al. 2012
Rod.–*Ae. speltoides* 5SL*	Introgression line Rodina-1 – *Ae. speltoides* 16-9-5SL	5BS · 5BL-5SL translocation (donor - *Ae. speltoides* k-389)	Adonina et al. 2012
Rod.–*Ae. speltoides* 6SL	Introgression line Rodina-1 – *Ae. speltoides* 17-7-6SL	6BS · 6BL-6SL translocation (donor - *Ae. speltoides* k-389)	Adonina et al. 2012
S29	*T. aestivum* Saratovskaya 29	-	-
S29–*T. timopheevii* 2A	Introgression line Saratovskaya 29 – *T. timopheevii* 832-2A-BC3	Introgression from *T. timopheevii* var. *viticulosum* in chromosome 2A	Timonova et al., 2013
S29–*T. timopheevii* 5B/5G	Introgression line Saratovskaya 29 – *T. timopheevii* 832-5B-BC3	Introgression from *T. timopheevii* var. *viticulosum* in chromosome 5B	Timonova et al., 2013

Results and discussion

Drought and salinity tolerance. Tolerance indices calculated for each cultivar/line is presented at Fig. 1. In all cases the difference between shoot weight of stressed and control plants was significant ($p \leq 0.05$). Among original wheat cultivars, drought tolerance decreased from S29 to N29: S29 – 84% (tolerant), Rod. – 69% (moderately tolerant), N29 – 33% (sensitive), while all three genotypes had moderate salinity tolerance (Fig. 1a). Evaluation of drought resistance has identified some positive influence of translocation T6BS • 6BL-6SL from *Ae. speltoides* and slightly negative effect of *T. timopheevii* introgressions in chromosome 2A (Fig. 1b). On the example of translocation T5BS • 5BL-5SL, it was shown that the same foreign fragment introgressed into different wheat genotypes can have different effects on resistance to osmotic stress depending on the drought tolerance degree of the initial wheat genotype: *Ae. speltoides* 5SL fragment improved significantly (30%) tolerance of the sensitive N29 cultivar, whereas the same introgression from the same *Ae. speltoides* accession didn't effect drought tolerance of the moderately tolerant Rodina-1 (Fig. 1b). Evaluation of salinity has identified positive influence of translocation T5BS • 5BL-5SL and *T. timopheevii* introgression in chromosome 2A, while the presence of *T. timopheevii* introgression in chromosome 5B and translocation T6BS • 6BL-6SL, on the contrary, reduced the resistance of wheat to salt stress. Comparison with known wheat salinity tolerance QTL locations (Lindsay *et al*., 2004; Ma *et al*., 2007; Genc *et al*., 2010) may suggest that the positive or negative effect of introgressions in all lines (with the exception of S29–*T. timopheevii* 5B/5G) can be related with elimination of, respectively, unfavorable or favorable wheat alleles.

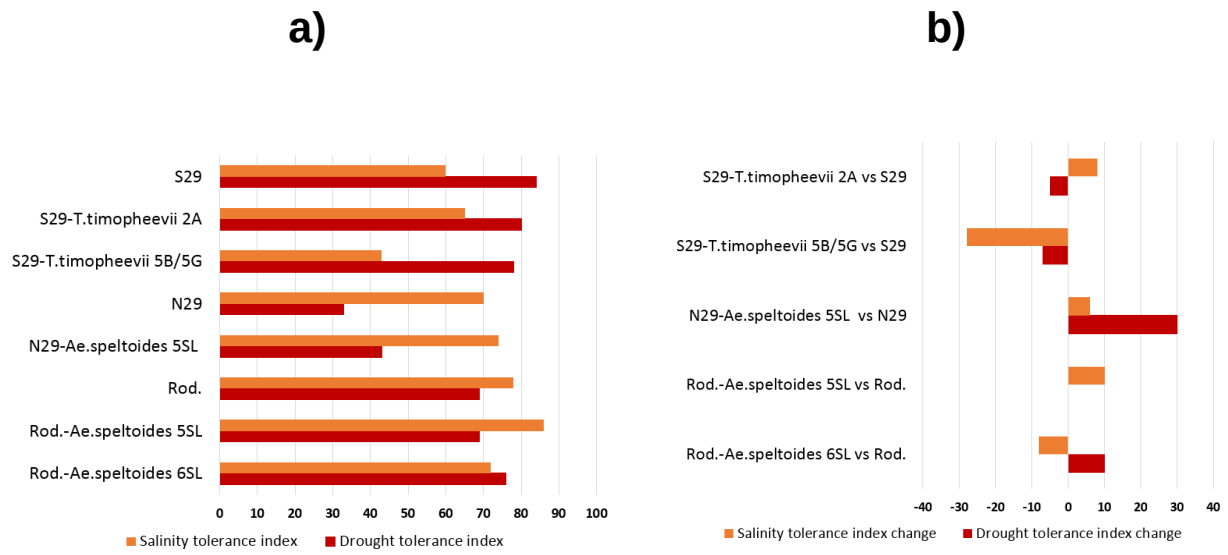

Fig. 1: Drought and salinity tolerance indices in the original wheat genotypes and introgression lines derived from them (% to control) (**a**). Change of the drought and salinity tolerance indices in the introgression lines in comparison to the original wheat genotypes (%) (**b**). Control – distilled water. Salinity – 150mM NaCl. Drought – 15% PEG. In all cases the difference between shoot weight of stressed and control plants was significant ($p \leq 0.05$).

Chi expression. Replacement of *Chi-B1* by *Chi-S1* (in line Rod-Aes5S) or by *Chi-G1* (in line S29-Tt5G) leads to decrease of the global levels of *Chi-1* transcription in the shoot (Fig. 2). The same was not the case in the root (Fig. 2), although this may simply reflect the smaller contribution made by *Chi-B1* to the overall level of *Chi* transcript compared to that made by both *-A1* and *-D1* (Shoeva et al. 2014). CHI is required for the synthesis of numerous flavonoid compounds which are important for the plant response to stress (reviewed in Khlestkina 2013; Khlestkina et al. 2015). Thus any decrease in its transcript abundance is likely to be undesirable.

Conclusions

The effect of certain fragments of *Ae. speltoides* or *T. timopheevii* chromosomes on wheat tolerance to drought and salinity is shown. In some cases alien chromosome effect can be related with the loss of certain wheat alleles after introgression/translocation.

The same foreign fragment introgressed into different wheat genotypes can have different effects on resistance to osmotic stress depending on the drought tolerance degree of the initial wheat genotype.

Introgression of *Ae. speltoides* or *T. timopheevii* fragment into 5BL region carrying *Chi* gene leads to undesirable decrease of the global levels of *Chi* transcription in the seedling shoot.

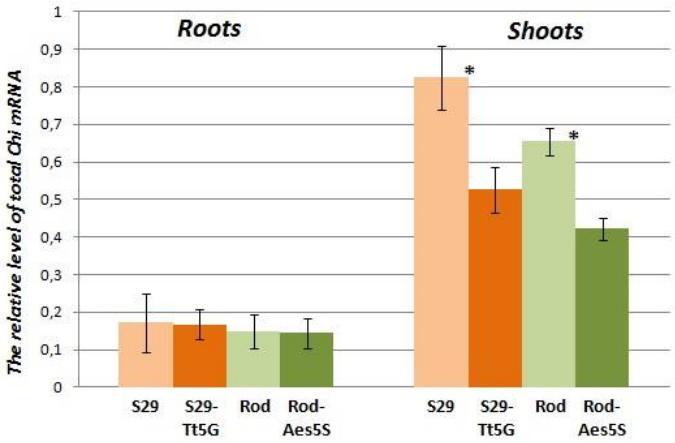

Fig. 2: *Chi* transcript abundance in the seedling root and shoot of 'Saratovskaya 29'-*T. timopheevii* 832-5B-BC3 (S29-Tt5G), 'Rodina'-*Ae. speltoides* 5SL (Rod-Aes5S) and their parental wheat cultivars 'Saratovskaya 29' (S29) and 'Rodina-1' (Rod). *: differences statistically significant at $p \leq 0.05$ (*U*-test).

Acknowledgements

We thank Mrs Olga V. Zakharova and Ms Galina V. Generalova for technical assistance.

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APPENDIX – Cereals Precise Genetic Stocks Holdings

John Innes Centre, Norwich, UK

General remarks:
Not all combinations exist in all classes. Further information on availability can be obtained online using our searchable database system Seedstor (www.seedstor.ac.uk). Use the "WPGS Search" pages to look for aneuploids and chromosomal addition / substitution lines, use free text search with the term "amphiploid" as species to interrogate the amphiploid set.

Pairing gene deletions
Chinese Spring: Pairing gene deletion: ph1b
Capelli DES35 - ph1c deletion

Wheat Aneuploids
Chinese Spring: Euploid
Chinese Spring: Nullisomics
Chinese Spring: Monosomics
Holdfast: Monosomics
Chinese Spring: Ditelosomics
Chinese Spring: Nullisomic –Tetrasomics (or Monosomic-Tetrasomics)

Wheat/Alien chromosomal addition and/or substitution lines
Chinese Spring (*Ae. comosa*)
Chinese Spring (*Ae. longissima*)
Chinese Spring (*Ae. mutica*)
Chinese Spring (*Ae. umbellulata*)
Chinese Spring (*Ae. uniaristata*)
Chinese Spring (*Ae. variabilis*)
Chinese Spring (*D. villosum*)
Chinese Spring (*H. chilense*)
Chinese Spring (*H. vulgare 'Betzes'*)
Chinese Spring (*H. vulgare 'Tulleen 346'*)
Chinese Spring (*S. cereale* 'Imperial')
Chinese Spring (*S. cereale* 'King II')
Chinese Spring (*S. montanum*)
Chinese Spring (*T. urartu*)
Chinese Spring (*Th. bessarabicum*)
Chinese Spring (*Th. elongatum*)
T. durum 'Langdon' (Chinese Spring)
T. aestivum 'Vilmorin 27'(*Th. intermedium*)
Holdfast (*Ae. bicornis*)
Holdfast (*S. cereale* 'King II')

Amphiploids
T. monococcum x *T. thaoudar*
T. thaoudar x T. *aegilopoides*

T. dicoccoides x *T. monococcum*
T. dicoccum x *T. monococcum*
T. turgidum x *T. monococcum*
T. paleocolchicum x *T. monococcum*
T. dicoccoides (Kew 2B) x *T. urartu D*
T. dicoccum x *T. aegilopoides*
T. durum (Indian Runner) x *T. urartu D*
T. paleocolchicum x *T. aegilopoides*
T. pyramidale x *T. aegilopoides*
T. dicoccoides x *T. thaoudar*
T. dicoccum x *T. thaoudar*
T. turgidum x *T. thaoudar*
T. paleocolchicum x *T. thaoudar*
T. durum (Kubanka) x *T. thaoudar*
T. timopheevi x *T. thaoudar*
T. dicoccoides x *T. timopheevi*
T. dicoccum x *T. timopheevi*
T. turgidum x *T. timopheevi*
T. timopheevi x *T. turgidum*
T. timopheevi x *T. durum* (Indian Runner)
T. timopheevi x *T. polonicum*
T. timopheevi x *T. pyramidale*
T. timopheevi x *T. turanicum*
T. carthlicum x *T. timopheevi*
Ae. longissima x *T. dicoccoides*
Ae. longissima x *T. dicoccum*
Ae. longissima x *T. paleocolchicum*
Ae. longissima x *T. carthlicum*
Ae. longissima x *T. turanicum*
Ae. longissima x *T. durum (Kubanka)*
Ae. longissima x *T. turgidum*
Ae. speltoides E x *T. diccocum*
Ae. speltoides D x *T. carthlicum*
Ae. speltoides A x *T. monococcum*
Ae. speltoides A x *T. aegilopoides*
T. urartu D x *Ae. bicornis*
T. dicoccoides Kew x *Ae. bicornis*
T. turgidum x *Ae. sharonensis*
T. dicoccoides (Kew 2D) x *Ae. umbellulata*
Ae. caudata A x *T. dicoccoides*
T. dicoccum x *Ae. caudata A*

Ae. caudata A x *T. dicoccum*
Ae. caudata A x *T. turgidum*
Ae. caudata A x *T. durum (Indian Runner)*
Ae. caudata A x *T. thaoudar*
Ae. cylindrica E x *T. dicoccum*
Ae. cylindrica x *T. durum*
T. dicoccoides (Kew 2B) x *Ae. ovata* A
T. timopheevi x *Ae.ovata* A
Ae. ovata A x *T. timopheevi*
Ae. ovata A x *T. aegilopoides*
Ae. biuncialis x *T. dicoccoides*
Ae. triuncialis x *T. durum* (Indian Runner)
Ae. comosa A x *T.turgidum*
Ae. longissima A x *T. aestivum* (Holdfast)
T. aestivum (Holdfast) x *S. cereale* (King II)
T. aestivum (Swedish Iron) x *S. cereale* (King II)
T. aestivum (Wilhelmina) x *S. cereale* (King II)
T. aestivum (Jubiligem) x *S. cereale* (King II)
T. aestivum (Soissonais) x *S. cereale* (King II)
T. aestivum (Druchamp) x *S. cereale* (King II)
T. aestivum (P.P.) x *S. cereale* (King II)
T. aestivum (Atle) x *S. cereale* (Pearl)
T. aestivum (Holdfast) x *S. cereale* (Pearl)
T. aestivum (Chinese Spring) x *S. cereale* (King II)
T. aestivum (Chinese Spring) x *S. cereale* (Petkus)
T. aestivum (Chinese Spring) x *S. montanum* R15
T. durum (Aziziah) x *S. montanum* R15
T. aegilopoides x *Ae. squarrosa*
T. monococcum x *Ae. uniaristata*
T. dicoccum x *Ae. squarrosa*
T. dicoccoides x *Ae. umbellulata*
T. dicoccoides x *Ae. caudata*
T. dicoccoides x *Ae. sharonensis*
T. dicoccoides x *Ae. uniaristata*
Ae. cylindrica x *Ae. umbellulata*
Ae. umbellulata x *Ae. uniaristata*
T. timopheevi x *Ae. bicornis*
T. durum x *Ag.elongatum*
T. dicoccum x *D. villosa*
T. timopheevi x *T. monococcum*
T. carthlicum x *T. timopheevi*

T.durum x *T. timopheevi*
T. aestivum (Chinese Spring) x *Ae. caudata* A
T. aestivum (Chinese Spring) x *Ae. sharonensis* A
T. aestivum (Chinese Spring) x *Ae. mutica* A
T. aestivum (Chinese Spring) x *Ae. umbellulata* A
T. aestivum (Chinese Spring) x *Ae. comosa* A
T. turgidum x *Ae. ventricosa* A
T. timopheevi x *Ae. squarrosa*
T. durum (Mexicali 75) x *Ae. speltoides* A
Ae. comosa A x *S. cereale* (Petkus) (spring)
T. aestivum (Chinese Spring) x *Th. bessarabicum*
T. turgidum x *T. aegilopoides*
T. aestivum (Chinese Spring) x *S. ancestrale*
T. durum (Nordum) x *Ag. Disticum*
T. durum (Calvin) x *Ag. Disticum*
T. dicoccoides x *Ae. squarrosa*
T. timopheevi x *T. monococcum*
TA 182 *dicoccoides* (Kew 2B) x *aegilopoides* Kew
TA 201 (Indian Runner) x *aegilopoides* Kew
T. aestivum (Chinese Spring) x *Ae. elongatum*
T. aestivum (Chinese Spring) x *Ae. caudata*
T. aestivum (Chinese Spring) x *S. cereale* (Imperial)
T. aestivum 'Chinese Spring' x *H. chilense*
T. aestivum 'Chinese Spring' x *Ae. longissima* 2150011

National Research and Development Institute, Fundulea, Romania

Monosomics
Favorit
Bezostaya 1A

Intervarietal substitution lines
Favorit/F.26-70 (not all lines verified for correctness of substitution)

Recombinant substitution lines (SCRL)
70 lines for chromosome 7B (Favorit/F.26-70 7B)

Mapping populations
85 DH lines F.132/G.603 (grain weight/grain size)
115 DH lines F.132-1-30/Martonvasari 9 (intergeneric crossability)

Mutant DH line populations (gamma irradiation, 200Gy)
77 DH lines in cv. Izvor

50 DH lines in advanced breeding line F00628G-34

Mutant/recombinant DH line population
(recurrent irradiation on seeds resulted from direct and reciprocal crosses of M1`s plants)
279 DH lines Izvor (M1) x F.00628G-34 (M1); 100 Gy
148 DH lines F.00628G-34 (M1) x Izvor (M1); 200 Gy

Synthetic wheats
38 synthetic hexa-amphiploids (winter wheat Romanian durum cultivars and breeding lines/*Aegilops tauschii* biotypes of diverse geographic regions)

Other amphiploids
F.132/*Agropyron junceum*
F.132/*Secale cereale*, cv. Harkovsckaia

Alien translocations
F.132/*Ae. comosa*
F.132/*Ae. caudata*

Alien substitution
G.615 Favorit/*Ae.variabilis*

Crop Research Institute, Prague-Ruzyně, Czech Republic

Monosomic series
Zlatka (monosmic status derived from Chinese Spring)
Chromosomes 1A, 1B, 1D, 2A, 2B, 2D, 3A, 3B, 3D, 4A, 4B, 4D, 5A, 5B, 5D, 6A, 6B, 6D, 7A, 7B, 7D)

Monosomic lines
Bezostaya1 5A
Bezostaya1 5B
Bezostaya1 5D
Fenman 3B
Jara 3B
Košutka 3B
Košutka 5A
Košutka 5B
Košutka 5D
Mironovskaya808 5A
Mironovskaya808 5B
Mironovskaya808 5D
Sandra 3B
Vala 3B
Vala 5A
Vala 5B
Vala 5D
Zdar 5A

Zdar 5B
Zdar 5D
Zdar 2A
Zdar 2B
Zdar 2D
Zdar 3B
Zlatka 3B

Substitution lines
(most of them have not been verified using molecular checks)
Bezostaya (Mironovskaya 5A)
Bezostaya (Mironovskaya 5A)
Bezostaya (Mironovskaya 5D)
Jara (Česká Přesívka 3B)
Jara (Zdar 5A)
Košutka (Chinese Spring 5A)
Košutka (Chinese Spring 5D)
Košutka (Zlatka 5A)
Košutka (Česká Přesívka 3B)
Košutka (Česká Přesívka 5B)
Mironovskaya (Bezostaya 5A)
Mironovskaya (Bezostaya 5B)
Mironovskaya (Bezostaya 5D)
Sandra (Česká Přesívka 3B)
Vala (Zlatka 5A)
Vala (Česká Přesívka 3B)
Vala (Česká Přesívka 5B)
Vala (Chinese Spring 5A)
Vala (Chinese Spring 5D)
Zdar (Chinese Spring 2A)
Zdar (Chinese Spring 2B)
Zdar (Chinese Spring 5D)
Zdar (Sáva 2D)
Zdar (Sonora 2D)
Zdar (Zlatka 5A)
Zdar (Česká Přesívka 3B)
Zdar (Česká Přesívka 5B)
Zlatka (Chinese Spring 2A)
Zlatka (Chinese Spring 2B)
Zlatka (Chinese Spring 5D)
Zlatka (Sonora 2D)
Zlatka (Zdar 5A)

Recombinant mapping populations
Mironovskaya (Mironovskaya 5A/Bezostaya 5A)
Sandra (Sandra 3B/Česká Přesívka 3B)
Zlatka (Zlatka 3B/Česká Přesívka 3B)

Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Stadt Seeland/OT Gatersleben, Germany

Substitution lines

At the 10th EWAC meeting in Viterbo (1997) three sets of intervarietal substitution lines were chosen to focus on in future research. These sets were:

Cappelle Desprez/Besostaya (Cap/Bes)
Chinese Spring/Synthetics (CS/Syn)
Saratovskaya29/Janetskis Probat (S29/JP)

The three series were checked for correctness by Korzun et al. (1997) Euphytica 95:149-155, Pestsova et al. (2000) Theor Appl Genet 101:95-99 and Salina et al. (2003) EWAC Newsletter, Proc of the 12th Int EWAC Conf Norwich, UK:28-31. Not all lines were correct.

Single chromosome recombinant DH lines for mapping

Chinese Spring/Synthetics 6A
Chinese Spring/Synthetics 1D
Chinese Spring/Synthetics 6D
Chinese Spring/Synthetics 3A
Chinese Spring/Synthetics 5A
Chinese Spring/Synthetics 3B
Chinese Spring/Synthetics 5B
Saratovskaya 29/Yanetzkis Probat 1A
Saratovskaya 29/Yanetzkis Probat 2A
Saratovskaya 29/Yanetzkis Probat 3A
Saratovskaya 29/Yanetzkis Probat 4A
Saratovskaya 29/Yanetzkis Probat 5A
Saratovskaya 29/Yanetzkis Probat 3B
Saratovskaya 29/Yanetzkis Probat 5B
Saratovskaya 29/Yanetzkis Probat 1D
Saratovskaya 29/Yanetzkis Probat 2D
Saratovskaya 29/Yanetzkis Probat 4D
Saratovskaya 29/Yanetzkis Probat 5D
Saratovskaya 29/Yanetzkis Probat 7D
Chinese Spring/Bezostaya 5A
Chinese Spring/Bezostaya 5B
Chinese Spring/Bezostaya 3D
Chinese Spring/Bezostaya 5D
Chinese Spring/*T. spelta* (7D)

D-genome introgression lines

84 Chinese Spring/*Aegilops tauschii* introgression lines for chromosomes:

1D (9 lines)
2D (20 lines)
3D (11 lines)
4D (7 lines)
5D (11 lines)
6D (13 lines)
7D (13 lines)

Isogenic lines

April Bearded	(tall control)
April Bearded	(*Rht-B1b*)
April Bearded	(*Rht-D1b*)
April Bearded	(*Rht-B1c*)
April Bearded	(*Rht-B1b* + *Rht-D1b*)
April Bearded	(*Rht-B1c* + *Rht-D1b*)
Bersee	(tall control)
Bersee	(*Rht-B1b*)
Bersee	(*Rht-D1b*)
Bersee	(*Rht-B1c*)
Bersee	(*Rht-B1b* + *Rht-D1b*)
Bersee	(*Rht-B1c* + *Rht-D1b*)
M. Huntsman	(tall control)
M. Huntsman	(*Rht-B1b*)
M. Huntsman	(*Rht-D1b*)
M. Huntsman	(*Rht-B1c*)
M. Huntsman	(*Rht-B1b* + *Rht-D1b*)
M. Huntsman	(*Rht-B1c* + *Rht-D1b*)
M. Widgeon	(tall control)
M. Widgeon	(*Rht-B1b*)
M. Widgeon	(*Rht-D1b*)
M. Widgeon	(*Rht-B1c*)
M. Widgeon	(*Rht-B1b* + *Rht-D1b*)
M. Widgeon	(*Rht-B1c* + *Rht-D1b*)

Institute of Field and Vegetable Crops, Novi Sad, Serbia

Variety/Line	Genes
Cappelle Desprez	*rht*
S.Cerros	*Rht1, Pa, Rg, Rht-D1b*
Bersee	*Rht1, Rht-D1b*
Tanori 71	*Rht1, Rht-D1b*
Highbury	*Rht1,Rg,Gai,Ra, Rht-D1b*
Lerma Rojo	*Rht1,Ra, Rht-B1b*
Norteno 67	*Rht1,Rht-D1b*
Inia 66	*Rht1, Ra, Rht-B1b*
Condor	*Rht1,Rht-D1b*
Cook	*Rht1,Rht-D1b*

Banks	*Rht1, Rht-D1b*
Saitama 27	*Rht1S,VrnA1,Rg, Rht-D1b*
Aobakomughi	*Rht2, Hg, Rg, Rht-D1b*
Galahad	*Rht2, Pm2, Rht-D1b*
TJB 990-15	*Rht2, Rht-D1b*
Dwarf A	*Rht2, Rht-D1b*
Durin	*Rht2,Rht-D1b*
Hobbit	*Rht2, Rht-D1b*
Brigant	*Rht2, Pm6, Rht-D1b*
Bounty	*Rht2, Pm2, Rht-D1b*
Sentry	*Rht2, Pm2, Rht-D1b*
Wizard	*Rht2, Rht-D1b*
Avalon	*Rht2, Rht-D1b*
Norman	*Rht2, Pm2, Rht-D1b*
Mithras	*Rht2, Rht-D1b*
Fenman	*Rht2, Pm2, Rht-D1b*
Sandown	*Rht2, Rht-D1b*
Longbow	*Rht2, Pm2, Rht-D1b*
Era	*Rht2, Rht-D1b*
Kite	*Rht2, Sr26, Rht-D1b*
Sonalika	*Rht2, Rht-D1b*
Buckbuck	*Rht2, Rht-D1b*
Norin 10	*Rht2, Gai1, Rht-D1b*
Norin10-Brev.14	*Rht6, Rht-D1b*
Olesen Dwarf	*Rht2, Rht-D1b*
UPI 301	*Rht2, Rht-D1b*
Suwon 92	*Rht2, Hg, Rg, Rht-D1b*
Bersee	*Rht1+2*
Yecora	*Rht1+2, Ra*
Cajeme 71	*Rht1+2*
Tom Thumb	*Rht3, Gai3, Ra, Rht-B1c*
Min.Dwarf	*Rht3, Gai3, Ra, Rht-B1c*
Bersee	*Rht3,Rht-B1c*
M.Huntsman	*Rht3, Rht-B1c*
D6899	*Rht3, Gai3, Ra, Rht-B1c*
Bersee	*Rht2+3*
Bersee Mutant	*Rht7*
Akagomughi	*Rht8+9, Rg*
Sava 2D	*Rht8*
Fortunato 2D	*Rht8, Rg*
Cap.Dep./Mara	*Ppd1, ppd2*
Podunavka	*Rht8*
NSR 1	*Rht8*
NSR 2	*Rht8*
Posavka 1	*Rht8*
Jugoslavija	*Rht8*
Una	*Rht8*
Žitnica	*Rht8*
Pomoravka	*Rht8*
Zvezda	*Rht8*
Rana niska	*Rht8*
NS 646	*Rht8, Rg*
Sanja	*Rht8*
Super Zlatna	*Rht8, Gai*
Sana	*Rht8*
Skopljanka	*Rht8, Gai*
Raduša	*Rht8*

Mara	*Rht8+9*
Acciaio	*Rht9*
Ai bian	*Rht10*
Tibet Dwarf	*Rht10*
Hira	*Ra*
Norin 50	*Rg*
Al-Kan-Tzao	*Rg*
Ching-Chang 6	*Rg*
San Pastore	*Rg*
Rusalka	*Sr7b+8a*
Biserka	*Rht8, Pan*
NSR 2	*Rht8*
NSR 5	*Rht8*
Nov. crvena	*Rg*
Kenya Gala	*Rg*
Arg.80/5216	*Rg*
Purd. 79406 I-26-2	*Pan*
NS 1/92	*Pan*
ND 516	*Pan, Rg*
ND 517	*Ra*
D 6899	*Rht3, Gai3*
Biserka	*Rht8, Pan*
Pergamino Gaboto	*Rg*
Resistente	*Rg*
S.174/72	*Rg*
Purd. Composite	*Ra*
Hope	*Pm5*
Agent	*Sr24*
ABE	*Pan, Pm6*
Vireo “S”	*Ra*
Sap”S”-Mon”S”	*Rg*
OK 75R 3645	*Hg, Ra*
Benni multifloret	*Rg*
M.Huntsman	*Rht1, Rht-D1b*
Suwon 92	*Rht2, Hg, Rg, Rht-D1b*
Nizija	*Rht8*
Jubilejnaja 50	*Pan*
Stepnjačka 30	*Ra*
Balkan	*Rht8*
Noe	*Ra*
Fontezuela Inta	*Rg*
Bolonjska	*Hg*
Forlani	*Rht9, Rg*
Mex.3	*Ra*
INTRO 7	*Rg*
Huequen	*Rg*
KM 2213-95	*Ra*
KM 2002-95	*Ra*
ZG 1008	*Rg*
ZG 1011	*Ra, Rg*
ZG 1020 A	*Ra*
ZG 8065	*Rg*
ZG K 2A/82	*Ra, Rg*
ZG K 3/82	*Rg*
ZG K 146/82	*Rg*
ZG K 238/82	*Ra*
ZG K 242/82	*Rg*

L-1/91	*Hg*
KM 2003-95	*Ra*
Mex. 17	*Hg*
INTRO 613	*Hg*
INTRO 29	*Hg*
L -152/89	*Hg*
L -154/89	*Hg*
L -156/89	*Hg*
L -159/89	*Hg*
L -160/89	*Hg*
NS 56/90	*Hg*
SST 101/A	*Sr36*
Zarija	*Pan*
PPG-186	*Ra*
Tr.Spelta	*Rg*
Tr.Spelta iz Mađarske	*Rg*
KM 1355-95	*Ra*
KM 2060-95	*Ra*
KM 2172-95	*Ra*
KM 2196-95	*Ra*
KM 1405-95	*Ra*
KM 2079-95	*Ra*
KM 2080-95	*Ra*
KM 2198-95	*Ra*
KM 1359-95	*Ra*
KK 8634-1-1-11	*Ra*
Baulander Spelz (Spelta)	*Pan, Rg*
Oberkulmer (spelta)	*Pan*
Stepnjaja	*Ne2*
KM 1514-95	*Ra*
KM 2161-95	*Ra*
KM 1449-95	*Ra*
Mex.3/ZG K 171/1/82	*Ra*
ZG K 172/82	*Ra, Rg*
KM 1405–95	*Ra*
Mara kontrola	*Ra*
Magnif 41	*Rht13, Sr8a+9b*
NS 62-20	*Rg*
NS 62-21	*Rg*
NS 7003	*Pan, Pc*
Tr.Sphaerococcum	*Rg*
Fisherect 4 A	*Rg*
UC 65680	*Rg*
UC 66052	*Rg*
UC 66206	*Rg*
UC 67052	*Ra, Rg*
UC 64246	*Ra, Rg*
Multibraun	*Rg*
Red coat	*Ra, Pan, Pm5*
Purdue 79406-1-26-2	*Pan*
Buckskin	*Pan*
NS 57/92	*Pan*
NS 97/95	*Pan*
NS 98/95	*Pan*
NSA 89-5126	*Ra*
Mironovska 808	*Pan*
Triple Dirk B	*Hg*

Triple Dirk E	*Hg*
ND. 516	*Rg*
Vel	*Pan*
Bean	*Pan*
Newton	*Pan*
Ruler	*Pan*
Frankenmuth	*Rg*
Oasis	*Pm6*
Kharkof	*Pan*
W 9785	*Pan*
Barb	*Rg, Pan*
Eider	*Rg*
Burr	*Rg*
Combo	*Rg*
CI 9321	*Pan*
Red Bear 22	*Ra*
Transved 6B	*Rg, Pm7*
W 238	*Pan*
L 39/91	*Hg, Ra*
Recital	*Ra*
UPI 301	*Rht2, Rht-D1b*
UC 66206	*Rg*
Rogosija	*Rg*
Velja pšenica	*Rg, Ra*
Caldwell	*Pm5*
ZG K 238/82	*Ra*
L 154/89	*Hg*
Kite + TIN	*Hg*
Duggan SS	*Ra*
Ana/Pobeda	*Pc*
Lr	*Lr*
Lr B Tc*6/V 336	*Lr*
Lr B Tc*6/Carina	*Lr*
Lr B Tc*6/PI 268316	*Lr*
Lr 37 Tc*8/VPM	*Lr*
Lr 38 Tc*6/Kohn	*Lr*
Lr 38 Tc*6/TMR 514-12-24	*Lr*
Lr 34 Tc*6/8404	*Lr*
Lr 44 Tc*/T.spelt	*Lr*
Triple Dirk B II	*Hg*
Triple Dirk D	*Vrn*
Triple Dirk C	*Vrn*
Abukuma-wase (original)	*Vrn*
Abukuma-wase (winter)	*Vrn*
Asakaze-komugi (original)	*Vrn*
Asakaze-komugi (winter)	*Vrn*

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