European Wheat Aneuploid Co-operative Newsletter

2008

Proceedings of the 14th International EWAC Conference

6 – 10 May 2007

Istanbul, Turkey

www.ewac.eu

Leiniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany

and

The John Innes Centre, Norwich Research Park, Colney, Norwich, UK

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Dedicated to the 75th Birthday of Colin Law

Edited by
A. Börner and J. W. Snape

Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany
and
The John Innes Centre, Norwich Research Park, Colney, Norwich, UK

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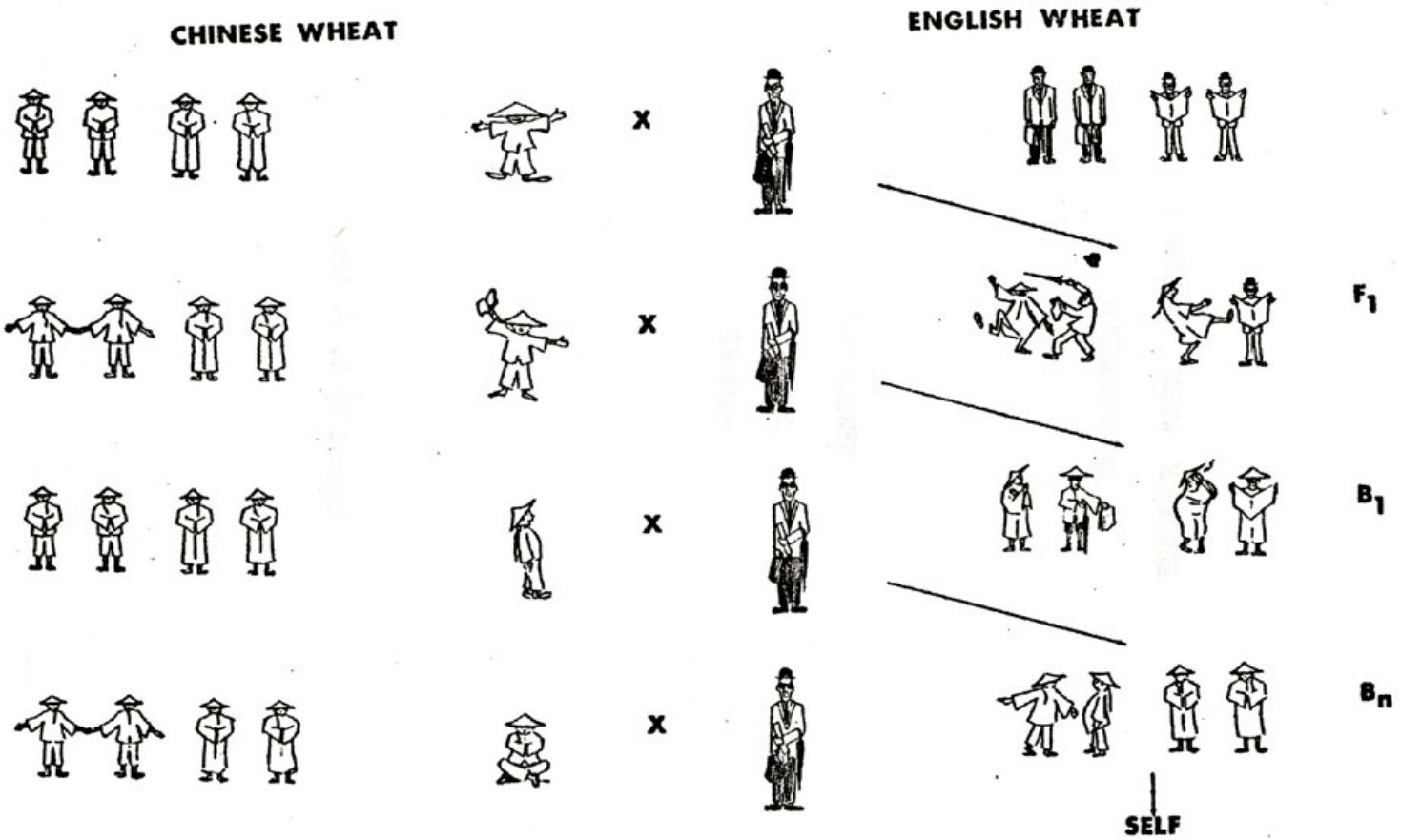

The development of inter-varietal chromosome substitutions

Cartoon published in the 1st EWAC Newsletter

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Preface

A. Börner (Secretary, EWAC)

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As agreed in Prague in 2005, the 14th EWAC Conference was organised in Istanbul, Turkey's most populous city, extending both on the European and on the Asian side of the Bosphorus, and being thereby the only metropolis in the world which is situated on two continents. The Conference was held from May 6 to 10, 2007 - 40 years after the founding of the Co-operative. More than 40 participants from 14 countries attended.

The Conference started with a review of 40 years of EWAC activities. Under the leadership of Colin Law the first EWAC meeting was held at the Plant Breeding Institute in Cambridge from 17 – 20 July, 1967. It was the consequence of a 'Proposal for the co-ordination of European work with wheat aneuploids' made by Ralph Riley and Colin Law at the 'Fifth Yugoslav Symposium on Wheat Research' held in Novi Sad, former Yugoslavia, in 1966.

The aims of EWAC were defined to ensure the following:

- The prevention of the duplication of efforts, either in the development of material or the extraction of information
- The exchange of information
- That cytologically verified stocks of material are available for exchange
- That joint work is undertaken, between geographically separate workers
- That channels are available for the exchange of fully developed material so that co-operative study and testing can be undertaken in a range of environmental conditions
- That arrangements can be made for training workers in the techniques and application of aneuploid stocks

(Riley and Law, 1968).

A photocopy of the programme of the 1st EWAC Meeting is shown in figure 1. The first EWAC Newsletter appeared in 1968 and included a nice cartoon showing the development of inter-varietal substitution lines created by Colin Law (see page 4). Thirteen more conferences followed through until 2007. The complete set of EWAC meetings is given in table 1.

At the last conference in Prague the participants decided to change the name of the co-operative into: 'EWAC – The European Cereals Genetics Co-operative'. Now it has become an excellent platform for researchers developing and using cereal stocks for genetic studies including the localisation and mapping of genes/QTLs by employing molecular techniques. The presentation of 20 oral lectures and more than 30 posters provided a nice overview about the ongoing research activities. For the first time an award for the best poster was made. The winners of the award were Elena Khlestkina and co-authors.

With respect to the place where, in fact, EWAC was born, the next conference will be organised by Boris Kobiljski in Novi Sad, Serbia in spring 2010. We look forward to that 15th EWAC Conference.

PROCEEDINGS OF THE FIRST EUROPEAN WHEAT ANEUPLOID CO-OPERATIVE MEETING

WATES CONFERENCE

Held at the Plant Breeding Institute, Cambridge, 17th-21st July, 1967

PROGRAMME

Tuesday, 18th July

Morning –	9.30	Greetings and welcome by Dr. G.D.H. Bell, Director, Plant Breeding Institute.
	9.45 – 10.30	Introductory talk 'The aneuploids of wheat' by Dr. E.R. Sears.
	Break for coffee	
	11.00 – 12.15	Chairman: Dr. R. Riley Description by participants of the work at present going on in wheat aneuploidy within Europe.
	Lunch	
Afternoon –	2.00 – 3.30	Discussion continued.
	4.00 – 5.00	Wheat aneuploidy in the U.S.A., Canada, and Australia, brief accounts given by Drs. E.R. Sears, J.W. Morrison, and I.A. Watson.
Evening –	7.30	Dinner at Emmanuel College.
	9.00	Informal gathering at University College.

Wednesday, 19th July — Chairman: Professor Dr. G. Fischbeck

Morning –	9.30 – 10.15	The development and use of monosomics. Dr. G. Röbbelen.
	Break for coffee	
	11.00 – 12.00	The development and use of inter-varietal chromosome substitutions. Dr. C.N. Law.
	Lunch	
Afternoon –	2.00	Demonstrations of techniques and experimental material at the Plant Breeding Institute.
Evening –	6.30	Dinner at Emmanuel College.
	7.30	River trip.

Thursday, 20th July — Chairman: Professor Dr. S. Borojević

Morning –	9.30 – 10.30	Genetic mapping in wheat. The mapping of genes using telocentric chromosomes. Dr. E.R. Sears.

		The mapping of factors controlling quantitative characters. Dr. C.N. Law.
	Break for coffee	
	11.00 – 12.15	The introduction of alien variation. Aneuploidy and the introduction of alien variation. Dr. G. Kimber.
		Introduction of genetic variation from *Aegilops comosa*. Dr. R. Riley.
	Lunch	
Afternoon –	2.00	Chairman: Dr. R. Riley
		Outline and discussion of co-operative proposals. (i) Information exchange. (ii) Joint work. (iii) The development of reciprocal substitutions on a co-operative basis. (iv) Exchange of developed material for testing in different environments. (v) The organisation of E.W.A.C.
Evening –	7.30	Farewell dinner at Emmanuel College.

Fig. 1: Programme of the 1st EWAC Meeting

Table 1: Years and venues of EWAC conferences

1st	1967	Cambridge	UK
2nd	1970	Weihenstephan	Germany
3rd	1974	Novi Sad	Yugoslavia
4th	1979	Cambridge	UK
5th	1981	Wageningen	The Netherlands
6th	1984	Versailles	France
7th	1987	Martonvasar	Hungary
8th	1990	Cordoba	Spain
9th	1994	Gatersleben	Germany
10th	1997	Viterbo	Italy
11th	2000	Novosibirsk	Russia
12th	2002	Norwich	UK
13th	2005	Prague	Czech Republic
14th	2007	Istanbul	Turkey

Reference

Riley R, Law CN (1968) EWAC Newsletter: 1-3

Oral Presentations

The history of the development of precise genetic stocks of bread wheat in Novosibirsk and their application for investigation of grain quality

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The history of precise genetic stocks of bread wheat in the Institute of Cytology and Genetics SB RAS (ICG), Novosibirsk, began at the end of 60th when a set of Chinese Spring monosomic lines were received from the USA and Canada. In 1966 R. Riley and C. Law suggested to call a special meeting to discuss the organization and coordination of the work with wheat aneuploids in Europe. Although no constant contacts existed between ICG and Plant Breeding Institute in Cambridge at that time the information about the first inaugural meeting reached Novosibirsk somehow. Several participants from the institute took part in the first EWAC Meeting and Dr. Olga Maystrenko was among them. At that moment she was a leader of the Laboratory of grain quality and had published several papers concerning the inheritance of the trait where the conventional genetic experiments were accomplished. She clearly understood the restrictions of this method of investigation of this quantitative trait and was interested to enlarge the scale of the work using a new kind of genetic material which had been developed outside the USSR. The organizers of the first EWAC Meeting delivered a special Questionnaire to the participants in order to know the state of the work on the development of precise genetic stocks in Europe. In the Questionnaire from Olga Maystrenko it was pointed that two sets of monosomic lines were began to be developed in two bread wheat cultivars Saratovskaya 29 (S29) and Diamant 2 (Dm2) with the help of CS monosomics as well as the substitution lines. The choice of the two parental cultivars was conditioned by their contrast bread-making qualities with S29 having a good quality but rather low gluten content in grain and Dm2 having very poor quality but gluten content about 40%. This work was fulfilled on the South of the USSR, in Uzbekistan on the fields of one of the Departments of VIR, Leningrad (now S-Petersburg). The favourable climate gave a possibility to obtain much grain for the technological analysis of aneuploids. Such work was begun since the first backcrosses during obtaining the new monosomic sets.

For the first time O. Maystrenko with colleagues investigated grain quality directly in ditelosomic lines of CS (Fig.1). In three-year experiment it was found that the absence of the different chromosome arms may cause both improvement and deterioration of physical properties of dough as measured by alveograph and farinograph. Both decrease and increase of dough strength was observed as well as changes in ratio of tenacity (P) and extensibility (L). The effects were explained in terms of presence of genes inhibiting or enhancing flour quality in CS genotype. These results were published in the Proceedings of 4th International Wheat Genetics Symposium (Maystrenko et al. 1973). Although the biochemical structure of gluten was rather intensively investigated in those years, practically nothing was known about the genetic basis of gluten formation. Nevertheless, the special important role of 1D chromosome was detected during the analysis of grain quality in subsequent backcrosses in the process of the development of monosomic lines. Was it the effect of the early generation

or the chromosome dose? Studies of mixing properties with Brabender farinograph have shown that dose is the main reason of deterioration (Fig.2). It was found that both in the second and in the fourth backcrosses just the hemizygous dose of 1D chromosome showed a significant effect on mixing parameters of high quality cultivar S29.

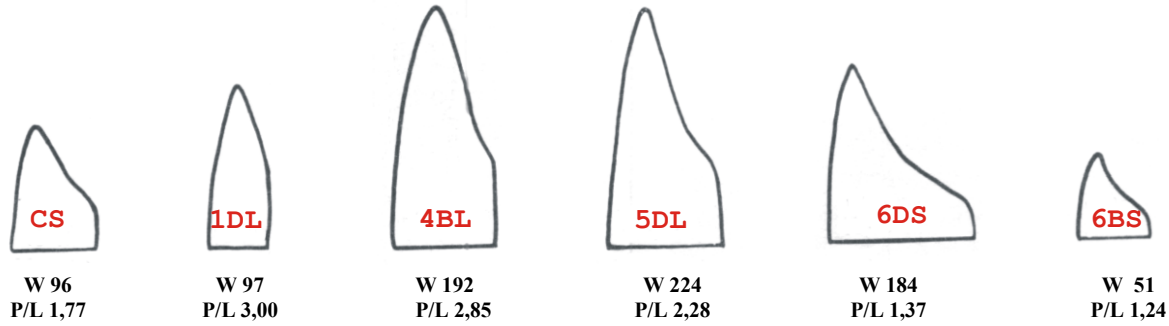

Fig. 1: Alveograms, dough strength (W) and P/L ratio of some ditelosomic lines of Chinese Spring (Uzbekistan, 1970-1972)

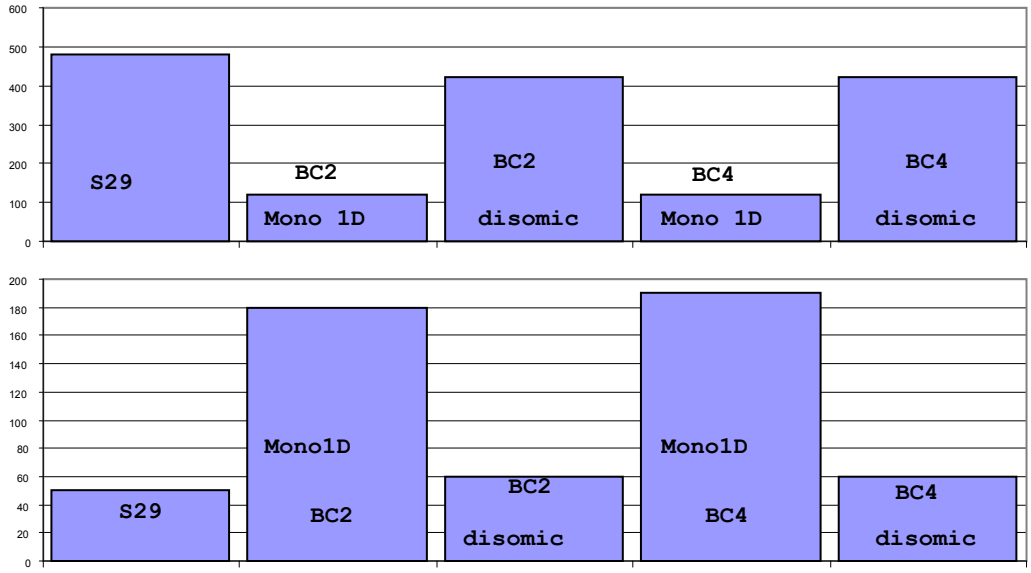

Fig. 2: Dough resistance to mixing (seconds) and dough thinning (units of farinograph) in monosomic and disomic populations selected in BC2 and BC4 of 1D monosomic line in S29

To additionally investigate this phenomenon a special experiment was elaborated using a gluten swelling test of monosomic and disomic populations obtained from the cross of CS mono 1D with several high-quality cultivars. It was again found (Fig. 3) that the dose of 1D chromosome has a crucial effect on gluten volume in lactic acid even on the heterozygous genetic background of the hybrids. But the background itself was also important. While disomic F_2 of the cross CS × Aurora slightly differed from the high quality cultivar Aurora in swelling volume, in the crosses with S29 and Skorospelka 35 disomic F_2 has substantially lower meanings. Further analysis of monosomic lines of cultivars S29 and Dm2 with contrast technological properties again demonstrated that the lack of one of the two homologous chromosomes 1D has a pronounced negative effect on physical properties of dough in both cases and loaf volume in S29 (Fig. 4, 5).

In 70-80th the development of the different sets of intervarietal substitution lines was accomplished and one of them was obtained specially for the investigation of grain quality.

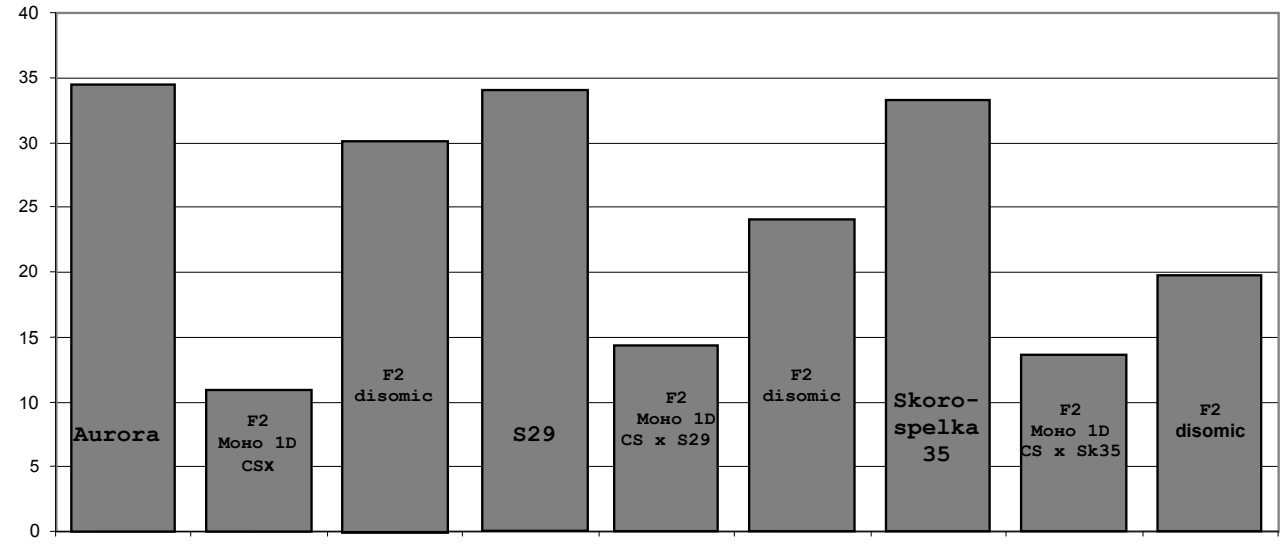

Fig. 3: Gluten swelling (0,02N lactic acid, ml) in monosomic and disomic F2 hybrids obtained from the crosses of mono 1D of Chinese Spring with high quality cultivars Aurora, S29 and Skorospelka 35

In this set the each chromosome of 1 and 6 homoeological groups of the low quality cultivar Dm2 were substituted for the homologue from the high quality cultivar Novosibirskaya 67. At this time it was known that the genes for biosynthesis of endosperm storage proteins participating in gluten formation are located in these chromosomes. It was found that the role of 1A chromosome is important; this substitution significantly increased dough strength and tenacity (Table 1) measured by alveograph. This data coincided with the earlier results obtained with the use of substitution line CS/Hope 1A (Payne et al. 1987). At the same time this substitution did not affected any mixing parameters. The most striking effect was found after the substitution of two chromosomes of the donor, 1A and 6D, in the genotype of the low quality recipient. In this case not only the alveograph parameters were improved but the mixing parameters also (Table 1).

Further investigations were fulfilled using a full set of substitution lines S29/Janetzkis Probat (S29/JP) where both the recipient and the donor have a good grain quality. This work is being continued now but preliminary results have showed that the intervarietal substitution in this case also influenced the separate physical properties of dough (Table 3). Substitution of 1D and 3A chromosome has an influence on the balance between tenacity and extensibility of dough, substitution of 4B chromosome significantly decreased extensibility and in the case of substitution of 4D chromosome all the alveograph parameters have changed.

In the last years a new genetic material presented by wheat recombinant inbred lines (ITMI population) was involved in the investigations of grain quality. The new technique of searching of associations between phenotypic variability of quantitative trait and molecular markers was used for investigations. We were able to identify several QTLs both connected with loci for glutenin biosynthesis in chromosomes of 1 homoeological group and located on the other chromosomes where no special genes relevant to grain quality have been detected (Pshenichnikova et al. 2006).

It is thought now that the key mechanism determining the rheological properties of gluten is provided by interactions between branching polymer protein chains. The most important are the interactions provided with covalent disulfide bonds (Shewry, Tatham, 1997) which number correlates with grain quality parameters. Their formation and decomposing are catalysed by the system of thiol-disulfide metabolism enzymes.

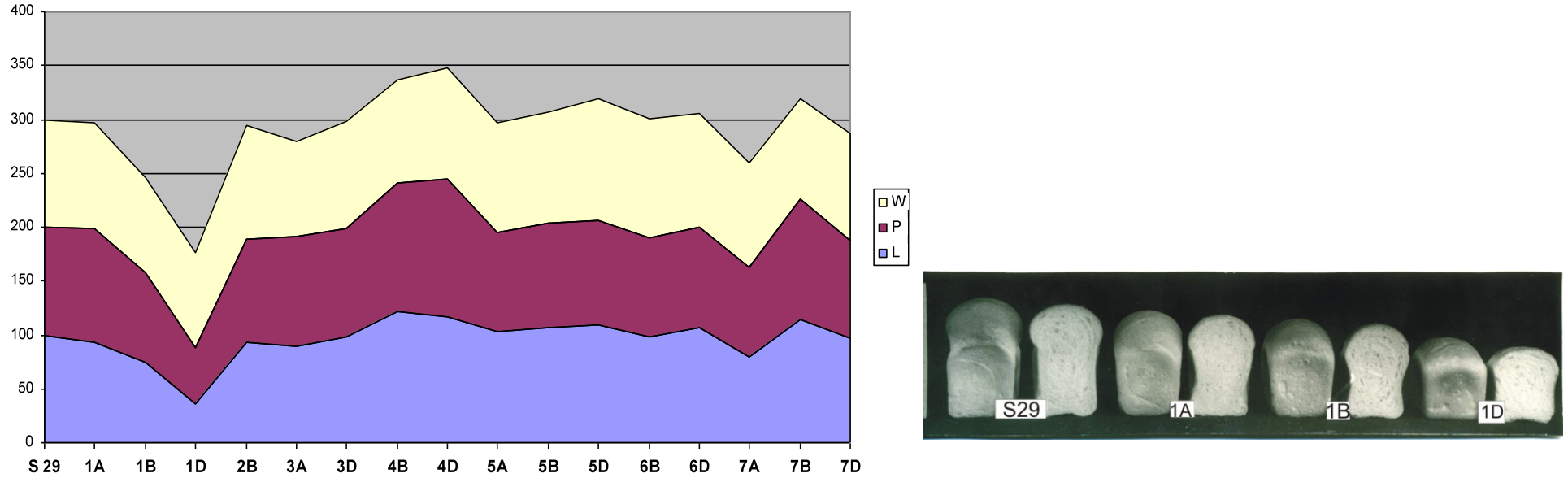

Fig. 4: Alveograph parameters (W-dough strength; P- tenacity, L- extensibility) of S29 monosomic lines and loaves baked from the flour of the monosomics of the 1 homoeological group (Novosibirsk, 1976-1979).

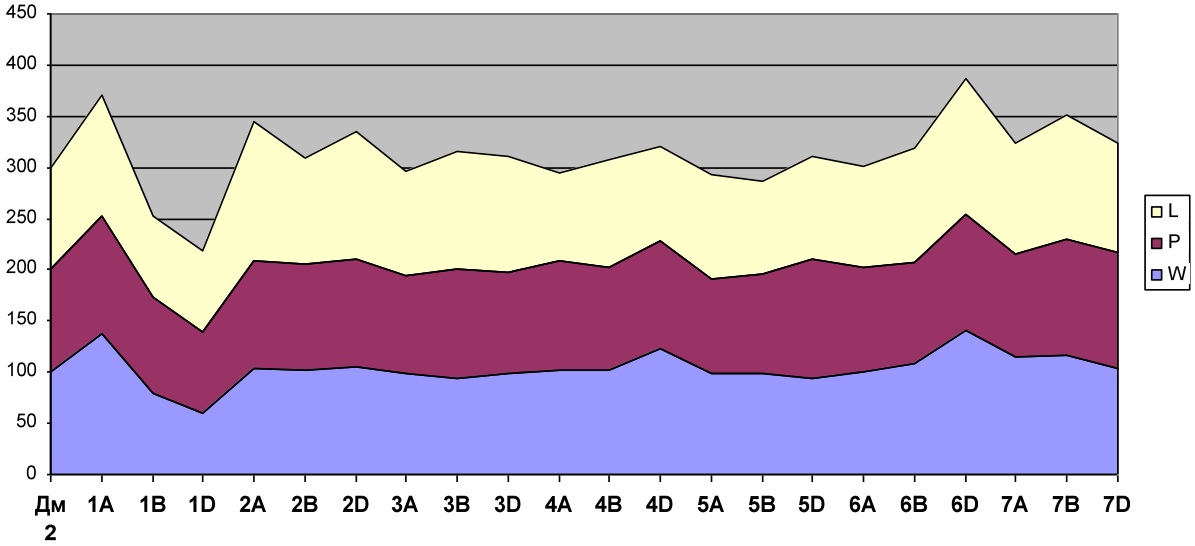

Fig. 5: Alveograph parameters (W- dough strength; P - tenacity, L - extensibility) of Dm2 monosomic lines (Novosibirsk, 1976-1979).

Table 1. Physical properties of dough in the intervarietal substitution lines Diamant 2/Novosibirskaya 67 (Dm2/N67)

Lines, cultuvars	Alveograph parameters				Farinograph parameters		
	Dough strength, u.a.	Tenacity, mm	Extensibility, mm	P/L	Resistance to mixing, sec	Stability during mixing, sec	Dough thinning, u.f.
Dm2, recipient	153	66	84	0,83	195	495	70
N67, donor	352*	114*	101	1,22*	420*	840*	13*
Dm2/N67 1A	233*	74*	118	0,69	360	795	35
Dm2/N67 1B	137	64	91	0,77	105	360	65
Dm2/N67 1D	147	71	82	1,04	90	345	61
Dm2/N67 6A	122	68	71	1,05	75	270	70
Dm2/N67 6B	162	67	94	0,79	75	300	64
Dm2/N67 6D	172	69	93	0,78	195	495	49
Dm2/N67 1A 6D	287*	76*	130*	0,63	507**	973**	35

* - P< 0,05 (comparing to the recipient)

Table 2. Physical properties of dough in the intervarietal substitution lines Saratovskaya 29/Janetzkis Probat (S29/JP)

Genotypes	Alveograph parameters			
	Flour strength, W, u.a.	Tenacity, P, mm	Extensibility, L, mm	P/L ratio
S29	513±15	156±6	108±2	1,4±0,03
JP	316±17*	101±7*	103±8	1,0±0,00*
S29/JP 1D	519±42	132±2	110±3	1,2±0,02*
S29/JP 3A	420±18	122±4	131±2	0,9±0,01*
S29/JP 4B	507±12	146±18	130±2*	1,1±0,15
S29/JP 4D	356±32*	97±2*	153±0,3*	0,6±0,01*

* - P< 0,05 (comparing to the recipient)

We investigated the activity of one of such enzymes – disulfide reductase, responsible for decomposing S-S bonds in gluten in ITMI population. It was found that most of QTLs detected for this parameter form cluster with QTLs earlier found for physical parameters of gluten. Such clusters were detected in 4A, 5D, 6A and 7D chromosomes. Although the genetic control of this enzyme is still unknown it may be supposed that one of the detected QTLs is attributed to the structural gene for disulfide reductase.

The new application of precise genetic stocks consists in using them for verification of the identified QTLs. Now we are fulfilling the introducing of chromosomes carrying the QTLs positively and negatively influencing on different technological characteristics from ITMI lines into genotypes of S29 and Dm2. The material is on different stages of backcrossing. In addition, the future investigations will include the use of recombinant substitution lines S29/JP for further mapping of genes for technological properties of dough.

The establishment of EWAC 40 years ago was initiated by two remarkable scientists, Ralf Riley and Colin Law and followed up by many scientists participated in the work of EWAC during these years. They brought in a big input in the development of wheat cytogenetics. The first of them was Tony Worland, EWAC Secretary, passed away so untimely. The main idea of the EWAC constituent documents - international cooperation and exchange with the genetic material and ideas – gave the impressive results. Before the first meeting only two monosomic sets had been obtained in Europe by Colin Law, for cultivars Koga II and Capelle-Desprez. On the 9th EWAC meeting it was communicated about 30 of monosomic sets not saying about other kinds if genetic material from different countries. The history of EWAC is first of all a history of close and friendly scientific and human cooperation.

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The Wheat Genetic Improvement Network (WGIN) populations at the John Innes Centre, Norwich, UK

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Project Outline

Department for the Environment Food and Rural Affairs (Defra) Wheat Genetic Improvement Network (WGIN) has been formed in the UK to enable a close working relationship between researchers, breeders and the end users of the UK wheat industry. Since 2003, its aim has

been to ensure the sustainable development of the UK wheat community and has ensured that UK wheat researchers have shared objectives in the promotion of the genetic improvement of UK wheat varieties.

The overall goal of the project has been to generate pre-breeding material, carrying novel traits. The project also provides access to advanced breeding technologies which will ultimately allow the targeting of new alleles and in turn advance the improvements for new varieties.

The JIC has focussed on the production of populations which represent the past, present and future. The A E Watkins population captured world wide varieties and landraces from the 1930s. The doubled haploid mapping population of Avalon x Cadenza has become a key reference point for the UK wheat community and the mutant populations, based on the UK spring variety Paragon, seek to exploit novel diversity. Together with the EU Gediflux collection of agriculturally important Northern European varieties from the 1940s to 2000, these populations represent an immense amount of genetic diversity in wheat.

The Future: Paragon Mutant Populations

Two separate mutant populations of Paragon have been developed using i) ethyl methane sulfonate (EMS) and ii) gamma sources of mutagenesis (Koebner 2001). It was determined that fifty percent germination would be satisfactory to ensure a large range of single base deletions in the EMS (Munroe 2000). Seven thousand individual seed were treated with 1% EMS for 3 hours and after washing and germinating on filter paper, these were sown into a peat and sand mixture. One bagged ear per plant was harvested and two seeds from this were sown, taking the population to around 7000 individuals. A single seed descent technique was used to develop the population to M_5, bagging ears at each generation to ensure self pollination. Notes were taken during the early generations, but due to the small pot size and close proximity of the plants, mutations could not be validated until the field trial stage.

The M_5 generation was sown under field trial conditions in spring 2006, with 30 seeds to a 1 metre row, being grown. Uniformity within lines was observed and confidence then placed in the subsequent interesting mutations that were noted and photographed. Twenty five lines were shorter than 50% Paragon control height, with 548 lines at least 10% shorter. Over 130 lines were taller than the average Paragon control, with five lines taller than the tallest Paragon. Four day earlier flowering times were noted and up to 25 day later variants were seen. Ear structure variations such as club type, laxness, tapered spelt type and awn suppressor knockouts were noted, along with seed shape, (*sphaerococcum* type), temperature sensitivity (zebra leaf effect), high and low tiller number, disease, and differing senescence times. The population represents a vast amount of diversity and recently a focus has been made towards the senescence variation and the ability to use this by backcrossing to varieties and checking segregation at the F_2 generation.

The collection is currently around 6500 validated lines and data from the 2006 trial are available at www.wgin.org.uk. Seed is freely available to researchers and can be sourced through simon.orford@bbsrc.ac.uk. Of equal interest, a population of gamma irradiated seed has also been developed to the M_3 generation. This collection of around 2000 lines shows larger deletions, occurring most notably at the 250 grays irradiation level (Al-Kaff 2007).

The Present: Avalon x Cadenza Mapping Population

The population consists of 202 doubled haploid lines developed using the maize cross technique. Over 330 markers have been used to construct the current map, based on major genes (*Glu-1, Rht-D1, Pin-D1, SSR and DArT.*) The map and genotypic data from this work is

available at www.wgin.org.uk but it is still very much work in progress and is constantly being updated.

The population was developed due to the wide range of trait diversification between the two parents. QTL analysis has already been carried out on a range of traits including grain colour, flowering time and straw wall thickness.

Table 1: Comparison of Avalon x Cadenza parental lines

Avalon Characteristics	**Cadenza Characteristics**
Maris Ploughman x Bilbo	Axona x Tonic
Winter type habit	Spring type habit
Pinb-D1b allele present	Pinb-D1c allele present
5B-7B translocation	Normal 5B-7B
Rht D1 dwarf	No dwarfing genes
1/6 + 8/2 + 12 HMW proteins	Nulll/14 + 15/5 +10 HMW proteins
Thin straw wall	Thick straw wall

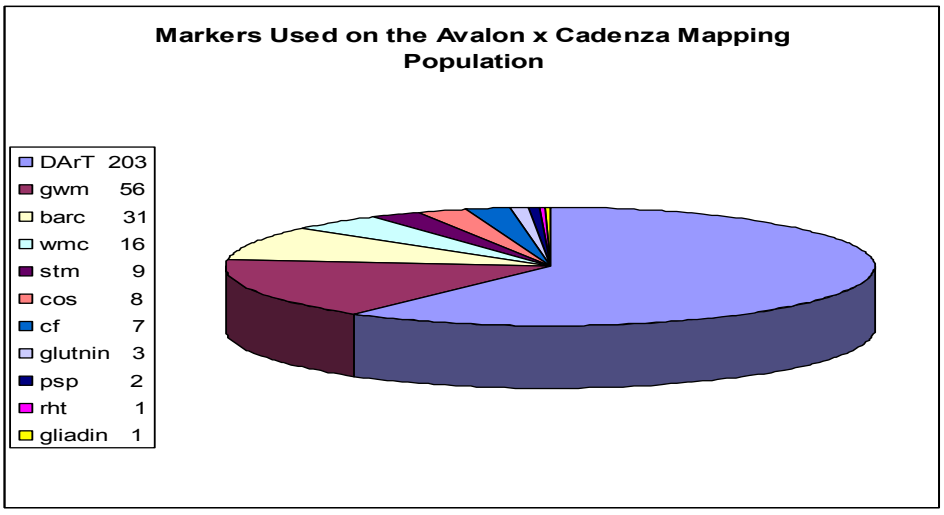

Fig. 1: Sources of markers used on the Avalon x Cadenza genetic map

The Past: A. E. Watkins Collection and Gediflux

Evaluation of core collections held within the National Wheat Collection at the JIC has shown the immense diversity of material held in the A. E. Watkins' collection. Watkins used his connections with the London Board of Trade in the 1930s to access the wheat markets of the world (Miller 2001). Important lines such as those from Burma reflect the importance of this collection. Due to the fact that wheat is no longer grown there, this area of diversity would possibly have been irretrievably lost.

Over 800 lines from 32 countries have been scored for ear emergence, 77-120 days, height, 50 to 150cm (with plant growth regulator) and winter or spring growth habit. Some accessions were thought to be of mixed origins. The accessions were therefore taken back to four single seeds and new accessions grown from these. The collection was grown in full, using the four newly formed accessions in the spring of 2006 to assess the homozygous nature within accessions as well as checking the diversity across the accessions. It was found that 80% of

the lines were phenotypically homozygous, with the remaining 20% now having a set of sub lines from the original accession numbers. Data recorded for the 2006 trial can be viewed at www.wgin.org.uk.

Table 2: Countries represented by the 1930s AE Watkins collection

Country	**Accessions**	**Country**	**Accessions**
Algeria	7	Crete	12
Canary Islands	13	Cyprus	2
Egypt	4	Finland	1
Ethiopia	2	France	21
Morocco	21	Greece	25
Tunisia	16	Hungary	8
Afghanistan	35	Italy	17
Burma	4	Poland	20
China	89	Portugal	39
India	130	Romania	7
Iran	52	Spain	101
Iraq	8	UK	3
Palestine	2	USSR	60
Syria	1	Yugoslavia	50
Turkey	17	Australia	32
Bulgaria	14	Brazil	1

As part of the EU Genetic Diversity Temporal Flux project (Gediflux), a collection of over 500 of the most agronomically important varieties from ten Northern European countries, from 1940 to 2000, was made. For inclusion, a variety needed to cover at least 5% of a country's wheat growing capability.

Nucleotide Binding Sites (NBS), retrotransposons and SSRs were used as DNA fingerprinting techniques to assess the degree of the allelic erosion or addition for each decade, as well as between recommended listings and national listings. Results showed no significant erosion over the time period (Reeves 2004). The collection has been maintained from the project and is available as a resource tool

Table 3: Countries represented by the Gediflux collection

Country of Origin	**Period Span**	**Number of Varieties**
Austria	1940 – 2000	40
Belgium	1960 – 2000	24
Germany	1990 – 2000	18
East Germany	1940 – 1990	30
West Germany	1950 – 1990	19
Denmark	1980 – 2000	5
France	1940 – 2000	34
United Kingdom	1940 – 2000	66
Netherlands	1940 – 1990	19
Sweden	1950 – 1980	26

Summary

The populations mentioned in this report are freely available for the wheat community's use and represent a large degree of genetic diversification. Any requests for genotypes can be made to simon.orford@bbsrc.ac.uk or at The John Innes Centre's address at the top of the report.

Acknowledgements

We would like to thank the Department for Environment, Food and Rural Affairs for their funding and support for this work

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Genetic stocks in wheat research – examples of successful co-operation

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At the Fifth Yugoslav Symposium on Wheat Research, Novi Sad, 1966, Ralph Riley and Colin Law presented a proposal for the development of an European Wheat Aneuploid Co-operative. The first inaugural conference was held one year later in Cambridge, 40 years ago. The initial aims of EWAC were the exchange of information and cytologically verified stocks but also the co-ordination of the development of new material and the co-operation in testing developed material in a range of environmental conditions. Consequently, series of genetic stocks including monosomics, chromosome substitution lines, alloplasmic lines, single chromosome recombinant lines, introgression lines, etc. have been created. Using those precise genetic stocks many qualitative and quantitative inherited traits were associated to certain chromosomes, chromosome arms or introgressed segments. When molecular marker techniques became available, the knowledge gathered from the stock investigations often was the prerequisite for the precise mapping. In our presentation we give two examples for the successful co-operation within the frame of EWAC. Data on molecular mapping of major genes and QTLs determining morphological and agronomically important traits are presented.

Trichomes – the hairy story

Outgrowths of the epidermis of plant organs, called trichomes or hairs are common in many plant species. It is supposed that the occurrence of hairs (pubescence) is positively associated with harsh moisture regimes. A layer of hairs will in most cases decrease the air movement next to the leaf (or any other plant organ), and thus create a special microclimate being some kind of buffer. In addition a thick hair cover also protects the leaf surface from intensive solar irradiation.

An improvement of drought tolerance in wheat due to leaf pubescence was suggested by Reynolds et al. (1999) or Skovmand et al. (2003). The reason may be a greater thickness of still air through which water must diffuse to the dryer outside under drought stress conditions. On the other hand a positive effect on cold tolerance was reported by Thretowan et al. (1998). In the Mexican highlands it was observed that pubescent spikes suffered less damage than non-pubescent ones following severe frosting at flowering. Here the microclimate on the leaf surface seems to reduce (delay) the harmful effects of cooling down.

The genetics of hairiness of several organs is well studied. The trait hairy glumes is controlled by the dominant gene *Hg*, mapped on the short arm of chromosome 1A (Blanco et al., 1998; Khlestkina et al., 2002; 2006). Performing F_2 monosomic analysis Maystrenko (1976) described a gene for leaf hairiness (*Hl1*) in three common wheat cultivars ('Saratovskaya 29', 'Saratovskaya 210', 'Milturum 553') to be located on chromosome 4B. Using 'Saratovskaya 29' as a tester line Maystrenko (1992) showed that nine cultivars bred in drought environments of Siberia, Kazakhstan and the Volga region carry one and the same gene for leaf hairiness. Another dominant gene controlling the hairiness of the auricles and designated *Pa* (*pubescent auricles*) was determined by Maystrenko (1992). Using ditelosomic lines, both *Hl1* and *Pa* were positioned on the long arm of chromosome 4B. Linkage between the Hl1 and *Pa* was calculated to be 30 cM. Another gene for leaf hairiness was discovered on chromosome 7BS applying monosomic and telosomic analysis (Taketa et al. 2002).

Beside of wheat genes for hairiness of leaves are described for barley or rye but also for the wild relatives of the cultivated cereals. In cultivated barley (*Hordeum vulgare* L.) a gene for pubescent leaf blade is located on chromosome 3HL (*Pub*) whereas another gene determining hairy leaf sheath (*Hs* syn. *Hsh*) is known to be located on the long arm of chromosome 4H pleiotropically linked to *Hn* syn. *Hln* determining the trait hairs on lemma (Franckowiak, 1997; Lundqvist, et al. 1997). A homoeologous gene determining hairy leaf sheath in *Hordeum bulbosum* L. and designated Hsb was described by Korzun et al. (1999).

Furthermore, Korzun et al. (1999) could show that the gene *Hp1*, determining pleiotropically the hairiness of the peduncle and leaf sheath in rye (*Scaele cereale* L.) and which is known to be located on the long arm of chromosome 5R nicely lines up with the barley genes *Hs* (*Hsh*) and *Hsb*. The co-linearity is due to a translocation with and a homoeology to the distal part of the 4L chromosomes of the other Triticeae members (Devos et al., 1993).

Pshenichnikova et al. (2006) identified a gene determining hairy leaves in Aegilops speltoides Tausch, a wild relative of cultivated wheat, in the wheat/*Aegilops speltoides* introgression line '102/00i'. Results of a monosomic analysis revealed that the introgression carrying the hairy leaf gene was located on chromosome 7B.

Within the frame of the EWAC organisation activities were initiated between the IPK Gatersleben and the IZG Novosibirsk in order to map genes determining hairy leaves in wheat. Mapping populations were created segregating for major genes on chromosomes 4B and 7B, originating from cultivated wheat (*T. aestivum* L.) and the wheat/*Aegilops speltoides* introgression line described above, respectively. Beside this, a QTL mapping approach was performed investigating the 'International Triticeae Mapping Initiative' (ITMI) mapping

population and considering the hairiness of leaves and auricles. Finally a test cross was carried out for testing the allelism between Hl2 (7BL) and the Hl gene of the wheat/*Aegilops speltoides* introgression line (7B).

The results of that co-operative work were summarised by Dobrovolskaya et al. (2007). Two major genes for leaf pubescence of wheat and a wheat/Aegilops introgression line were mapped on chromosomes 4BL (*Hl1*) and 7BS ($Hl2^{Aesp}$), respectively, together with QTLs determining leaf and auricle pubescence on the long arms of chromosomes 4B (contributed by Opata) and 4D (contributed by Synthetics, i.e. *Ae. tauschii*). Because the positions of the QTLs for hairy leaves and auricles were highly comparable on both chromosomes, it may be concluded that both traits are inherited pleiotropically. However, linkage of two different loci can not be excluded.

Considering the data obtained by Korzun et al. (1998, 1999) and using the consensus linkage map of barley published by Langridge et al. (1995), the homoeologous group 4 wheat (*Ae. tauschii*) genes/QTLs *Hl1, QHl.ipk-4B, QPa.ipk-4B, QHl.ipk-4D* and *QPa.ipk-4D* line up with the barley pubescence genes *Hln/Hsh* and *Hsb* as well as the rye gene *Hp1* (Figure 1). It was concluded that the locus seems to be pleiotropically responsible for the pubescence of different plant organs in different species of the Triticeae.

A second homoeologous series seems to be present on the short arms of the homoeologous group 7 chromosomes at least in wheat (7B) and Aegilops speltoides. For this, clear indication is given by the result of the test cross between 'Hong-mang-mai' and '102/00i' analysed.

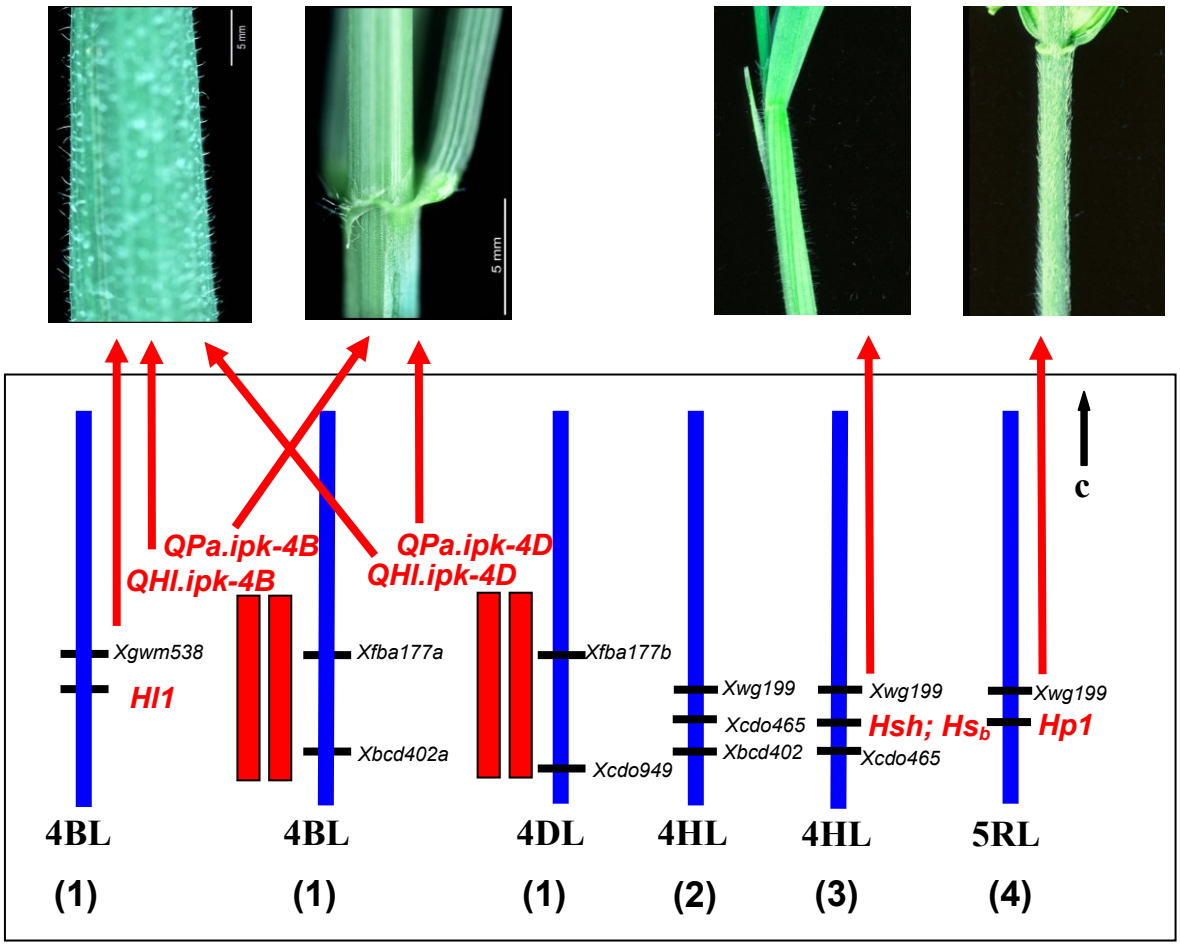

Fig. 1: Comparative molecular mapping of genes determining hairiness of different plant organs in wheat (*Aegilops tauschii*), barley and rye. The mapping data were originated from (1) Dobrovolskaya et al. 2007; (2) Langridge et al. 1995; (3) Korzun et al. 1999; (4) Korzun et al. 1998. c = centromere position, L = long arm

Biotic stress resistance

The second example presented here is a co-operative study including the National University La Plata, Argentina, the IPK Gatersleben, Germany and the IZG Novosibirsk, Russia. A set of 84 wheat (*T. aestivum*)/*Aegilops tauschii* introgression lines was developed by backcrossing the seven wheat 'Chinese Spring/Synthetic 6x' D genome chromosome substitution lines with 'Chinese Spring' ('CS'). The 'Synthetic 6x' used for the creation of the substitution lines had been obtained from a cross between tetraploid emmer (*Triticum dicoccoides*) and *Aegilops tauschii* (McFadden and Sears, 1947) and, therefore, the material available contains different segments of individual chromosomes of *Aegilops tauschii* in the 'CS' background (Figure 2). The introgressed segments of the individual lines were determined by using microsatellite markers (Pestsova et al., 2001; 2006).

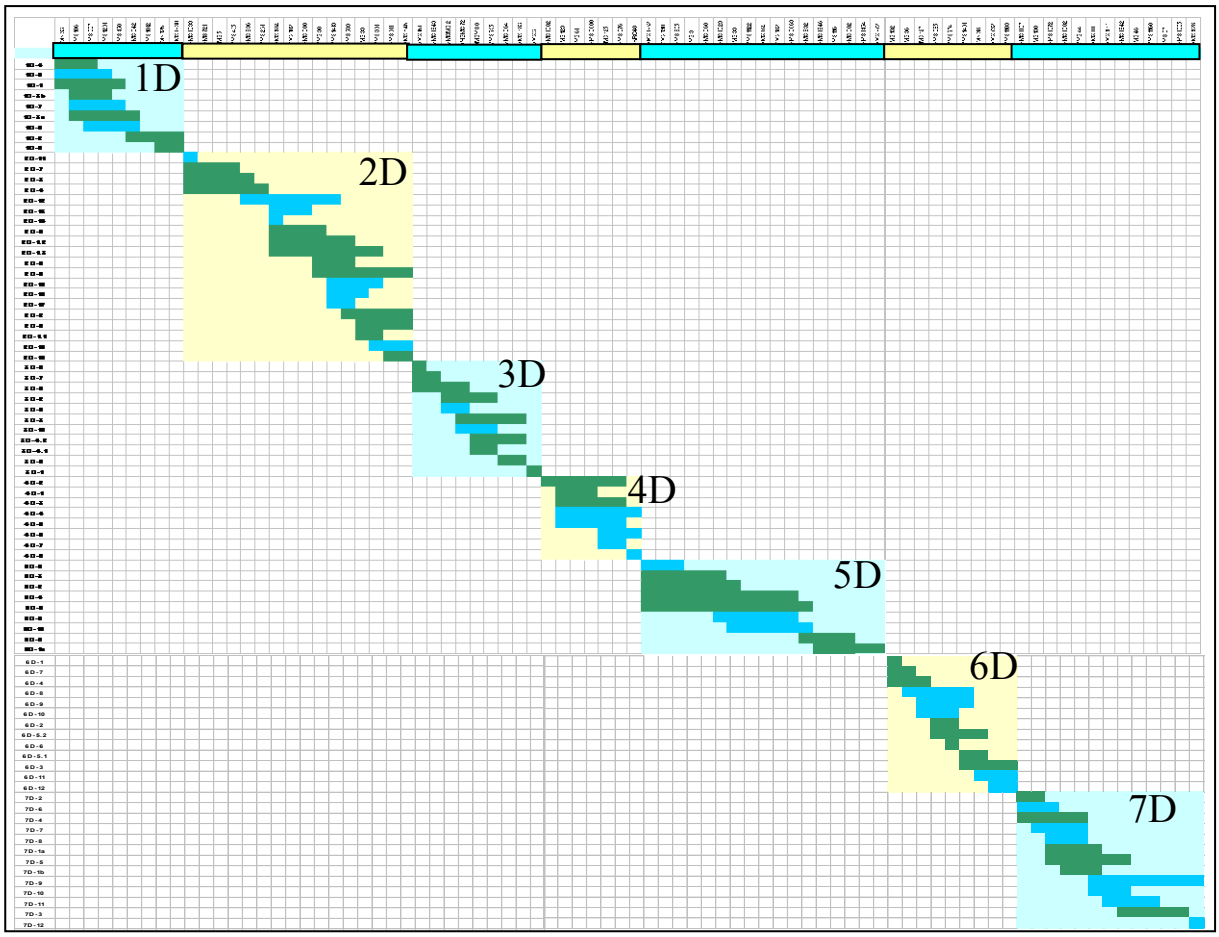

Fig. 2: The set of 84 wheat (*T. aestivum*)/*Aegilops tauschii* introgression lines

Analysing several sets of single chromosome substitution lines including the 'CS/Synthetic 6x' series, Simon et al. (2001; 2005) identified chromosome 7D of 'Synthetic 6x' to be almost complete resistant to two virulent Argentinean isolates of the disease Septoria tritici blotch, designated IPO 92067 and IPO 93014. Both isolates were used to phenotype thirteen chromosome 7D wheat/*Aegilops* introgression lines by Simon et al. 2007).

A summary is given in figure 3. 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 is present in the centromeric region of the short arm of chromosome 7D. It was shown, that the resistance is acting against both isolates used and in both developmental stages, although the effects were more pronounced at the seedling stage.

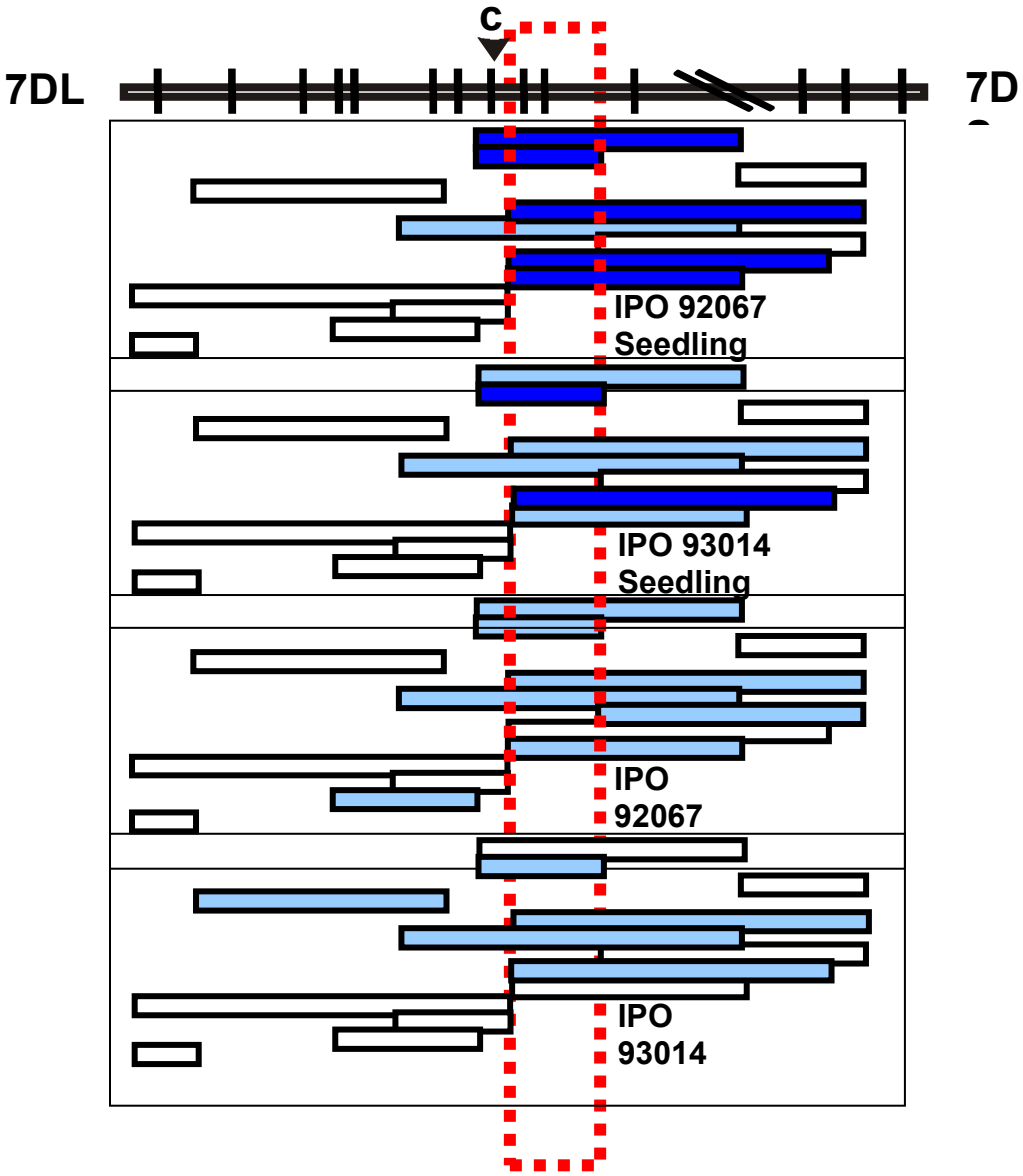

Fig. 3: Wheat/*Ae. tauschii* chromosome 7D introgression lines inoculated with septoria tritici blotch isolates IPO 92067 and IPO 93014 at seedlings and adult plant stages. Lines significant different to 'CS' in at least two or even four independent experiments are given in light blue and dark blue colour, respectively. Boxes in broken lines indicate the position of the resistance locus. L = long arm, S = short arm, C = centromere position.

The position of the locus detected here is highly comparable with that, described by Arraiano et al. (2001), investigating single-chromosome recombinant lines developed on the basis of the 'CS/Synthetic 6x' chromosome 7D substitution line. *Stb5* was mapped distal to the marker *Xgwm44*, also included for the identification of the introgression lines (Pestsova et al., 2006) and, therefore, present in our studies. We suppose that we have tagged the major gene *Stb5* by

applying the 'Introgression Mapping Approach'. If so, it may be concluded that *Stb5* causes resistance against *M. graminicola* isolates from Europe (Portugal, the Netherlands) but also from South America (Argentina) which is of special interest to plant breeders of that area. At least to some extend the resistance seems to be isolate non-specific.

This example nicely indicates that the present set of *Triticum/Aegilops* introgression lines is a suitable tool for the detection of genes/QTL originated from *Ae. tauschii*, the progenitor of the D genome of hexaploid wheat.

The whole set of introgression lines is available on request for any kind of further investigation.

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Genetic mapping of the genes and development of near-isogenic lines in durum wheat

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Since Watanabe (1994), more than forty near-isogenic lines were developed in durum wheat cultivar LD222. The genes to be introduced are located on the specific chromosome and mapped in the linkage maps using the aneuploid stocks of LD222 and Langdon, Landgdon D-genome chromosome substitution lines, and microsatellite markers.

Genetic mapping of the genes in durum wheat

We contributed the mapping of the genes for long glumes on chromosomes 7AL and 7BL (Watanabe et al. 1996, 1999, 2002; Watanabe & Imamura 2002), brittle rachis on chromosomes 3AS and 3BS (Watanabe et al 2002, 2006) and liglulesness on chromosome 2BL (Watanabe et al 2004). Two mutations for sphaerococcoid seed (MA16219) and compact spike (MA 17648) isolated from M_3 progeny of a durum wheat cultivar, Altaiskaya Niva. The gene for sphaerococcoid grain, s^{16219}, was allelic to *S2*, which is located on the centromeric region of chromosome 3B in hexaploid wheat. The gene for compact spike, C^{17648} is located on the chromosome 5A. It was observed that C^{17648} was different from the *Q* locus. Using microsatellite markers, the gene order was *Xbarc319* - C^{17648} - *Xgwm179* - *Xgwm126* - *Xgwm291* - *B1* (Kosuge et al. *Euphytica in press*).

Development of near-isogenic lines in durum wheat

The near-isogenic lines for sphaerococcoid seed and compact spike were established as ANW 22A and ANW 11D. The near-isogenic lines for GA-sensitive *Rht* genes (*Rht 14*, *Rht 16*, *Rht 18* and *Rht 19*) were developed, although their chromosomal locations have not been determined. The multiple alleles at *Rht-B1* locus were introduced into the genetic background of cv. LD222. *Triticum polonicum* IC 12196 may be considered as new source of *Rht* gene (Watanabe, 2002). The effort to develop near-isogenic lines was extended to introduce taxonomy-related traits such as spelt, squarehead and awn on the glumes. The table below summarizes presently available near-isogenic lines of durum wheat cultivar LD222. Several near-isogenic lines are available upon request. The information is also available at the website, http://seimei.agr.ibaraki.ac.jp/ibaraki_public_html/catalogue.htm.

Code	Character	Allele	Donor
Chromosome 1A			
ANW 1A	Black glume	*Bg*	*T. durum reichenbachii*
ANW 1B	Black glume, Hairy glume	*Bg,Hg*	*T. carthlicum* #521
ANW 2A	Hairy Glume	*Hg*	*T. durum melanopus*
Chromosome 3A			
ANW 9A	Red grain	*R-A1b*	LDN(DIC 3A)
ANW 10A	Brittle rachis	*Br2*	LDN(DIC 3A)
ANW 11B	Sphaerococcoid	*S3*	MS 1453, a mutant of Saratovskaya 29 (2n=42)

Code	Character	Allele	Donor
Chromosome 5A			
ANW 16C	Reduced height	*Rht 12*	Mv 17(Karcagi 522 5A) (2n=42)
ANW 22A	Compact spike	C^{17648}	MA17648, A mutant of Altaiskaya Niva
Chromosome 7A			
ANW 5A	Long glume	*P1*	*T. polonicum vestitum*
ANW 5C	Long glume	*P1*	*T. petropavlovskyi* Maystrenko's line (2n=42)
ANW 5D	Long glume	*P1*	*T. polonicum ssp. abyssinicum*
ANW 5E	Long glume	*P1*	*T. petropavlovskyi* k44126
ANW 5F	Long glume	*P1*	*T. aestivum* PI 191834
ANW 5G	Long glume	*P1*	*T. aestivum* AUS 20561 (2n=42)
ANW 7A	Chlorina	*cn-A1d*	CDd6, a mutant of Langdon
Chromosome 2B			
ANW 3A	Non-glaucousness	*W1I*	*T. durum pyramidale*
ANW 3B	Non-glaucousness	*w2*	AUS 2499
ANW 12A	Ligulelessness	*lg1*	A variant of Marvroullos
Chromosome 3B			
ANW 9B	Red grain	*R-B1b*	LDN(DIC 3B)
ANW 10B	Brittle rachis	*Br3*	LDN(DIC 3B)
ANW 11C	Sphaerococcoid	*S2*	MSK 2454, a mutant of Skala (2n=42)
ANW 11D	Sphaerococcoid	S^{16219}	M-16219, a mutant of Altaiskaya Niva
Chromosome 4B			
ANW 4A	Reduced height	*Rht-B1b*	*T. durum* Cando
ANW 4B	Reduced height	*Rht-B1c*	A near-isogenic line of Maringa (2n=42)
ANW 4C	Reduced height	*Rht-B1d*	*T. aestivum* Saitama 27 (2n=42)
ANW 4D	Reduced height	*Rht-B1e*	*T. aestivum* Krasnodari 1 (2n=42)
ANW 4E	Reduced height	*Rht-B1f*	*T. aethiopicum* W6824D
ANW 4F	Reduced height	*Rht-B1h*	*T. polonicum* IC 12196
ANW 4G	Reduced height	*Rht-B1f*	*T. aethiopicum* W6807C
ANW 14A	Hairy peduncle	*Hp*	Hp-S615, a near-isogenic line of S615 (2n=42)
ANW 20A	Blue grain	*Ba2*	UC66049
Chromosome 7B			
ANW 5B	Long glume	*P2*	*T. ispahanicum*
ANW 7B	Chlorina	*cn-B1b*	CDd2, a mutant of Langdon
ANW 6A	Purple culm	*Pc*	CS(Hope 7B)
ANW 13A	Chocolate black chaff	*cc*	Vic 'CBC' mutant
Location unknown			
ANW 8A	Yellow leaf	digenic	Yellow mutant (15:1)
ANW 11A	Sphaerococcoid	digenic	Sphaerococcoid mutant
ANW 16D	Reduced height	*Rht 14*	Castelporziano
ANW 16F	Reduced height	*Rht 16*	Edmore M1
ANW 16G	Reduced height	*Rht 18*	Icaro
ANW 16H	Reduced height	*Rht 19*	Vic SD1 line b

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Molecular cytogenetic characterization (GISH/FISH) of wheat-*Aegilops biuncialis* hybrids, amphiploids, addition and translocation lines

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The *Aegilops* (goatgrass) species, which are the most closely related to wheat, exhibit great genetic diversity, the exploitation of which has been the subject of experimentation for more than a century. *Aegilops* genus consists of 11 diploid, 10 tetraploid and 2 hexaploid species. Species belonging to the genus *Aegilops* have been evaluated for their resistance to diseases and pests. A large number of genes have been transferred from *Aegilops* species to the cultivated wheat genome, including leaf rust resistance, stem rust, yellow rust and powdery mildew and for resistance to various pests (cereal cyst nematode, root knot nematode, Hessian fly, greenbug).

Aegilops biuncialis Vis., an annual, allo-tetraploid species (2n = 4x = 28) with a $U^bU^bM^bM^b$ genome, originated from a cross between *Aegilops umbellulata* (2n=2x=14, UU), as the female parent and from *Aegilops comosa* (2n=2x=14, MM) as the male parent. *Ae. biuncialis* grows in the Mediterranean and Western Asiatic regions, being abundant in generally dry locations (225-800 mm annual rainfall), but also occurring in areas with 1250 mm rainfall (Van Slageren 1994). The great genetic variability of *Ae. biuncialis,* perhaps caused by its adaptation to different conditions and by its diverse geographical origin, may explain its good tolerance to drought (Molnár et al. 2004) and salt stress. Moreover, resistance against barley yellow dwarf luteovirus, leaf rust, yellow rust, brown rust and powdery mildew has also been reported. There are still many untapped gene resources in the *Aegilops* species (for example *Ae. biuncialis*) which could be used as resistance sources for plant breeding. The aim was to produce wheat- *Ae. biuncialis* addition and translocation lines and their analysis using *in situ* hybridization (ISH) in order to transfer agronomically useful traits from *Ae. biuncialis* into wheat.

1. Production and molecular cytogenetic identification of *Triticum aestivum-Ae. biuncialis* disomic addition lines

Ae. biuncialis (MvGB642- from Martonvásár Gene Bank) was crossed as a male parent with the winter wheat line Martonvásári 9 kr1 and F_1 hybrids were produced. These were treated with colchicine and the amphiploid plants obtained were backcrossed with wheat (Logojan and Molnár-Láng 2000). Six different lines with 44 chromosomes were selected. These showed 22 bivalents in metaphase I in meiosis, demonstrating that they were disomic additions of *Ae. biuncialis* in the wheat genome. Fluorescent *in situ* hybridization (FISH) was carried out on mitotic chromosome preparations of these lines using the DNA clones pSc119.2 (labelled with fluorogreen) and pAs1 (labelled with fluorored) to identify the *Ae. biuncialis* chromosomes.

To facilitate the exact identification of the *Ae. biuncialis* chromosomes in these *Triticum aestivum-Ae. biuncialis* disomic additions it was necessary to analyse the FISH pattern of *Ae. biuncialis*, comparing it to the diploid progenitors (*Ae. umbellulata* and *Ae. comosa*). In order to identify the *Ae. biuncialis* chromosomes, FISH was carried out using two DNA clones (pSc119.2, pAs1) on *Ae. biuncialis* and its two diploid progenitor species. Differences in the

hybridization patterns of all chromosomes were observed between the four *Ae. umbellulata* accessions (TA1829, TA1831, TA1835 – from WGRC, Manhattan, KS, USA and MvGB420 – from Martonvásár Gene Bank), the four *Ae. comosa* accessions (TA2101, TA1965, TA1968 and TA1975– from WGRC, Manhattan, KS, USA) and the three *Ae. biuncialis* accessions (MvGB642, MvGB382 and MvGB376 – from Martonvásár Gene Bank) analysed. Most of the diploid *Ae. umbellulata* and *Ae. comosa* chromosomes showed hybridization patterns similar to their standard karyotypes, but some differences were observed between lines of different origin.

In all the *Ae. umbellulata* accessions analysed the pSc119.2 probe mostly hybridized to the telomeric regions on all the chromosomes (on chromosome 7U an additional interstitial site was observed). Chromosomes 5U and 6U always had pAs1 hybridization sites, while on chromosomes 1U and 7U they were present only occasionally.

The hybridization patterns of the *Ae. comosa* accessions were more variable, with pSc119.2 sites on five chromosomes. The pAs1 probe hybridized to all chromosomes except in *Ae. comosa* accession TA1968, which had only six chromosomes with pAs1 sites. Chromosome 4M was the most polymorphic. The hybridization pattern of the two varieties (*Ae. comosa* var. *comosa* TA2101, TA1968 and *Ae. comosa* var. *subventricosa* TA1965, TA1975) was different, thus allowing them to be distinguished.

The hybridization patterns of the *Ae. biuncialis* accessions were also variable, and differences were observed not only between the *Ae. biuncialis* accessions but also between *Ae. biuncialis* and its diploid progenitors.

Five different wheat-*Ae. biuncialis* addition lines were produced from the wheat-*Ae. biuncialis* amphiploids produced earlier in Martonvásár. The 2M, 3M, 7M, 3U and 5U chromosome pairs were identified with FISH using three (pSc119.2, pAs1 and pTa71) repetitive DNA clones in the disomic chromosome additions produced.

Line No. 76.010130 was identified 3U addition on the basis of the telomeric pSc119.2 hybridization sites on both chromosome arms and on the basis of the arm ratio.

Line No. 629.020905 carried an extra satellited chromosome pair, which was identified as 5U disomic addition. On the 5U chromosome there was a strong pTa71 hybridization sign below the satellite on the short arm.

Line No. 80.010130 was identified as a 2M disomic chromosome addition. The arm ratio of the added chromosome in this addition corresponded to the 2M chromosome (which is more submetacentric than the 3M chromosome).

Line No. 77.001102 carried an extra 3M chromosome pair originating from *Ae. biuncialis*. This chromosome resembles the 2M chromosome, but is less acrocentric.

Line No. 63.010130 was identified as a 7M disomic chromosome addition. The 7M chromosome is one of the few nearly metacentric chromosomes of *Ae. biuncialis.*

Line No. 49.001102 had a small submetacentric *Ae. biuncialis* chromosome pair, which belongs to the M genome according to the Genomic *in situ* hybridization (GISH) analysis.

GISH was applied to all the addition lines and the *Ae. biuncialis* chromosomes were differentiated from the wheat chromosomes. The difference in the intensity of labelling confirmed the presence of the *Ae. biuncialis* chromosomes, but no chromosome rearrangements were detected (Schneider et al. 2005).

Some of the morphological features of the wheat-*Ae. biuncialis* disomic addition lines were unique to specific homeologous groups, but all spikes of the Mv9 kr1 -*Ae. biuncialis* disomic addition lines were seen to be bear a greater resemblance to those of Mv9 kr1. All the disomic addition lines had different morphological features and showed reduced seed set.

The spikes of the 3U disomic addition line resemble those of wheat, being small and loose, with scurs. The stems and spikes of the plants are thinner than those observed in the other addition lines, and the leaves are yellowish green. No powdery mildew infection was observed, but *Helminthosporium* was to be found in patches on the plants.

The spikes of the 5U wheat–*Ae. biuncialis* addition line are awnless and have anthocyanin coloration, like those of *Ae. biuncialis*. The plants are moderately tall, with impaired fertility.

The 2M wheat–*Ae. biuncialis* addition line has a compact spike with scurs, which is thicker than that of the 7M addition line. It has the broadest leaves of all the addition lines and an ash green stem. A small amount of powdery mildew and *Helminthosporium* was observed on the plants in the nursery.

The spikes of the 3M addition line are compact, with scurs; the spikes are broader than those of the other addition lines, but taper towards the top. Below each spikelet there tends to be another rudimentary spikelet. The plants were moderately infected with powdery mildew, and *Helminthosporium* was observed on the lower part of the stem, while many of the leaves were infested with cereal leaf beetles. This was the least healthy of all the addition lines.

The 7M addition line had long, loose spikes with scurs towards the tip. The plants were ash green and were the healthiest of all the addition lines, showing no traces of either powdery mildew or *Helminthosporium*.

All the lines grown in the nursery (3U, 5U, 2M, 3M, 7M) were infected with leaf rust, which was rife in the nursery.

2. Detection of intergenomic chromosome rearrangements in irradiated wheat/*Ae. biuncialis* amphiploids by multicolor genomic *in situ* hybridization

The main objective was to produce wheat-*Aegilops biuncialis* translocations with the aim of improving abiotic and biotic stress tolerance of bread wheat. Wheat-*Aegilops biuncialis* amphiploids were produced by crossing winter wheat genotype Mv9 kr1 and *Ae. biuncialis* accessions MvGB470 and MvGB1112 (from Martonvásár Gene Bank). Dry seeds of the third selfed amphiploid generation were irradiated by ^{60}Co γ-ray with 5, 50 and 100 Gy dosage. Unirradiated seeds were used as control. The irradiated and control seeds were germinated, and grown plants were allowed to self-pollinate. Both the mutagenised generation (M_0) and the self-pollinated progenies (M_1) were analysed by multicolor genomic *in situ* hybridization (mcGISH) to identify intergenomic translocations involving wheat and *Ae. biuncialis* U^b and M^b genome chromosomes. Biotinilated and digoxigenilated total genomic DNA of *Ae. comosa* and *Ae. umbellulata* were used as M^b and U^b genome probes, respectively. Unlabelled durum wheat genomic DNA was used as block.

Using differentially labelled probes the U^b- and M^b-genome chromosomes of *Ae. biuncialis* were clearly discriminated as having red and green fluorescence, respectively, while the unlabelled A-, B- and D-genome chromosomes of wheat had brown colour in the wheat-*Ae. biuncialis* amphiploids. According to the colour discrimination it was possible to detect intergenomic chromosome rearrangements between wheat and U^b, wheat and M^b and U^b and M^b chromosomes. The following chromosomal aberrations were found: dicentric chromosomes, terminal translocations, interstitial translocations, centric fusions and fragments. The chromosomal aberrations involving U^b and M^b genome chromosomes were classified as U- and M-type aberrations, respectively.

There was no significant difference between the frequencies of U^b and M^b chromosomes involved in detectable chromosomal changes in the non-irradiated amphiploids. However, the γ-irradiation induced a significant increase in the total U-type aberrations at 5 and 50 Gy doses, while the M-type aberrations reached the level of the U-type aberrations only at the

highest, 100 Gy dose. These significant differences in the ratio of the total U- and M-type aberrations could be attributed to the U-type centric fusions which were significantly more abundant than the M-type centric fusions at all the irradiation doses.

There was no significant difference between the total U- and M-type aberrations in the progenies of untreated and γ-irradiated plants. In the M_1 plants of the 5 and 50 Gy γ-ray treatment the predominant U-type aberration was the centric fusion (77 and 63 % of the total), while terminal (5 and 50 Gy) and interstitial translocations (50 Gy) were also relevant within the aberrations involving the M^b genome. In the progenies of the 100 Gy treated plants, the significant decrease in the total U- and M-type aberrations was attributed mainly to the elimination of the dicentrics, but small decreases in terminal translocations, centric fusions and fragments were also detected.

McGISH evidenced that the U^b genome of *Ae. biuncialis* was involved in more aberrations than M^b genome at 5 and 50 Gy, which suggests that U^b genome chromosomes are more sensitive to the irradiation. It can be hypothesized that differences in their heterochromatic content may account for the distinct radiosensitivity of the U^b and M^b genomes. The higher radiosensitivity of U^b genome chromosomes seems to particularly affect their centromeric and near-centromeric regions as evidenced by the higher number of U^b-wheat than M^b-wheat centric fusions at all irradiation treatments. The imbalance between U- and M-type aberrations induced in M_0 amphiploids is fairly well maintained in the M_1 generation, although some statistical differences have disappeared. This suggests that the mitotic and/or meiotic processes which are responsible for their transmission rates do not discriminate between U- and M-type aberrations but act independently on their genome composition. The fertile offspring of the irradiated amphiploids produced and analyzed here and their selfed progenies represent a very interesting material in wheat back-crossing programs including further selection for *Ae. biuncialis* traits of agronomic value (drought tolerance, disease resistances, etc.). Centric fusions and, to a lesser extent, internal translocations are expected to be predominant among the chromosome rearrangements stabilized in subsequent BC generations, which enable successful introgression of alien traits whose chromosomal location makes it unlikely to be stably incorporated into wheat by homoeologous recombination-based strategies.

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Approach to comparative mapping of structural genes in polyploid wheat and rye

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Unlike many morphological genes that have already been mapped in wheat, structural genes, coding for enzymes or other proteins required for a cell's structure or metabolism, cannot be mapped based on phenotypic variability within mapping populations. Moreover, in most cases several similar copies of structural genes are present in the polyploid wheat genome, which also complicates mapping of these genes. Structural wheat genes can be mapped based on DNA polymorphisms that may occur between the individual genomes and between different cultivars in non-coding parts of genes or in coding sequences (synonymous or non-synonymous nucleotide substitutions). In the present study the genes for one of the key enzymes of the flavonoid and anthocyanin biosynthesis pathway – flavanone-3-hydroxylase (F3H) – were mapped in hexaploid wheat (*Triticum aestivum*, AABBDD), tetraploid wheat (*T. timopheevii*, AAGG), and rye (*Secale cereale*, RR) using wheat deletion lines (Endo and Gill 1996), wheat-rye addition lines (Driscoll and Sears, 1971), introgression lines *T. aestivum* x *T. timopheevii* (Leonova et al. 2002), and also using a large selection of mapping populations available from former mapping studies in wheat (Khlestkina et al. 2002, 2004, Salina et al. 2006, Leonova et al. 2002, Dobrovolskaya et al. data upublished, etc.). Earlier four copies of the *F3H* gene were isolated from *T. aestivum*, two copies - from *T. timopheevii*, and one copy - from *S. cereale* (Khlestkina et al. data unpublished). Gene-specific PCR markers were developed for each of the *F3H* genes cloned. PCR analysis of the *T. aestivum* nulli-tetrasomic lines showed that one gene copy is located on the chromosome 2A, one copy - on the chromosome 2D, and the other two copies locate both on the chromosome 2B (Khlestkina et al. data unpublished). In the present study we applied the *F3H* gene-specific markers for physical and genetic mapping the flavanone 3-hydroxylase genes in wheat and rye. The *F3H* genes were mapped relative to SSR (GWM) markers analysed either in previous studies or in the current study according the methods described by Röder et al. (1998). Touchdown PCR with the *F3H* gene-specific markers was performed as was described earlier for SNP-markers by Somers et al. (2003). Linkage maps were constructed with MAPMAKER 2.0 (Lander et al. 1987).

Results

PCR analysis of the *T. aestivum* deletion lines showed the location of the *F3H* genes in the distal bins of the long arms of the chromosomes 2A, 2B and 2D. We added SSR markers to the bins, where the *F3H* genes were mapped. Both *F3H* sequences on the chromosome 2B appeared in one deletion region. It remained unclear whether these 2 copies belong to the two different loci or appear to be different alleles of the same locus. Further physical mapping performed by PCR analysis of the introgression line *T. aestivum* x *T. timopheevii* confirmed that the two *F3H* gene copies on chromosome 2B belong to two different loci. The proximal locus was designed *F3H-2B1*, the distal one – *F3H-2B2*. The loci on chromosomes 2A and 2D were designed *F3H-2A* and *F3H-2D*, respectively. Using the same introgression line we were able to locate the G-genome *F3H* gene on the chromosome 2G. This locus was designed

F3H-2G. By analysis of a set of wheat-rye addition lines with gene-specific markers for the rye *F3H* gene we determined the location of this gene on chromosome 2RL.

The further suggestion was that SNPs may appear in the same positions where the homoeologous sequence nucleotide polymorphisms were found. That means that the gene-specific markers could be used also as allele-specific markers and therefore could be applied for mapping the target genes in some mapping populations without any additional sequencing, as polymorphism within cultivars would be detected as presence-absence of the amplification product. Suggesting that, we have analysed parents of the twenty five wheat and four rye mapping populations using the *F3H* gene-specific primer pairs. Six polymorphic populations were selected and used for genetic mapping of the flavanone 3-hydroxylase genes. Genetic map positions of the *F3H* genes were in agreement with those determined by the physical mapping. The gene *F3H-2B2* was mapped at the distal end of the chromosome 2BL, closely linked to the marker *Xgwm1027*. The other five *F3H* genes (*F3H-2A, F3H-2B1, F3H-2D, F3H-2G, F3H-2R*) were mapped about 40 cM proximal from this position, and close to the homoelogous SSR loci *Xgwm1067* (2A, 2B) or *Xgwm0877* (2B, 2R). We conclude that we have mapped the homoeologous set of the *F3H* genes in wheat and rye together with one non-homoeologous duplication of the flavanone 3-hydroxylase gene in the B genome. This duplication may have appeared as early as in the diploid ancestor of the genomes B and S.

Gene-specific PCR-markers appeared to be useful for both the physical and genetic mapping of structural genes in polyploid wheat and rye. By using the proposed approach for genetic mapping one can escape preliminary sequencing in parents of the mapping populations. Availability of a large selection of mapping populations turned out to be an important factor facilitating genetic mapping of structural genes.

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Marker assisted selection - a fast track to increase genetic gain in cereals breeding

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Cereals make an important component of daily diet of human population, so that survival mainly depends on the cereal grain production, witch should match the growing human population. Due to remarkable efforts of plant breeders and geneticists cereal production in the past observed a continuous grown. However, conventional cereal breeding is time consuming and very depended on environmental conditions. Breeding a new variety typically takes between six and eight years and even then the release of an improved variety cannot be guaranteed. Hence, breeders are extremely interested in new technologies that could make this procedure more efficient. Molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improving the selection strategies in cereal breeding.

Trait tagging and marker-assisted selection

A large number of cereal studies have used markers as a tool to identify major genes, QTLs, or to introduce new characters in elite germplasm. In wheat, for example, molecular markers have been identified that are associated with around 40 traits of economic importance (Gurta et al., 1999; Landjeva et al., 2007). Similarly in barley, a large number of QTLs and genes for disease resistance, grain quality and physiological traits have been identified (http://www.barleyworld.org/NABGMP/qtlsum.htm).

Knowing the location of these genes/traits and specific alleles offers the possibility to apply marker-assisted selection (MAS) in cereals, because one of the main objectives of plant breeding is the introgression of one or more favourable genes from a donor parent into the background of an elite variety.

Example 1: Application of molecular markers for male fertility restoration in Pampa CMS in breeding of rye

Hybrid rye breeding and seed production require a cytoplasmic male sterility (CMS) system as a hybridisation mechanism. On the other hand, for the complete restoration of pollen fertility, effective, nuclear encoded restorer genes for CMS-inducing cytoplasm are indispensable. Partial restoration of male fertility causes a reduction in the amount of viable pollen, thus encourages infection by the ergot fungus (*Claviceps purpurea*). Ergot infection contaminates rye grains with scerotia containing toxic alkaloids. To reduce or avoid this risk, rye hybrids need effective restorer genes.

In recent times, a new restorer source was found in IRAN IX, an Iranian primitive rye population. This exotic material displays a significantly higher level of restoration than the currently used European lines (Geiger and Miedaner, 1996). However, despite the excellent restoration ability, the material contains many undesirable agronomic characters. In this case the breeding process can only be hastened by applying molecular markers for developing new material, which should combine an excellent pollen restoration with high agronomic performance. Recently, tightly linked markers to the restorer gene have been found and specific PCR-based assays have been developed (Stracke et al., 2003). This marker allow to improving the selection strategies in rye breeding (Figure 1). Recenty, two new hybrids

POLLINO and VISELLO has been breed at Lochow–Petkus by using a new restore gene and application of marker assisted selection (for details see: www.pollenplus.de).

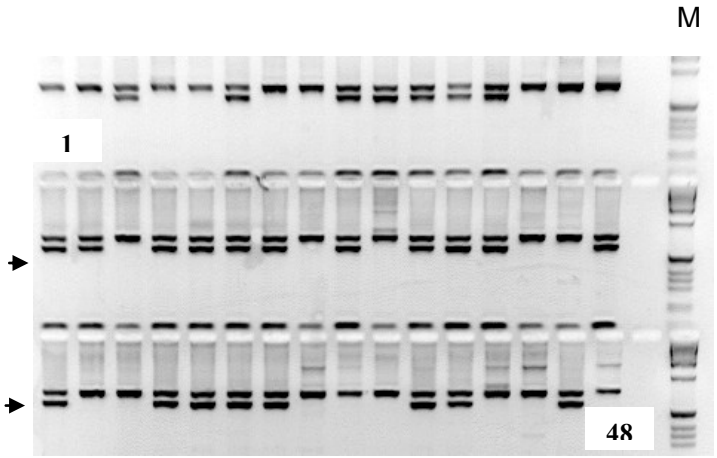

Fig. 1: Application of PCR-based marker SCxx04 for selecting fertile plants. M – 1 kb ladder; lanes 1-48 rye plants, fertile plants detected by two fragments.

Example 2. Application of molecular markers in breeding for resistance to Barley yellow mosaic virus

Barley yellow mosaic virus disease – caused by *Barley mild mosaic virus* (BaMMV) and *Barley yellow mosaic virus* (BaYMV) – has to be considered as one of the most important diseases of winter barley in Europe and East Asia (Ordon et al., 2005). Because of the transmission by the soil-borne fungus *Polymyxa graminis*, chemical treatments to control the disease are neither efficient nor economic. Therefore, breeding for resistance to this disease is of special importance. However, field selection for resistance genotypes is often difficult to perform because of unpredictable environmental conditions. Consequently, the application of closely linked PCR-based markers for the transmission of resistance gene against barley yellow mosaic virus is now successful and efficient (Table 1).

Example 3. Application of molecular markers in breeding for resistance to Fusarium head blight in wheat

Fusarium head blight, also called heat head scab, is a serious disease of wheat (*Triticum aestivum* L.) in humid and semi humid areas of the word (Waldron et al., 1999). In Central Europe, severe natural epidemics of *Fusarium* head blight (FHB) occur once or twice in a decade and can sharply reduce yield and quality of susceptible genotypes. At the same time, deoxynivalenol (DON) contaminated grain is less palatable to livestock and may cause emesis, depressed feed intake and feed refusal in pigs (D'Mello et al., 1999). These mycotoxins are also harmful to humans, because they are highly heat stable and cannot be eliminated totally once they entered the food chain.

Evaluation of Fusarium head blight resistance is time consuming, laborious and costly because the inheritance of resistance is complex and phenotypic expression is significantly affected by environmental factors. Molecular mapping has been successfully used to explain the quantitative trait loci for FHB resistance. The most important QTL was localised on chromosome 3BS and explained 15% of the total variation. Molecular markers closely linked to the major QTL involved in FHB resistance have recently been found (Buerstmayer et al.,

2002, Schmolke et al., 2005) and raise the possibility of using MAS for introducing resistance alleles into elite wheat varieties as have been confirmed also by us (Wilde et al., 2007). However, do to the multifactorial nature of FHB resistance, the combination of MAS on the major QTL during seedling stage with phenotypic selection on the particular plants after flowering stage could be at the moment more sufficient and safety strategy in breeding of a new varieties combining a high level of yield performance and high level of resistance to Fusarium head blight.

Table 1. Application of PCR-based marker *MG3H001* for detecting resistant plants allele. 169 bp corresponds to the susceptible plants, alleles 146 bp and 150 bp to the *ym4* or *ym5* resistant plants, respectively

Nrs	**Plants**	**Allele**	***MG3H1*_146**	***MG3H1*_150**	***MG3H1*_169**	**Resistance**
1	Ge0742_001	*MG3H001*_146	1			*ym4* resistance
2	Ge0742_002	*MG3H001*_169			1	susceptible
3	Ge0742_003	*MG3H001*_146	1			*ym4* resistance
4	Ge0742_004	*MG3H001*_169			1	susceptible
5	Ge0742_005	*MG3H001*_146	1			*ym4* resistance
6	Ge0742_006	*MG3H001*_146	1			*ym4* resistance
7	Ge0742_007	*MG3H001*_169			1	susceptible
8	Ge0742_008	*MG3H001*_169			1	susceptible
9	Ge0742_009	*MG3H001*_150		1		*ym5* resistance
10	Ge0742_010	*MG3H001*_150		1		*ym5* resistance
11	Ge0742_011	*MG3H001*_150		1		*ym5* resistance
12	Ge0742_012	*MG3H001*_169			1	susceptible

Example 4. Application of molecular markers in breeding for malting quality in barley

Apart from yield malting/brewing quality is the most important trait in any spring barley breeding program. Recent development in barley genomics has led to the identification of genes and markers that have been involved in many different malting quality traits and has produced a large quantity of markers that are today routinely used in plant breeding. A prominent example of a successful haplotype analysis of a key enzyme for the malting process in barley is the identification of thermostable forms of ß-amylase and the correlation to haplotypes of the respective gene (Erkkilä et al., 1998; Ma et al., 2001). Two SNP sites were identified that determine four isoforms with different thermostabilities of the ß-amylase enzyme (Paris et al., 2002). Again in this case, as similar to previous examples, the use of molecular marker is accelerate of plant material development in the expected way.

Conclusion

Although some examples of utilization of MAS are available in cereals, but the promise of marker-assisted selection at large scale in crop breeding still remains but achieving practical benefits is taking longer than expected. The main reasons for this delay are the insufficient quality of markers (with respect to their predictive and diagnostic value), inadequate experimental design, high costs and complexity of quantitative traits. Only close interactions between breeders and scientists will accelerate the effective implementation of marker-assisted selection in cereal breeding programmes.

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Mapping genes for earliness in wheat

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Wheat is grown as a crop from the sub-tropics of Africa to the high latitudes in countries such as the UK, and is probably the widest adapted crop in the World. Maximising yield potential in any of the many geographic regions where it is grown requires that the wheat plant maximises the use of resources, for example, water, mineral supply and radiant energy, and avoids stress conditions during growth and grain filling. To do this, the timing of the life cycle is paramount. Appropriate adjustments to the different phases can avoid winter damage from cold temperatures, avoid cool temperatures at flowering which can result in male

sterility, and escape drought and high temperatures in the warmer summer months. The major components of the life cycle are the periods between sowing, germination and emergence; the period after emergence when the crop grows vegetatively before the onset of floral initiation; the length of the period of floral initiation to terminal spikelet; the period between terminal spikelet and heading; and finally, the time of flowering through grain filling to maturity. Although the timing of sowing is farmer determined, following germination, all of the other growth periods are under genetic control since they differ between varieties. Understanding the genetical control of these components of the life cycle will enable plant breeders to fine-tune growth and development to the demands of any particular environment.

Analyses over the last thirty years has shown that nearly all chromosomes of wheat carry genes controlling this character (Snape et al. 2001), and there are, broadly speaking, three groups of genes which control the duration of the life cycle: vernalization (*Vrn*) genes, photoperiod (*Ppd*) genes, and genes that control the developmental rate independent of vernalization and photoperiod, so called 'earliness *per se*', *Eps*, genes. Both *Vrn* genes (Yan et al. 2003) and *Ppd* genes (Turner et al. 2005, Beales et al. 2007) have now been cloned. The next step is to map and understand the locations and modes of actions of *Eps* genes. These latter genes are thought to modify the duration of the different phases once the environmental pattern is set and have certain characteristics i) they do not respond to environmental stimuli (but maybe temperature dependent?), ii) responsible for changes in development and/or phenology?, iii) adjust flowering time by a small number of days, iv) widely dispersed across the genome. As a start to such an analysis, we have carried out a meta-analysis of *Eps* QTL in European germplasm by studying the many different mapping populations that we have developed over the last seven years, grown in different environments, and analysing their flowering time differences.

Materials and methods

Mapping populations and map development: We have developed several recombinant doubled haploid mapping populations based on crosses between adapted key European winter wheat varieties, and also some CIMMYT populations. In total, ten populations are currently being studied in detail – Table 1.

These populations have been mapped using mainly publicly available SSR markers, but recently, we have started to use the Diversity Arrays Technology (DArT) extensively and many of the populations now have DArT markers on their map. Because DarT is able to search for several hundred polymorphisms at once, we find that it can both fill gaps in SSR scaffold maps, link independent linkage groups on the same chromosome together, and extend the ends of linkage maps.

Phenotyping: The populations were grown in randomised, replicated field experiments to evaluate performance in sites with diverse environments, over different years, so that a large body of data is now available for QTL analysis within individual years/sites, but also for meta-QTL analysis across data sets, allowing comparisons of QTL incidence and location across all data. In each environment, flowering time was measured in days from the 1st May in a particular year, when 50% of the plot had ears emerged from the flag leaf.

Table 1: Mapping populations being studied to discover *Eps* genes

Cross	Population size and type	Cross type
Beaver x Soissons*	65 DH lines	W x W
Spark x Rialto	144 DH lines	W x W
Charger x Badger	99 DH lines	W x W
Renesansa* x Savannah	177 DH lines	W x W
Trintella x Piko	158 DH lines	W x W
Rialto x Savannah	132 DH lines	W x W
Avalon x Cadenza	203 DH lines	W x S
Lynx x Cadenza	171 DH lines	W x S
Buster x Charger	128 DH lines	W x W
Weebil* x Bacannora*	106 DH lines	S x S

W = Winter wheat, S = Spring wheat, * = photoperiod insensitive

Results and discussion

QTL for earliness were found to be segregating in all crosses when grown in different environments and over different years (see Table 2). Where QTL were found on the same chromosome in different crosses, usually, they could be mapped, within experimental error, to the same regions of the chromosome, implying that they are probably homologous loci. For example, the QTL on chromosme 3A in the Spark x Rialto, Charger x Badger and Avalon x Cadenza populations mapped into a homologous location around the centromere near to consensus map markers *barc19* and *gwm2*, and could be presumed to be homoalleles. An exception was for chromosome 3B where there may be two QTL segragating, one each in different crosses. These could be cross referenced to the Czech chromosome recombinant lines for the earliness genes on 3B of the Czech alternative wheat variety Česká Přesívka (Kosner and Pankova 2002) – see Figure 1. Many of the QTL found confirmed chromosomal locations found in studies of anueploids and chromosome substituion lines (Snape et al. 2001). In particular, *Eps* QTL had been previously been predicted on homoeologous Group 1, 3, 6 and 7 chromosomes.

It was thought that the *Eps* QTL on chromosme 1D might relate to a predicted *Ppd-D2* locus on this chromosome. However, comparative mapping with barley indicates that the *Eps* locus is much too distal on the long arm of 1D and does not align with the *Ppd-H2* position, but interestingly, alignes with a location for the early flowering mutant locus *eam8* in barley.

Conclusions

Consistent earliness QTL are found segregating in European winter wheat germplasm, particuarly on the Group 3, 6 and 7 chromosomes. This implies that European wheat breeders are modulating flowering time adaptation in their material by using different loci and alleles, hence maintainng polymorphisms for developmental stages. This suggests that there still exists plenty of variation to adapt the crop further to particular environments and regions should climate change bring about changes in particular regions needing longer or shorter durations of the wheat life cycle.

Table 2: QTL meta-analysis: summary of consistent QTL for earliness *per se* found over different crosses, years and environments, 2001-2006 (years in brackets)

Cross	1A	1D	3A	3B	6A	6B	7A
Beaver x Soissons	04, 06	04.05, 06	04, 05			04	
Spark x Rialto	01, 02, 03		01, 02, 03	02, 03		02, 03	01, 02, 03
Charger x Badger			01, 02, 03	02, 03	01, 02, 03	01, 02, 03	01, 02, 03
Trintella x Piko					01, 02, 03	01, 02, 03	
Avalon x Cadenza		06			06		

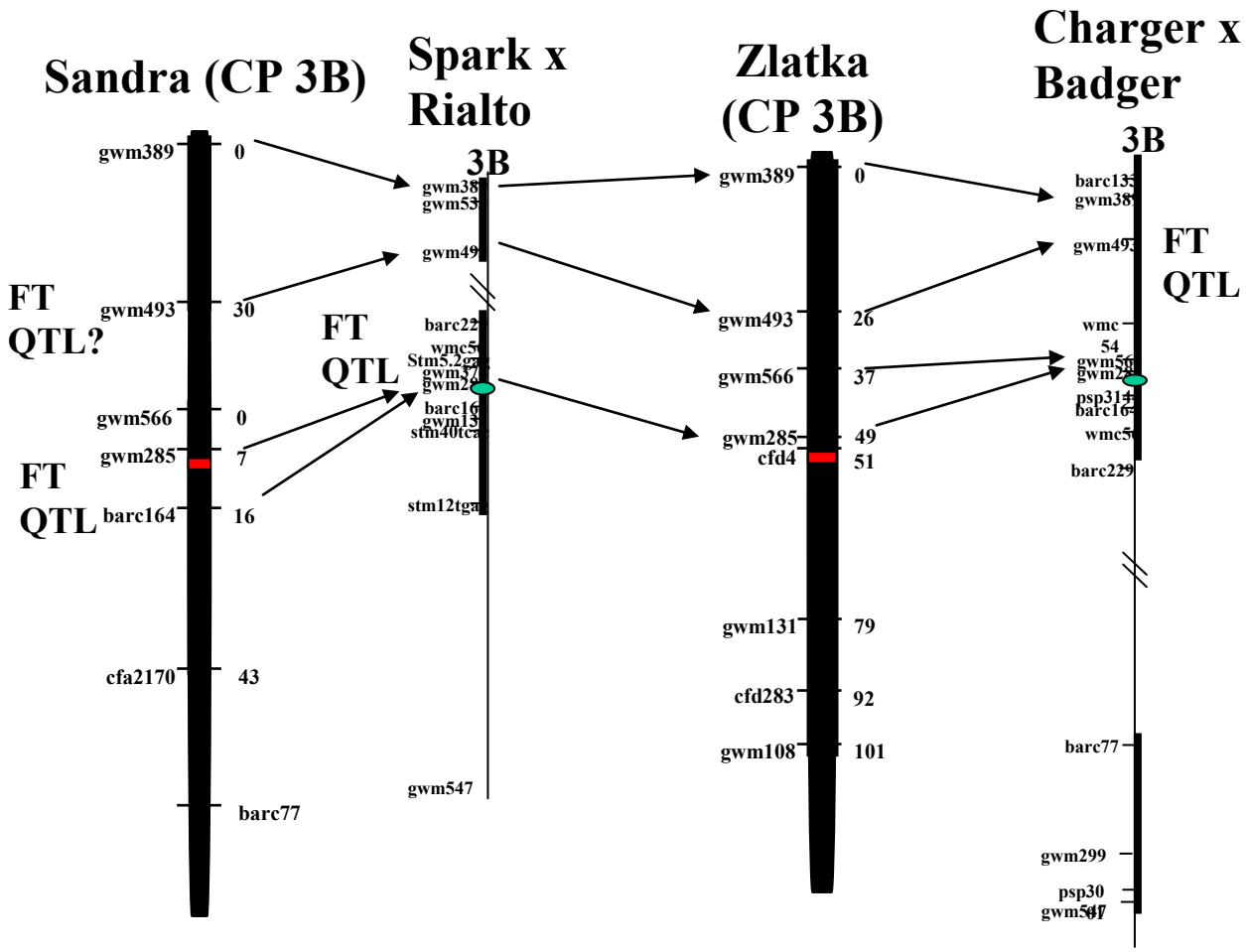

Fig. 1: comparative maps for chromosome 3B for UK and Czech populations segregating for Eps QTL

Acknowledgements

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Flowering time gene(s) on wheat chromosome 3B – characterization and location

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Recent research on wheat flowering time control has brought about important achievements, particularly on the genetics, physiology and molecular biology of the *Vrn* and *Ppd* genes that control vernalization and photoperiod responses, respectively. There is also growing evidence of the importance of *eps* (earliness *per se*) genes that mainly occur as QTL which control flowering time independent of environmental conditions. *Eps* genes appear to be scattered throughout the wheat genome, having been found on chromosome groups 2, 3, 4, 6 and 7. Their influence is supposed to control the number or rate of floral primordia initiation (Slafer, Rawson 1994, Worland 1996, Islam-Faridi et al.1996).

The presence of a flowering time gene(s) on chromosome 3B was indicated by the delayed flowering of wheat plants with substitutions of chromosome 3B from the alternative wheat variety Česká Přesívka (CP3B) into the background of a spring variety Zlatka, and the analysis of this effect revealed a probable effect of *eps* gene(s) (Košner 1987, Košner, Pánková 2001).To describe the phenotype more precisely, substitution lines and their parental varieties were grown under different photoperiod (natural long day /12 hours photoperiod) and vernalization (8 weeks / 0 weeks) regimes. Photoperiod had the strongest impact in all experiment treatments. Vernalization influenced the time to heading and lowered the differences in heading, but remained significant even under long days in the winter wheat materials. The study of this effect, aiming at mapping of these *eps* gene(s), has been continued using a set of CP3B substitution lines of Zlatka, Sandra, Zdar and Vala.

Materials and methods

Materials: Substitution lines of two spring wheat cultivars, Zlatka (CP3B), Sandra (CP3B) and two winter wheat cultivars, Zdar (CP3B) and Vala (CP3B) produced by RICP Prague, were verified using SSR DNA markers, and these, together with the parental cultivars were used in a more detailed study of the effects of the CP3B substitutions.

Dynamics of apical development: Apical development and differentiation were assessed in plants of Zlatka (ČP3B) and its parental cultivar Zlatka. The plants were sown into a shaded field plot on 20 April and grown under a short day (12 hours) regime. Morphological changes of the apices were analysed under the microscope at weekly intervals. Parallel checks of time to heading of the plants were carried out. Both dynamics of apical development and checks of time to heading were assessed in two seasons, 2006 and 2007.

Winter survival: The pot-culture method (Prášil and Rogalewicz 1989) under natural conditions was used to test winter survival (mainly the effect of frost) of the substitution lines Zlatka (CP3B), Sandra (CP3B), Zdar (CP3B) and Vala (CP3B) and their parental cultivars. Plants were grown in wooden boxes (40 × 30 × 15cm) filled with soil and placed on the ground for the whole winter. In spring, plant survival as a percentage score was assessed for 60 plants of each line. Winter survival was tested over two winters – 2006, 2007. As the winter of 2007 was extremely mild, the tested plants were also put through a laboratory frost test: they were subjected to four (decreasing) temperature treatments for 24 hours and percentage survival was calculated.

Mapping populations and molecular fingerprinting of chromosome CP 3B: Mapping populations of single chromosome recombinant lines resulting from backcrosses between Zlatka (CP3B), Sandra (CP3B) and Zlatka, Sandra, respectively, were obtained for mapping chromosome CP3B using SSR markers.

Monosomic recombinant substitution lines of Sandra (CP 3B/Sandra 3B) derived from 30 previous crosses at JIC of Sandra (CP 3B/Sandra 3B) F_1 (male) onto Sandra monosomic 3B (as female) have been grown for phenotypic screening and molecular marker analysis as a preliminary to more detailed experiments to map the CP 3B flowering time gene. SSR molecular fingerprinting of the Zlatka (Zlatka 3B/CP 3B) single chromosome recombinant F_3 lines was also carried out to develop a comprehensive map of chromosome 3B. The data obtained were processed using JoinMap computer software.

Results and discussion

The earlier commencement of the individual stages of spike development, on average by 5 to 7 days, was detected in the variety Zlatka compared to Zlatka (CP 3B) when grown under short days, and it took place throughout the period of reproductive development (Fig. 1). This suggests the effect of the flowering time gene(s) takes place from the very beginning of growth and development of the plant. A more marked effect on flowering time - up to 19 days, had been observed between Vala (CP 3B) and Vala (Pánková et al. 2006). The impact of chromosome CP3B substitutions on heading time had been analysed in more detail to reveal that separate from photoperiod, there was an important influence of interactions between genotype and vernalization (Košner & Pánková 2002).

A better winter survival had been referred to by Košner (1987) in Zlatka (CP3B) compared to Zlatka. However, the test of frost tolerance under natural winter conditions combined with the laboratory frost test in the winters 2006, 2007, respectively, only revealed enhanced survival of the substitution line Vala (CP 3B) compared to the original variety Vala, while the other tested substitution lines (Zlatka (CP 3B), Sandra (CP 3B) and Zdar (CP 3B) were unchanged in their sensitivity to frost (Table 1).

Table 1: Winter survival of wheat lines with CP3B substitutions, including their parental cultivars. Field / laboratory frost test, winter 2006/2007

Line	-8,2	-10,2	-12,2	-14,3	-16,8	% survival	LT_{50}
	% survival					t 1 - t 4	^0C
VALA (ČP3B) 21/04 SL-22	92	85	83	50	0	**77,5**	**-14,1**
VALA (ČP3B) 2/05 SL- 22	92	83	50	0	0	**56,3**	**-12,2**
VALA 1/02	92	73	69	8	0	**60,5**	**-12,5**
VALA 21/04	91	82	54	18	0	**61,3**	**-12,3**
ČESKÁ PŘESÍVKA 04	100	75	71	31	0	**69,3**	**-13,1**
ČESKÁ PŘESÍVKA 24/04	100	64	64	25	0	**63,3**	**-12,4**
ZDAR (ČP3B) 10/06 SL- 7	82	42	42	0	0	**41,5**	**-10,5**
ZDAR (ČP3B) 22/04 SL- 7	55	46	23	16	0	**35,0**	**-9,6**
ZDAR 02	80	58	27	8	0	**43,3**	**-10,6**
ZDAR 11/04	75	54	15	0	0	**36,0**	**-10,1**
ZLATKA (ČP3B) 11/05 SL- 21	50	45	10	0	0	**26,3**	**-9,0**
ZLATKA (ČP3B) 28/04 SL- 21	40	40	27	0	0	**26,8**	**-8,8**
ZLATKA 01	36	18	8	0	0	**15,5**	**-7,8**
ZLATKA 22/04	58	40	9	0	0	**26,8**	**-9,1**
SANDRA (ČP3B) 27/04 SL- 25	38	17	0	0	0	**13,8**	**-7,9**
SANDRA (ČP3B) 11/05 SL- 25	42	40	21	0	0	**25,8**	**-8,7**
SANDRA 1/00	17	8	0	0	0	**6,3**	**-7,3**
SANDRA 22/05	42	25	8	0	0	**18,8**	**-8,2**

SSR molecular fingerprinting of the Sandra (CP 3B/Sandra 3B) and Zlatka (Zlatka 3B/CP 3B) single chromosome recombinant lines was carried out to develop a comprehensive map of the 3B chromosome (Tables 2 and 3) and to carry out preliminary QTL analysis. New genetic maps of chromosome 3B have been developed and compared with other 3B maps available at the JIC based on recombinant doubled haploid populations of UK winter wheats Spark x Rialto and Charger x Badger. The genetic maps were all co-linear and indicated that the genetic maps available for the CP 3B chromosomes should provide sufficient coverage to map the genes for flowering time and other traits. Two markers were common between the Zlatka/CP 3B and Sandra/CP 3B, which also enabled alignment of QTL data. Using preliminary flowering time data on the recombinant lines, a flowering time QTL was provisionally mapped into a genomic region near the centromere. More detailed phenotyping and analysis using additional data will be needed to confirm these results (Snape et al. 2008, in press).

Acknowledgements

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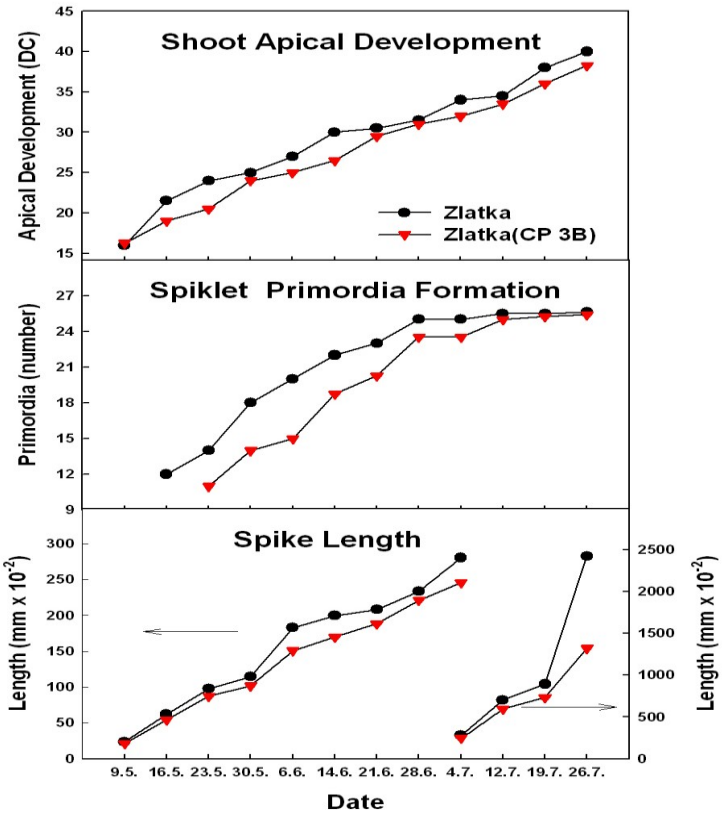

Fig. 1: Shoot apical development of wheat plants under short day conditions (12 hours) was delayed by 5 - 7 days in the substitution line Zlatka (CP3B), compared to the parental variety Zlatka

Table 2: Sandra (CP 3B) RSLs – Map and preliminary QTL analysis:

Single Marker ANOVA: Marker Positions and Trait Means: considered as one linkage group using the consensus map

Marker	Pos.	Add. eff.	mean(11)	n(11)	mean(22)	n(22)	F	P
gwm389	0.0	-0.09	86.11	19	86.3	14	0.0010	0.9756 NS
gwm493	30.0	-0.07	86.51	17	86.66	18	0.0010	0.9769 NS
gwm566	72.0	-1.57	84.88	17	88.02	18	2.7040	0.1096 NS
gwm285	79.0	-1.52	85.15	17	88.19	17	2.4600	0.1266 NS
barc164	88.0	-1.74	84.54	17	88.01	19	3.5060	0.0698 (*)
cfa2170	115.0	-0.7	85.68	18	87.07	18	0.5200	0.4757 NS
barc 77	130.0	-1.89	85.61	24	89.39	10	3.2990	0.0787 NS

QTL Mapping by Marker Regression: QTL located at 94.0 cM

Additive effect = -2.0205677

Source	df	MS	F	P
Add Reg	1	370.3	14.67	0.131
Residual	5	11.36	0.45	0.556
Error		29	25.24	

Table 3: Zlatka (CP 3B) RSLs – Map and preliminary QTL analysis

Single Marker ANOVA: Marker Positions and Trait Means: considering as one linkage group using consensus map

Markers	Pos.	Add.eff.	Dom.eff	mean(11)	n(11)	mean(12)	n(12)	mean(22)	n(22)	F	P	
barc147	0.0	-0.45	0.12	76.9	108	76.33	58	77. 23	92	0.853	0.4275	NS
gwm 493	10.0	-0.14	-0.49	76.84	123	76.49	73	77.12	83	0.315	0.7303	NS
cfd4	59.0	-0.17	-0.16	76.66	125	76.67	66	77.00	93	0.146	0.8643	NS
wmc307	63.0	-0.24	-0.93	76.78	137	76.08	59	77.26	82	0.987	0.3741	NS
barc164	76.0	-0.4	-1.69	76.89	132	75.6	80	77.69	70	3.504	0.0314	*
wmc326	159.0	-0.3	0.29	77.13	101	76.54	67	76.55	114	0.463	0.6297	NS

QTL Mapping by Marker Regression: Result Linkage Group: 1, Trait: 1
QTL located at 94.0 cM
Additive effect = -0.5248453
Dominance = -3.7866082

Source	df	MS	F	P
Add Reg	1	32.23	1.37	0.626
Res Add	4	11.71	0.5	0.576
Dom Reg	1	257.38	10.96	0.051
Res Dom	4	9.13	0.39	0.667
Error	282	23.48		

Simulated QTL position is 75.091 +/- 31.584
Simulated Additive QTL effect is -0.559 +/- 0.758
Simulated Dominance QTL effect is -4.054 +/- 3.823

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Mapping of a gene (*Vir*) for a non-glaucous, viridescent phenotype in bread wheat derived from *Triticum dicoccoides*, and its association with yield variation

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Wheat yields in the UK have significantly increased over the last 30 years. The challenge for wheat breeders is to sustain this trend. The introgression of desirable genes or alleles from the wild relatives of hexaploid wheat can be a valuable source of genetic variation for breeders to enhance modern varieties. The UK Group 1 premium bread making variety Shamrock is an example where the introgression of genetic material from wild emmer (*Triticum dicoccoides*) has been used to develop a modern cultivar, from a cross between a *T. dicoccoides* derivative (Comp Tig 323-1-3M) and adapted UK germplasm (CWW 4899/25 – Moulin x Monopol). Although having a lower yield potential and slightly softer endosperm than equivalent bread-making wheats, Shamrock exhibits some of the beneficial qualities of wild emmer. It is relatively stable and insensitive to changes in environment or agricultural practices, with consistent performance over a range of conditions. Its hardiness and adaptability means that it performs well when grown with low or no fungicide inputs relative to other varieties. A striking character of Shamrock is its unique viridescent colour compared to other UK wheats, a trait that coincides with a non-glaucous phenotype. A doubled haploid population (Shamrock x Shango) segregating for the trait was examined, to map the location of *Vir*, and analyse any associated pleiotropic effects.

Materials and methods

Mapping population and map development

A recombinant doubled haploid population of 102 lines of Shamrock x Shango was developed. Of this 87 lines were genotyped. The framework genetic map was primarily developed using publicly available SSR markers aiming for a marker density of one every 10-20cM. To further improve map density, DNA of the population was sent to Triticarte Pty Ltd, Australia (www.triticarte.com.au/) for Diversity Arrays Technology (DArT) genome profiling which greatly increased marker density by filling gaps and extending linkage groups.

Field trials and trait phenotyping

The population was grown in randomised, replicated field experiments at Church Farm, Norwich, UK, over two seasons, 2004/05 and 2005/06. The lines were sown in large-scale yield plots (1 x 6m) and standard farm pesticide and fertiliser applications were made to reproduce commercial practise. Additional trials were sown in conjunction with Syngenta in 2004/05, and RAGT Seeds in 2003/04 and 2004/05, at sites across East Anglia.

A simple visual assessment was made of the viridescent phenotype, with the population segregating 42 to 45, within statistical probability for a 1:1 ratio expected for a single gene controlling the trait (Fig. 1). Segregation in levels of plant glaucosity was also observed, with the 'Shamrock viridescent' phenotype exhibiting absence or very low levels of waxiness.

A major interest in the viridescence trait is to investigate the pleiotropic effects on performance and productivity. Consequently, growth traits, yield components such as crop

height, plant biomass, spikelet number, spike yield, 1000 grain weight, harvest index and grain number, as well as plot yield, were recorded on the John Innes Centre trials.

Fig. 1: Classification of the Shamrock 'viridescent' phenotype. The bright green, non-glaucous Shamrock plots are easily identifiable from the waxy Shango types.

Results

Genetic linkage map

The genetic map contained 263 loci covering a distance of 1337 cM. With the viridescent trait showing a 1:1 segregation pattern, it was possible to map this as a major gene to a precise chromosomal location. The gene was mapped to the short arm of chromosome 2B, close to microsatellite marker X*gwm614*, Figure 2.

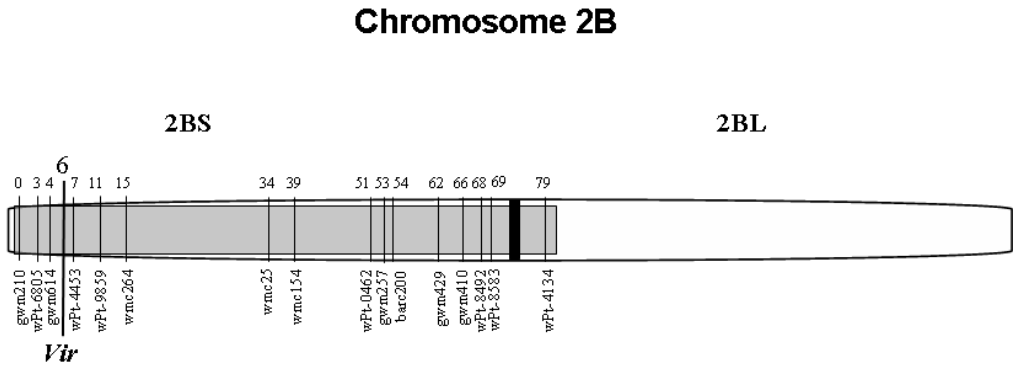

Fig. 2: Genetic linkage map of chromosome 2B for the Shamrock x Shango population (distances are in centimorgans).

Phenotypic / QTL Analysis

Despite the parents showing a similar time duration to full plant senescence, there was transgressive segregation within the population for this trait. The effect of the *Vir* genotype was observed when lines were classified by their respective alleles at the *Vir* locus (Figure 3a). A clear distinction was seen, with lines containing *Vir* showing an increase in days to full senescence, essentially an increased grain filling period. However, when the effects of flowering time were removed in calculating the grain fill period, the effect of *Vir* was more evident. Of the parents, Shango exhibited the longest period of grain filling; however, as a trend, lines with the Shamrock allele at *Vir*, achieved a longer grain fill period (Figure 3b).

A similar pattern was observed for yield. Shamrock has a low yield potential and yielded over 1 t/ha less than Shango (Church Farm 2005). However, when the population was sorted by *Vir* genotype, the higher yielding lines contained the Shamrock allele (Figure 3c).

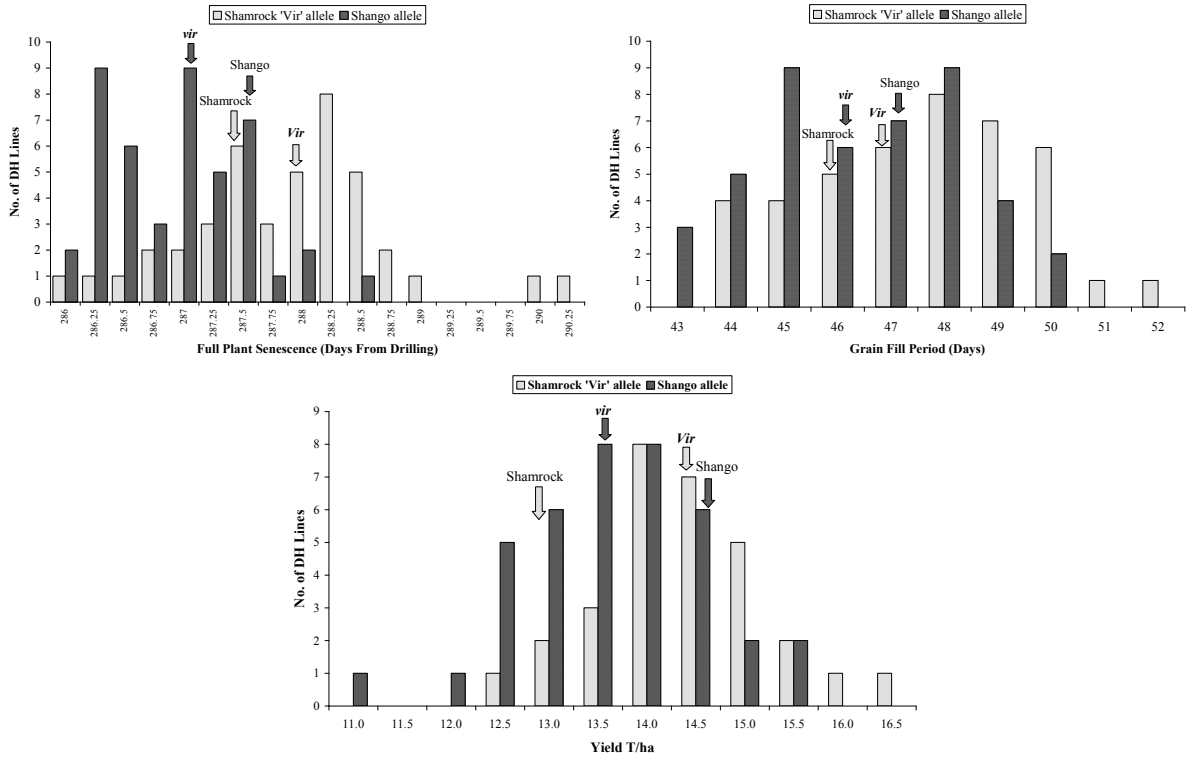

Fig. 3: Trait distributions for the Shamrock x Shango population, classified by their respective alleles at the *Vir* locus for senescence date, Church Farm 2006 (Fig. 3a), grain fill period, Church Farm 2006 (Fig. 3b) and yield at Church Farm 2005 (Fig. 3c). Arrows indicate the means of the parents and the segregating population, classified as having either the viridescent allele (*Vir*) or the Shango allele (*vir*).

Significant yield increases were associated with the *Vir* locus for four of the five locations by single marker ANOVA (Table 1). At its greatest, the effect produced a yield increase of 0.7 T/ha at Church Farm 2005. This effect explained 26% of the variation in yield. Through marker regression , all environments, apart from JIC 2006, produced a significant yield QTL (>0.05) at the *Vir* map location. QTL analysis also highlighted the significant effects on senescence and grain filling period at the same chromosomal location (Table 1).

Table 1: QTL output (Single Marker ANOVA) showing the levels of significance at each marker locus on Chromosome 2B for yield, days to full senescence and grain fill period.

		RAGT		Syngenta	John Innes Centre			
Marker	Pos.	2004	2005	2005	2005	2006	Senescence 2006	GFP 2006
gwm210/1	0	*	***	***	**	*	****	**
wPt-6805	3		***	*	**		****	*
gwm614	4	*	***	**	**		****	*
Vir	**6**	**(0.016)	***(0.001)	**(0.007)	**(0.001)		**** (0.000)	** (0.008)
wPt-4453	7	*	***	**	**		****	**
wPt-9859	11	*	***	**	***		****	*
wmc264	15	*	***	*	****		****	
wmc25	34				**		***	
wmc154	39		*		***		**	

Mapping the T. dicoccoides contribution

To confirm that *T. dicoccoides* was the source of the Shamrock viridescence, DNA samples of CWW4899-25, Shamrock and Shango were genotyped using the microsatellite marker X*gwm614*, which mapped 2cM from *Vir*. A sample of the *T. dicoccoides* parent was unavailable. However, the results confirmed that at this locus, Shamrock contains an alternative allele to CWW4899-25, suggesting that the viridescence was derived from a different source, presumably *T. dicoccoides*.

Discussion

The *Vir* gene was mapped to the distal end of the short arm of chromosome 2B, 2cM proximal to microsatellite marker X*gwm614*. Previous studies (Driscoll 1966) indicated that a dominant gene for wax production, *W1*, is located in this region. The physical properties determining the intense greenness of *Vir* have yet to be measured. It is possible therefore that *Vir* is either closely linked to *W1*, or is the same gene. It should be noted, however, that historically, glaucousness has been found to have a positive relationship with yield, especially under drought stressed conditions, whereas in the present study, the converse was found. Additionally, W1 is located 4cM distal to both Lr16 and Sr23 (http://wheat.pw.usda.gov/GG2/index.shtml), which are in turn located a further 12cM from *Xgwm614*.

QTL analyses confirmed that lines with *Vir* exhibited a significant delay in leaf senescence and a prolonged grain fill period. The ability to stay-green for an extended grain fill period has obvious benefits, as the plant can photosynthesise for longer periods. Significant yield increases associated with the *Vir* allele were consistently observed across years and environments. At its strongest the QTL accounted for a quarter of the variation for yield within the population, amounting to an increase of up to 0.7 t/ha.

To confirm the stay-green and yield QTL on chromosome 2B, a backcrossing program has been initiated whereby the Shamrock viridescence will be transferred into a series of UK recommended wheat varieties. A series of near isogenic lines will be produced and grown in the field to validate the pleiotropic effects of *Vir*.

The yield and stay-green advantages associated with *Vir* emphasise the value of introducing desirable, novel alleles, from wild wheat relatives such as *T. dicoccoides*. This research adds further credence to the utilization of such relatives in breeding programs, as they can provide us with vast amounts of genetic diversity, to be exploited for the continued improvement of cultivated wheat.

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Genetic analysis of osmotic stress tolerance in early stages of plant development in two mapping populations of wheat

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In continental climate areas, winter wheat is frequently subjected to early season drought stress. The vulnerability of germination and the early seedling stage to the drought-provoked osmotic stress affects the subsequent plant development and causes yield reduction (Slavov and Alexandrov 1994). Key traits contributing to drought tolerance at the critical early developmental stages, which can be included in breeding programmes targeting drought tolerance, are deep rooting, which may help plants get deeper water thus avoiding the soil surface water deficit; long coleoptiles which may benefit seedling emergence in cases when deeper sowing is practiced for more efficient use of the scarce soil water reserves; and early seedling vigour to guarantee adequate crop establishment. By applying the QTL-mapping approach (Collard et al. 2005) it is possible to dissect the genetic basis of these quantitative traits and to identify the responsible chromosomal regions. Analysis of plant performance in model systems simulating a specific target environment is of particular value for the evaluation of potential tolerance related traits in large segregating populations. The aim of our study was to perform a QTL analysis of the growth response under PEG-induced osmotic stress at the early seedling stage to identify which chromosome regions are associated with the ability of seedlings to produce longer roots, coleoptiles and shoots under drought stress.

Material and methods

Two segregating populations were tested: 1) 114 lines of the International Triticeae Mapping Initiative (ITMI) population released from a cross between a synthetic wheat 'W7984' (*Ae. tauschii* x 'Altar 84' durum wheat) and the spring cultivar 'Opata 85'; 2) 85 D-genome introgression lines (ILs), developed recently in the wheat 'Chinese Spring' background by backcrossing the D-genome substitution lines 'Chinese Spring' /'Synthetic 6x' (*T. dicoccoides* var. *spontaneovillosum* x *Ae. tauschii*) with the wheat parent and subsequent selfing (Pestsova et al. 2006). The latter represents a population of single chromosome recombinant lines suitable for a high resolution QTL mapping in wheat.

Drought was simulated by 12 % PEG (PEG 6000) treatment. Distilled water was used as control. Eight (ITMI) or 10 (ILs) seeds per line were germinated in covered plastic transparent boxes on two layers of filter paper moistened with PEG solution or distilled water. Germination and seedling growing was performed in a growth chamber at 21±1 ^{o}C in the dark for the first 3 d, and under 12 h light/12 h dark regime for the next 5 d. Measurements of root, coleoptile and shoot length were obtained on 5 (ITMI) or 8 (ILs) seedlings per line on day 8th. Root / shoot length ratio (RSR) was determined. The tolerance index (TI) was calculated for all the traits as a ratio between the mean trait value obtained under stress and the corresponding trait value under control. For each set of lines, three independent replications of the experiments were conducted.

The QGene software programme (Nelson 1997) was used to calculate the QTLs. The detected QTLs were classified as major (LOD > 3.0) and minor (2.0 > LOD > 3.0). The relatively low LOD threshold allowed to differentiate between 'vigour QTLs', detected either in controls, or

under stress but overlapping with QTLs for the same trait in controls, and 'tolerance related QTLs', detected only under stress conditions.

Results and discussion

ITMI population

In total, 35 QTLs affecting seedling growth traits were identified, of which 9 were major ones. The contribution of individual QTLs to the total variation of a trait was ranging from 7 to 17%. The QTL were distributed over 10 chromosomes, mostly on D-genome (16), followed by B (10) and A (9) genome (Table 1). Almost half of QTLs (16) were detected in controls, 17 under osmotic stress conditions and two QTLs were determined for TI. Vigour QTLs were detected on 8 chromosomes (1A, 1B, 2A, 2D, 3D, 5B, 6B and 7D), and tolerance-related QTLs were found on 4 chromosomes (1A, 6D, 7D and 2B). The majority of QTL alleles enhancing root and coleoptile were derived from 'Opata 85', and those with positive effects on shoot characteristics originated from the synthetic parent. All alleles of the recognized tolerance-related QTLs were contributed by 'Opata 85'.

Examples of 'tolerance-related QTLs'

For the trait 'root length', six tolerance-related QTLs were detected on chromosomes 1A, 6D and 7D (Fig. 1a), which altogether explained 63 % of trait variation. The region on 1AL affecting the trait was relatively large, comprising 4 QTLs. The highest LOD score (3.93) in this region was associated with marker *Glu1A,* accounting for 16 % variation. The same region carried QTLs for 'TI-root length'. Influential regions for the traits 'RSR' under PEG-simulated stress, and the 'shoot length-TI' were identified on chromosomes 6D and 2B, respectively (Fig. 1a). In other Triticeae members, QTLs for drought tolerance component traits have been previously localized at rather similar positions on the groups 1 and 7 chromosomes, and at various positions on the groups 2 and 6 chromosomes (Cattivelli et al. 2002). These latter QTLs were identified at a later developmental stage, and may therefore operate via a different set of tolerance mechanisms to explain the tolerance.

Examples of 'vigour QTLs'

Many of the loci controlling the same growth traits under stress and in controls mapped to common chromosome intervals, such as *Xcdo426-Xksue18D* on 1AS, *Xcdo1173-Xbcd12* on 1BL, *Xcdo1379-Xcdo405a* on 2DS, *Xabc164-Xbcd1140* on 5BL and *Xwg341-Xksug30* on 6BL. The big cluster on 2DS, comprising 10 vigour QTLs (Fig. 1b), is within the region that contains major *Ppd* genes controlling plant adaptation to day length (Worland et al. 1997), QTLs for heading and flowering time (Börner et al. 2002) and QTLs for yield components under various stresses (Quarrie et al. 2005). This suggests that the region on 2DS between markers *Xcdo1379* and *Xcdo405a* carry long-term manifested genes governing plant development and adaptation at different stages. The second big cluster of vigour QTLs was found on 5BL in close linkage with marker *Xwg889* (Fig. 1b). The key role of group 5 is widely recognized among Triticeae members, carrying QTLs and major genes controlling plant adaptive traits (heading time, frost resistance). The region on the long arm of group 5 chromosomes proximal to the centromere is also associated with tolerance to drought, salt and cold stress (Cattivelli et al. 2002; Quarrie et al. 2005).

D-genome introgression lines

In response to PEG stress considerable segregation among the ILs was observed only with respect to the trait 'coleoptile length'. The analysis with QGene detected three tolerance related QTLs on chromosome 4D within the marker interval *Xgdm129 – Xgwm3000* (Fig. 1a). The contribution of chromosome 4D to the overall variation of the final coleoptile length has been established earlier by chromosome substitution analysis (Matsui et al. 1998). Large regions of chromosome 4D, comprising almost the whole long arm, were reported to affect the yield component traits in drought and salt stress environments (Quarrie et al. 2005).

Ten vigour QTLs, affecting shoot length and RSR, were detected on chromosome 5D, associated with marker loci *Xgdm99*, *Xgwm1122*, *Xgwm174*, *Xgwm182* and *Xgwm3063* (Fig. 1b).

Understanding the physiological and biochemical basis of tolerance and revealing the association between QTLs for drought tolerance component traits and the stress related genes could help the dissection of plant behaviour under stress. Key role in the drought tolerance is ascribed to the osmotic adjustment and accumulation of ABA. In wheat, a major locus controlling osmotic adjustment was placed on wheat chromosome 7AS (Morgan and Tan 1996) and a major QTL associated with drought-induced ABA accumulation was reported on 5AL (Quarrie et al. 1994). However, in our study we did not map QTLs on chromosomes 7A and 5A.

The association between QTL regions for drought tolerance and stress-related DNA-regions is currently not well characterized. The research findings in this respect suggest that the molecular basis explaining a QTL involves either a gene that directly governs a particular biochemical pathway underlying the target phenotype, or a transcription factor, controlling the expression of many genes. Plant cell response to osmotic stress involves induction of several genes controlling accumulation of osmolytes which play important role in osmotic adjustment and maintaining the cell turgor (Taylor 1996; Chen and Murata 2002), cell membrane repair and induction of antioxidant enzyme system (Bakalova et al. 2004). For example, it has been reported, that genes coding for L-isoaspartyl methyltransferase (an enzyme involved in the repair of damaged proteins) and betaine aldehyde dehydrogenase (enzyme involved in the biosynthesis of glycine betaine, one of the most effective osmoprotectants) are up-regulated in barley and wheat plants, subjected to drought (Mudgett and Clarke 1994; Ishitani et al. 1995). In Triticeae, a number of stress-related genes of known or unknown function have been isolated and characterized (Cattivelli et al. 2002). For example, in barley, a stress responsive gene (*Srg6*) was mapped to chromosome 7H within a region that previously had been associated with osmotic adaptation (Malatrasi et al. 2002).

In conclusion, the large number of detected QTLs suggests that the stress growth response have a complex genetics, which is not surprising as numerous genes are affected by dehydration (Bartels and Souer 2003), that makes it difficult to determine genes and/or chromosome regions of priority in conferring stress tolerance.

Acknowledgements

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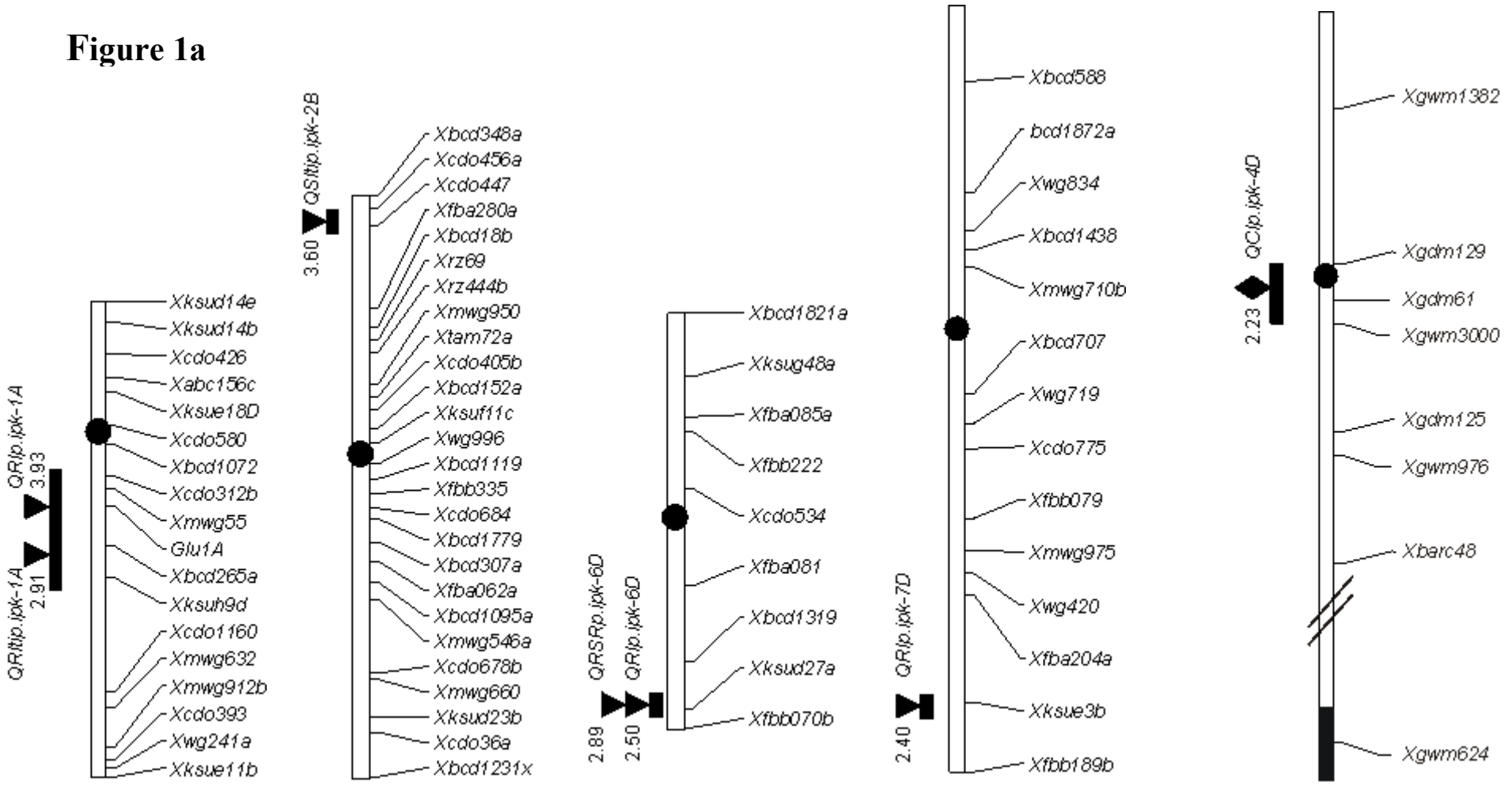
Table 1: A summary of detected QTLs affecting seedling growth traits determined in controls (distilled water) and under osmotic stress (12 % PEG) identified using ITMI mapping population and a set of 85 D-genome introgression lines.

Trait	Treatment	Number	Chromosome	QTL type
ITMI population				
Root length	Control	1	5B	Vigour
	PEG	6	1A, 6D, 7D	Tolerance-related
Coleoptile length	Control	5	1A, 1B, 6B, 7D	Vigour
	PEG	5	1A, 1B, 6B	Vigour
Shoot length	Control	5	2D, 5B	Vigour
	PEG	4	2D, 5B	Vigour
Root-shoot length ratio	Control	5	2A, 2D, 3D	Vigour
	PEG	2	2D 6D	Vigour Tolerance-related
Tolerance Index		2	1A, 2B	Tolerance-related
D-genome introgression lines				
Coleoptile length	PEG	3	4D	Tolerance-related
Shoot length	Control	5	5D	Vigour
Root-shoot length ratio	Control	5	5D	Vigour

ITMI population **D-genome introgression lines**

Figure 1a



ITMI population **D-genome introgression lines**

Figure 1b

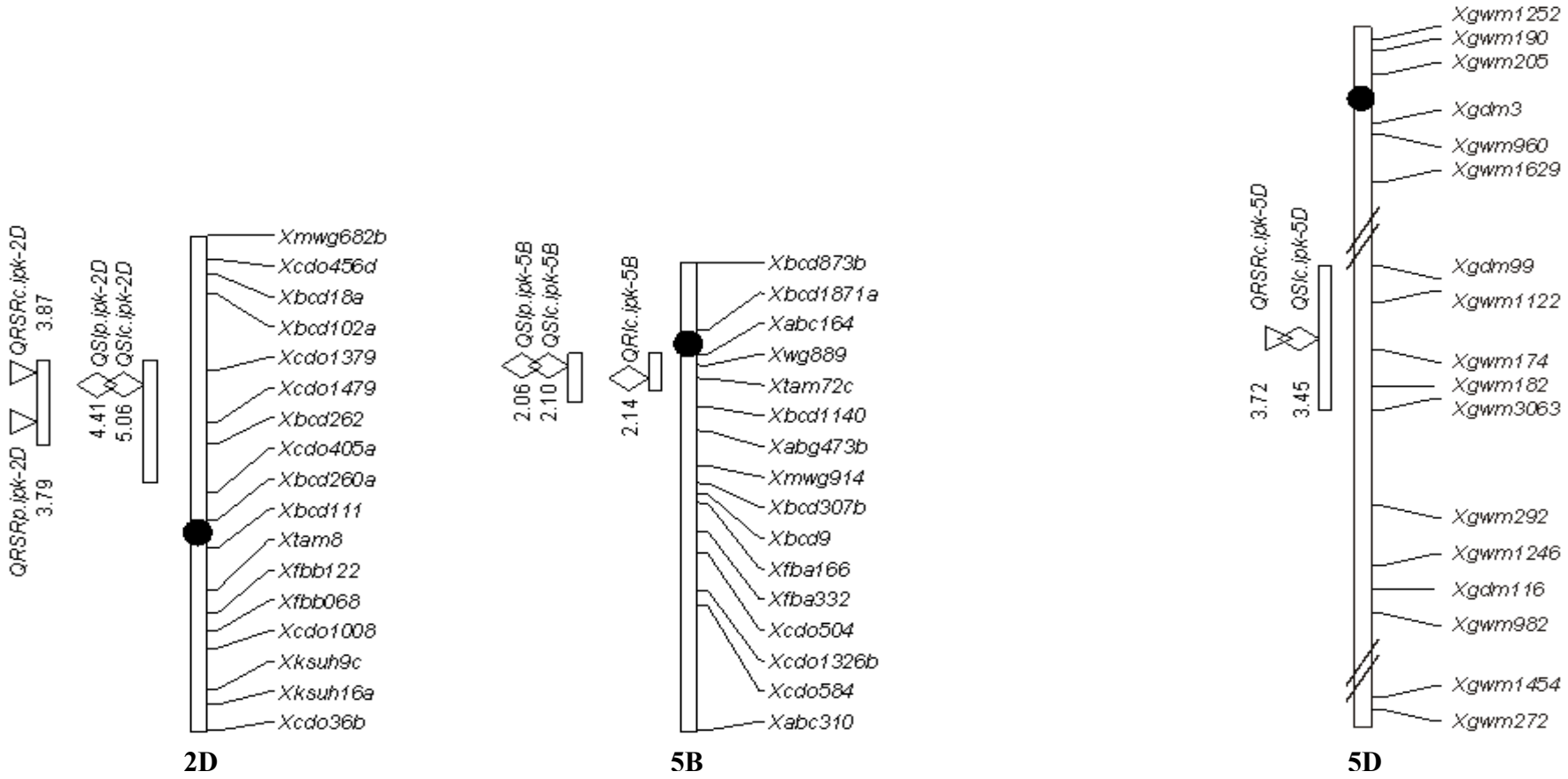


Fig. 1: Most representative examples of QTLs affecting wheat seedling growth traits in controls (distilled water) and under osmotic stress (12 % PEG) identified using ITMI mapping population and a set of 85 D-genome introgression lines. (**a)** 'tolerance-related' QTLs; **(b)** 'vigour' QTLs. The contribution of the wheat mapping parents 'Opata 85' or 'Chinese Spring' is indicated by a *triangle*, and that of 'W7984' or 'Synthetic 6x' by a *diamond*. (*Rl*, root length; *Cl*, coleoptile length; *Sl*, shoot length; *RSR*, root-shoot length ratio; *c*, control; *p*, PEG stress; *tip*, tolerance index under PEG stress

The study of inheritance of pollen grain osmoregulation using aneuploid analysis

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The gene for osmoregulation (expressed in leaves) was reported by Morgan (1991) to be on chromosome 7A. Simultaneous observation of osmoregulation in leaves and pollen grains in some varieties and lines gave rise to a hypothesis that osmoregulation in leaves and pollen grains is controlled by the same gene (Morgan 1999). Assuming the gene *or* for osmoregulation to be on chromosome 7A, pollen grains from plants monosomic for chromosome 7A should segregate for the osmoregulation gene. This is because some pollen grains produced by monosomic plants have 20 (n-1) and some have 21 (n) chromosomes and both groups have equal viability (Khush 1973). The proportion of n and n-1 pollen produced by a monosomic plant depends upon the frequency of lagging and misdivision of the univalent chromosome which differs between hemizygous chromosomes. The aims of present study were to compare some wheat varieties differing in their response to drought for pollen grain osmoregulation under pot conditions and to investigate whether the gene for osmoregulation is located on chromosome 7A using monosomic analysis.

Materials and methods

Experiment 1: Five varieties including Oxley, Falchetto, Falat, Zagros and CS were assessed in this experiment. Monosomic lines are available in Cs and Oxley and varieties Zagros and Falchetto are reported to be drought tolerant and Falat to be drought susceptible. In this study, the relative area (i.e. area of stressed pollen grains/ area of control pollen grains) of pollen grains was used for a preliminary comparison between the varieties. Then osmotic adjustment was calculated using the method of Morgan (1999) for each variety and used for final comparison between the varieties.

Experiment 2: This experiment was conducted with plants monosomic for chromosome 7A of the varieties Oxley and CS during April-June 2001. 10 plants from each monosomic line were grown under the conditions described in Exp 1. Pollen grains from 3 monosomic plants of varieties Oxley and CS were subjected to PEG solutions and the number of shrunken and normal pollen grains was recorded. These results were statistically tested with data obtained from 3 disomic plants of each variety.

Results and discussion

According to the present results, pollen grain osmoregulation occurred in Oxley, CS and Falat while no evidence for this character was observed for Falchetto and Zagros (Table 1). Morgan (1983) studying F_4 breeding lines derived from a cross between the cultivar Songlen and a line called C/3Ag14, reported that osmoregulation in leaves is associated with higher LRWC and higher grain yield under water-stress conditions. Since it is reported that leaf osmoregulation happened in pollen grains as well (Morgan 1999), the varieties Oxley and CS having high pollen grain osmoregulation were expected to produce high LRWC and high tolerance to water-stress. But, the results obtained by Mohammady-d et al (2005) indicated that Oxley, despite producing pollen grains with a positive response to osmotic stress, had low

LRWC and high drought susceptibility index and CS showed only moderate tolerance to water-stress.

Table 1: Mean ±SE of pollen grain areas in 30 and 50% solutions of PEG, area ratios (50/30%) and the amount of osmoregulation ($\Delta\pi$) of pollen grains of 5 cultivars

Variety	Area 30% ($mm^2 \times 10^{-4}$)	Area 50% ($mm^2 \times 10^{-4}$)	Relative area 50/30 (%)	$\Delta\pi$ (Mpa)
Oxley	21.623±1.11[a]	19.840±0.84[a]	0.9228±0.023[a]	1.845±0.06[a]
CS	17.91±0.75[b]	16.88±0.51[b]	0.9482±0.019[a]	1.950±.09[a]
Falchetto	17.12±0.39[b]	11.180±0.31[c]	0.6930±0.025[b]	0.797±0.14[b]
Zagros	17.18±0.35	12.03±0.41[c]	0.7058±0.02[b]	0.811±0.19[b]
Falat	19.34±0.21	18.01±0.23[ab]	0.9324±0.1[a]	1.893±0.12[a]

In each column, means followed by a common letter are not significantly different at 1% level of probability

Assuming the method used by Morgan (1999) for identification of high pollen grain osmoregulation is correct, the most likely reason for the above difference is that in the present study more severe water-stress was applied to the plants in comparison with Morgan's work so that the extending stress period and the excessive loss of water by Oxley caused severe damage by the end of water-stress period. Aggressive consumption of water by high osmoregulation genotypes has been reported by Ludlow and Muchow (1990). They believe that osmotic adjustment promotes root growth and exploration and consequently greater soil water extraction particularly from the lower part of the soil. They also concluded that enhanced root and better soil water extraction could not be expressed in pots. In this situation, therefore, leaf water potential drops quickly and this can cause leaf and plant death if soil water is exhausted (Ludlow and Muchow 1990).

Since osmoregulation reduces osmotic potential in leaf cells, therefore it can change the osmotic balance between the soil and plants in favour of plants and, in this situation, osmotic adjustment enables the plant to continue to acquire water from soil at low soil water potentials (Lambers *et al.* 2000). At the beginning of the water-stress period, when there is enough water in the soil, the water could be absorbed by high osmoregulation plants due to a more negative gradient in these plants. Conversely when the majority of water is absorbed and a severe water shortage happens in the soil because of extending the drought, osmotic potential of the soil solution drops sharply so that osmotic adjustment is not a powerful character to decrease osmotic potential of plants. In this situation, reduction in osmotic potential of plant cells happens due to water loss which consequently decreases LRWC of the plants.

A close study of Morgan's work reveals the accuracy of the above justification. In the experiments carried out by Morgan (1983) the relationship between LRWC and water potential was measured and used for estimating osmoregulation. The genotypes with high osmoregulation were identified if their relative water content showed little reduction with a decrease in water potential and low osmoregulation genotypes were identified if their LRWC decreased significantly with a reduction in water potential. In a study (Morgan 1991) carried out with the variety CS (high osmoregulation) and the variety Red Egyptian (low osmoregulation), he reported LRWC for the high osmoregulation genotype decreased to 0.5 at

a water potential of –4 Mpa. At this level of water potential, Red Egyptian had a higher LRWC. This implies, contrary to the definition of osmoregulation, that genotypes with low osmoregulation are likely to have better drought survival possibly due other adaptation mechanisms such as reduction in leaf area and conductance. In another method, Morgan estimated leaf osmoregulation from a linear relationship which was performed between *ln* osmotic potential (π) and *ln* LRWC at -2.5 Mpa of osmotic potential (Morgan 1991). This amount of osmotic potential happens under moderate water-stress (Morgan 1999). Therefore, as Morgan himself has mentioned, his calculation of osmotic adjustment reflects the response of plants, at most, to moderate water-stress. In one of his studies (Morgan 1991), water-stress was so low that the osmotic potential of only 67 out of 72 genotypes under study went below -2 Mpa, a level which was insufficient to distinguish between high and low genotypes.

Monosomic analysis

The accumulative number of shrunken and normal pollen grains in the three disomic and 3 monosomic plants is presented in Table 2 for varieties Oxley and CS. The segregation ratio of normal, shrunken and aborted pollen grains in monosomic plants was not significantly different from that in disomic plants under both 30% (control) and 50% (stress) PEG solutions for both Oxley and CS. Both monosomic and disomic plants indicated small proportions of shrunken and aborted pollen grains. These abnormal pollen grains seem to be due to environmental and developmental effects but not due to the effect of PEG solutions. Such abnormal pollen grains have been reported during the study of pollen grain viability of wheat varieties under normal conditions (Briggs *et al.* 1999; Saini and Aspinall 1981; Welsh and Klatt 1971).

Table 2: The accumulative number of normal, shrunken and aborted pollen grains of 3 plants monosomic for 7A and 3 dismomic plants of Oxley and CS at 30 and 50% of PEG solutions and χ^2 test for departure from the disomic segregation ratio.

			30%					50%		
Oxley	TN	Nor	Shr	Abo	χ^2	TN	Nor	Shr	Abo	χ^2
Monosomic	460	422	20	18	0.996^{ns}	533	481	19	33	2.59^{ns}
Disomic	495	448	24	23	-	516	470	23	23	-
			30%					50%		
CS	TN	Nor	Shr	Abo	χ^2	TN	Nor	Shr	Abo	χ^2
Monosomic	398	361	18	21	0.083^{ns}	573	468	65	40	1.397^{ns}
Disomic	422	368	19	19	-	479	400	49	30	-

TN: total number
Nor, Shr and Abo: normal, shrunken and aborted, respectively
Ns: not significant

The majority of pollen grains in the 9 F_2 backcross reciprocal plants having chromosome 7A from Oxley were normal at 50% PEG. These plants therefore can be recorded as high osmoregulation (Morgan 1999). However some shrunken pollen grains were also observed in each plant. From the 10 F_2 reciprocal plants having chromosome 7A from Falchetto, 8

segregated for normal and shrunken pollen grains but the majority of pollen grains in the other 2 plants were shrunken.

Assuming the gene for pollen grain osmoregulation to be on chromosome 7A, pollen grains with 21 chromosomes (having gene *or*) are expected to maintain their volumes when they are subjected to high concentrations of PEG.

On the other hand, pollen grains with 20 chromosomes (deficient for gene *or*) are expected to be shrunken because of their inability for osmotic and water loss adjustment. Thus it was expected that the majority of pollen grains from monosomic plants are shrunken (about 70%) and only about 30% of them remain normal. This segregation in monosomic plants did not occur in the present experiment. These results indicate that the reported recessive gene for high osmoregulation is possibly a null allele of a dominant inhibitor causing low osmoregulation because absence of the gene in monosomic plants of CS and Oxley did not show any effect on the number of shrunken pollen grains.

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Detecting loci for abiotic stress tolerance via segregation- and association-based mapping in barley

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Abiotic stress as drought and salinity can strongly reduce agriculture's yield all over the world. Wrong irrigation in dry areas is the reason why twenty percent of the cultivated land is affected by salt. The predicted increase of lower rainfall in big parts of today's cultivation centres makes the search for drought and salt tolerant genotypes urgent. Even though drought and salinity share mechanisms there are also differences (Munns 2005) and a drought tolerant genotype must not be also salt tolerant and vice versa. Conventional breeding methods are unfortunately very time-consuming and the screening for stress tolerance is anyway a very complicated process which needs many replications because of the strong environmental influence.

The quantitative trait analysis (QTL) which became possible after the development of molecular marker techniques raised hope to speed up the breeding process via marker assisted

selection (MAS). But the success for abiotic stress tolerance until now is only very little. A main reason for failing success is the use of only two genotypes as parents of a mapping population. It can only be detected the tolerance loci the parents are segregating for. For finding more relevant loci it is necessary to screen several populations which cost time and effort. From human genetics comes a new approach the so called association mapping (Hirschhorn 2005). Here it is possible to detect more loci at once because a mapping population of several hundred unrelated individuals is genotyped with molecular markers. More advantages are the higher mapping resolution and less research time because there is no need to cross parents and several traits can be investigated in the same population as it shows high genetic diversity (Yu and Buckler 2006). The higher mapping resolution is a benefit of the natural crossovers happened in the population history. In a conventional mapping population just a few meiosis took place and so also markers far away from the QTL (eg. 10 cM) are still associated with it. But in collection of different genotypes the natural recombination has removed the association between a given QTL and any marker not tightly linked to it and therefore the locus can be detected very precisely. A hidden genetic population structure in the genotype collection can result in false positives and must be investigated before the QTL analysis. If subpopulations are present and a marker allele is overrepresented in one of them then this allele will be associated with the trait without a real association. To detect this genetic structure Pritchart et al. (2000) developed statistical models which are implemented in the software s*tructure*.

We investigated salt and drought tolerance at the germination stage in barley with conventional mapping populations and with association mapping to compare the approaches.

Material and methods

Plant material

We investigated the Steptoe x Morex (SM) and the Oregon Wolfe Barley (OWB) populations, which were developed under the US Barley Genome Project and are both doubled haploid. The barley collection (BC) used for association mapping consists of 227 different barley genotypes including cultivated barley (*Hordeum vulgare* L.) and wild barley (*Hordeum spontaneum* Koch). The accessions come from thirty different countries but the main part is from the Near East and North Africa. The genetic map for association mapping is very dense. It contains over 800 molecular markers mapped to all seven barley chromosomes. Most of the markers are DArT markers but there are also SNPs and SSR markers included. With the software *structure* we tested for population structure and found that the collection consists of five subpopulations. The wild material is clearly forming one separate group, a second group is formed by the two-row *H. vulgare* and two subgroups are formed by the six-row material, where the geographical origin is the reason for the division (one group coming from Central Asia, the other from North Africa and the Near East). The last group is very inhomogeneous as two and six-row barleys from different origin are included, so the reason for clustering together is not obvious.

Germination salt tolerance test

We used ten seeds per line and treatment. The treatments were 0% NaCl as a control to check germination per se, 1.5, 2.0 and 2.5% NaCl for the salt treatments. Filter paper was placed in a transparent plastic box and moistened with solution. The boxes were kept for ten days at 20°C and a 12 hours light / 12 hours dark period in a climatic chamber. After a modified scheme from Mano et al. (1996) the seedlings were scored for their salt tolerance (scale from 0 = not germinated to 9 = good developed seedling) in the three NaCl treatments. The score points from all seedlings per line were summed and the QTL analysis was performed with QGene

software (OWB, SM) or with a mixed model including the subgroup membership probability from *structure* (BC).

With the OWB and SM three independent replications were performed, the AM results rely on one experiment so far.

Germination drought tolerance test

The experimental design was the same as in the salt test. Osmotic stress was induced by moistening the filter paper with 15 % polyethylene glycol (PEG 6000). Also here we used as a control Aqua dest. After eight days we measured on five plants per line the root and the shoot lengths of both treatments. With the mean value we calculated the tolerance index (TI = (trait under stress/trait under control)*100)) and performed the QTL analysis with QGene.

This test was only conducted in the OWB and SM.

Results and discussion

Both mapping populations showed a good transgressive segregation for salt and drought tolerance. Morex is more salt and drought tolerant and the same is true for the recessive parent of the OWB. The association mapping BC shows differences between the subgroups (Fig. 1). The *spontaneum*-subgroup is clearly the most sensitive one (nearly no germination under salt, especially for the higher concentrations) and the six-row barleys are more tolerant than the two-rowed.

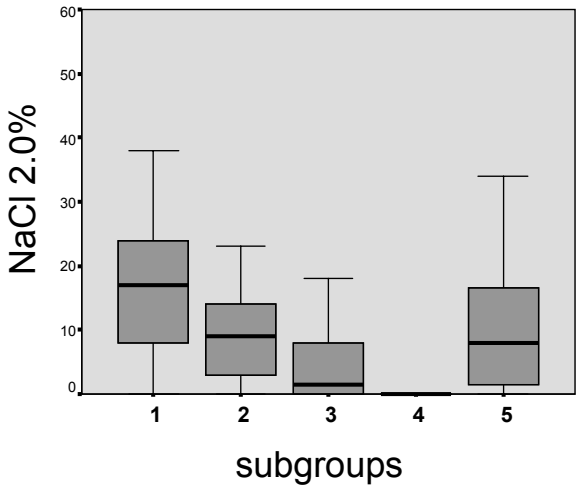

Fig. 1: Boxplots of the subgroups of the BC for treatment with 2.0% NaCl, 1 – subgroup with six-row barleys from Central Asia, 2 – inhomogeneous subgroup, 3 – two-rowed barley (H. *vulgare* L.), 4 – wild barley (H. s*pontaneum* Koch), 5 – six-row barley from North Africa and Near East

QTLs analysis germination salt tolerance

In the SM two loci exist for salt tolerance, one on the long arm of 5H close to the centromere and another one on the end of the short arm of 4H. LOD-value for 5H was over 3 in all three replications while for the 4H-locus a LOD receiving a value over 2 was only detected for 1.5% NaCl. But this locus was also found from Mano and Takeda (1997) in the SM. Also in the OWB is a locus on 5H but the location is on the short arm close to the centromere. Regardless it could be the same locus because the QTL interval is quite large and one cannot

find the exact position. Another locus is on 7H again at the centromeric region. For all replications and NaCl concentrations the LOD-values exceeded 3.

With the association mapping approach more loci could be detected (Tab. 1). Again we find loci on 5H and 7H but also on 1H, 2H and 3H. Only the 4H locus in the SM could not be detected via association mapping. The high resolution power of AM is also shown in the table; the map positions of the loci are very precise. There seem to be genetic differences for the three salt concentrations. Some loci were found for low or high levels and only one locus was detected for all three concentrations. This implements that at different stress levels different mechanisms can be active to deal with the salt.

Table 1: QTL positions in the three NaCl-treatments detected by association mapping

Chromosome	Map position	1.5% NaCl	2.0% NaCl	2.5% NaCl
1H	132.7	X		
2H	116.9 – 117.1		X	X
2H	168.4		X	X
3H	165.6		X	X
3H	211.0	X		
5H	89.75 – 89.88			X
7H	72.1	X	X	X
7H	155.8 – 156.1	X		

QTLs analysis germination drought tolerance

The QTLs found for salt tolerance overlap with QTLs for drought tolerance in both mapping populations. Salt has two effects on plant growth, the osmotic effect and the salt-specific effect. Overlapping QTLs indicating that at this very early stage of development the osmotic effect has the higher influence. For early drought tolerance additional QTLs were found on 1HL (OWB, SM), 5HL (OWB) and 1HS (SM). It is possibly that the different evaluation of salt and drought tolerance (scoring, measuring growth parameters) is a reason for finding more QTLs for drought than for salt tolerance.

We will next screen drought tolerance with the association mapping approach to see if also here all salt loci overlap with the drought loci and if we find also more drought tolerance QTLs than in salt. Also higher developmental stages will be investigated.

Conclusions

QTL mapping for stress tolerance with the new association mapping approach is a more effective way to detected QTLs than with conventional mapping populations. The higher mapping resolution could be confirmed making association mapping perhaps a more powerful tool for MAS.

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Pyramiding of dwarfing genes in the winter bread wheat varieties from the South of Ukraine

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Dwarfing or reduced height (*Rht*) genes have been associated with large increases in the yield potential of cereals and have been a key component of the Green Revolution since they were introduced in the wheat and rice breeding programs 40-50 years ago (Evans 1993).

From the Sixties of the last century, the dwarfing genes have been involved in breeding programs in the South of Ukraine. By extensively use of Bezostaya 1 in breeding programs for the development of semi-dwarf and non-lodging wheat varieties, the *Rht8* gene from Akakomugi was introduced into Ukrainian wheat cultivars. Developed by chemical mutagenesis from Bezostaya 1 the Russian cultivar Krasnodarsky karlik 1 was used as a source of *Rht-B1e* dwarfing gene. At the eighties the different sources of dwarfing genes: Red River 68 (USA), Ciano 79, Lerma Rojo, Veery S5 (CIMMYT), Zlatna Dolina (Bulgaria) were also involved in crosses to the adapted varieties.

The aim of the work was to analyze the distribution of dwarfing genes in the genetic pool of bread wheat varieties in South of Ukraine.

Materials and methods

In the period of 1912-2002 cultivated and in Ukrainian breeding programs used winter wheat varieties were chosen from collections of Plant Breeding and Genetics Institute (Odessa), Mironovskiy Institute of Wheat (Kiev region), the State Commission for Testing and Protection of Plant Varieties of the Ukraine and Genebank of the Institute of Plant Genetics and Crop Plant Research (Gatersleben, Germany). The list of the varieties was presented by Chebotar et al. (2006).

One hundred eleven RILs F_5 from the cross of Odesskaya 16 (*Rht8a*) x Bezostaya 1 (*Rht8c*) were tested with the *Xgwm261* marker and in the field experiment with the aim to identify a number of morpho-physiologycal cracteristics. The frost resistance was estimated in seedlings by Musich and Nagulak (1987) and by Gavrilov (2003). The winter hardiness was estimated by counting plants in October and those that survived during the winter in spring. The statistical parameters of the data were calculated by using standard methods (Rokitsky, 1973).

PCR analysis of *Xgwm261* was performed as described by Korzun et al (1998) and gibberellic acid test did according to Börner et al. (1987). PCR with allele-specific primers to *Rht-B1a, Rht-B1b* and to *Rht-D1a, Rht-D1b* were developed by Ellis et al. (2002).

Results and discussion

We have analysed the changes in allele distribution at locus *Xgwm261* within the set of wheat varieties used in Ukrainian breeding programs during 1912-2002 (Fig. 1). Earlier it was demonstrated that microsatellite *Xgwm261* was linked (0.6 cM) to the gibberellin sensitive dwarfing gene *Rht8* (2DS) (Korzun et al., 1998). The 192 bp allele at locus *Xgwm261* correlated with *Rht8c,* that decreased plant height (by 7-8 cm), and it had no pleiotropic effects on other agronomic traits. The 174 bp allele (*Rht8b)* was neutral with respect to plant height. The 164 (165) bp allele (*Rht8a)* correlated with an increase in plant height by 3-4 cm (Worland *et al.* 1998; 2001).

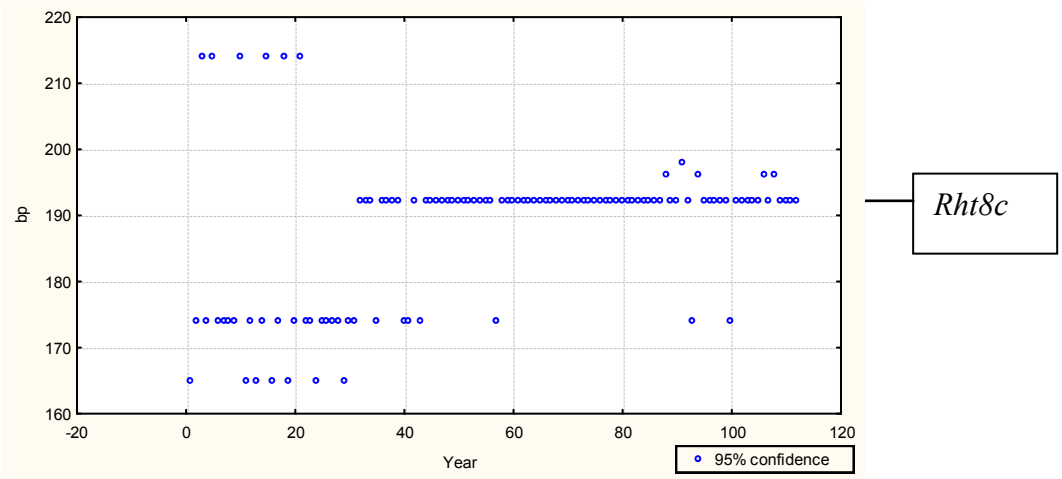

Fig.1: Scatterplot of allele distribution at locus *Xgwm261* among Ukrainian wheat varieties during the period of 1912-2002

Microsatellite analysis with *Xgwm261* marker demonstrates that *Rht8c* gene is present in 98% of modern wheat varieties from Plant Breeding and Genetics Institute (PBGI, South Region).

At the same time the test for sensitivity to gibberellic acid has shown that almost all modern varieties of PBGI are insensitive to GA_3. Allele-specific PCR with primers to *Rht-D1* (4D) and *Rht-B1* (4B) have shown that *Rht-D1b* allele is present in 82% of modern bread wheat of this Institute. We have not revealed *Rht-B1b* with a high frequency in the tested genetic pool.

According to analysis of biochemical test data and the pedigree the *Rht-B1e* gene has to be also present in the genotypes of modern wheat varieties of PBGI.

Contrastingly the varieties from the Mironovsky Institute of Bread Wheat (Kiev Region) have not carried gibberellin insensitive genes and only in 50% of tested genotypes we have detected *Rht8c.*

In order to answer the question, what advantages wheat genotypes with *Rht8c* may have, if this gene has high frequency in the South step region, we analyzed 111 RILs F_5 from the cross of Odesskaya 16 (*Rht8a*) x Bezostaya 1 (*Rht8c*). In the two years field experiment we did not find analogous confirmation of mind that the prevalence of the 192 bp allele of locus *Xgwm261*, tightly linked to the *Ppd-D1* gene determining photoperiod insensitivity (Worland et al., 1998), in Bulgarian wheat germplasm is probably a result of the selection for earliness, a trait of major importance to escape spring and summer drought (see Table 1). Summer period is mostly characterized by high positive temperatures and moisture deficit (total spring-summer precipitation about 100 mm).

Table 1: Characteristics for recombinant inbreed lines different for *Rht8c* and *Rht8a* alleles

Characteristics	2004		$LSD_{0,05}$	2005		$LSD_{0,05}$	Average		$LSD_{0,05}$
	Rht8a	*Rht8c*		*Rht8a*	*Rht8c*		*Rht8a*	*Rht8c*	
Plant height (cm)	129	121	3,0	116	111	1,2	122,5	116	1,8
Period to heading from 1st of May, days	23,0	22,0	0,65	22,7	26,1	-	22,9	24,1	-
Grain weight per ear, g	0.55	0.59	-	0.97	0.97	-	0.76	0.78	-
Yield, kg/m2	0.33	0.40	0.02	0.54	0.55	-	0.43	0.47	-

At the same time carring different *Rht8* alleles (*Rht8a, Rht8b, Rht8c*) three groups of wheat varieties have demonstrated significant differences for analyzed characteristics (Fayt et al., 2007; Table 2).

Table 2: Average characteristics of wheat varieties with different alleles of *Rht8* gene

Characteristics	*Rht8a*	*Rht8b*	*Rht8c*	$LSD_{0,05}$
Plant height (cm)	118	110	86	4
Period to heading from 1st of May, days	25,0	20,0	17,0	1
Grain weight per ear, g 2002/2003	0,59	0,66	0,65	-
Grain weight per ear, g 2001/2002 and 2004/2005	0,68	0,84	1,17	0,08
Yield, kg/m2 2002/2003	0,17	0,14	0,11	0,02
Yield, kg/m2 2001/2002 and 2004/2005	0,31	0,44	0,53	0,04
Average yield, kg/m2	0,26	0,34	0,38	0,01

The South of Ukraine is the region with risky agriculture due to in snowless winters, temperatures can vary from above 0 to -15^0C, and often to -22^0C within a short period, and plants are damaged by frost. According to Lyphenko et al. (1980) and Litvinenko, (1998) the introduction of dwarfing genes into Ukrainian wheat varieties have decreased their winter hardiness and frost resistance.

Three groups of wheat varieties with different alleles of *Rht8* gene revealed some differences in their levels of frost resistance (Table 3).

Table 3: Average frost resistance of wheat varieties with different alleles of *Rht8* gene

Characteristics	Wheat varieties with allele			$LSD_{0,05}$
	Rht8a	*Rht8b*	*Rht8c*	
Frost resistance, % at the beginning of winter	93	88	81	4
Frost resistance, % in the middle of winter	90	88	85	-
Frost resistance, % at the end of the winter	81	77	66	6
Test for frost resistance of seedlings	68	53	45	7
Winter hardiness 2002/2003	64	55	43	7

At the same time we did not reveal significant differences in frost resistance between two groups of recombinant inbreed lines with alleles *Rht8a* and *Rht8c* (Table 4).

Table 4: Frost resistance of recombinant inbreed lines different for *Rht8c* and *Rht8a* genes

Characteristics	2004		$LSD_{0,05}$	2005		$LSD_{0,05}$	Average	
	Rht8a	*Rht8c*		*Rht8a*	*Rht8c*		*Rht8a*	*Rht8c*
Frost resistance, % at the beginning of winter	91,6	94,8	0,13	97,1	96,3	-	94,4	95,0
Frost resistance, % in the middle of winter	-	-	-	86,2	89,7	-	-	-
Frost resistance, % at the end of the winter	96,6	96,1	-	83,65	84,8	-	90,1	90,5
Test for frost resistance of seedlings	28,9	27,3	-	82,5	78,0	-	55,7	52,7

Conclusion

We have detected changes in allele distribution at locus *Xgwm261* within the sets of Ukrainian wheat varieties and wheat varieties that were used in Ukrainian breeding programs during the period of 1912-2002. Pyramiding of the dwarfing genes *Rht8c* + *Rht-B1b*, *Rht8c* + *Rht-D1b*, *Rht8c* + *Rht-B1b*+ *Rht-D1b*, *Rht8c* + *Rht-B1e* took place in the South Ukrainian wheat varieties. *Rht8c* does not show effect on the frost resistance. "Perfect markers" are only fit to identify *Rht-B1b* and *Rht-D1b*, but when "perfect markers" are used for *Rht-B1e*, missed classification of its carriers is possible as tall wheat. Additional markers are necessary for detection of *Rht-B1e* and *Rht-B1d* presence.

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Allelic variation at the dwarfing gene *Rht8* locus in Serbian wheat varieties and advanced lines

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In the vast majority of modern wheat varieties height reduction is achieved by the incorporation of one or sometimes both of a pair of semi dwarfing GA-insensitive genes *Rht-B1b* and *Rht-D1b* (Worland *et al*., 2001). Both originate from the old Japanese variety Norin 10 and do not appear to promote the same agronomic benefits of associating a reduction in height with increases in fertility and yield under southern European environment as they do in a majority of wheat growing areas worlwide (Worland et al., 1990; Worland et al., 1998a).

Breeders from Mediterranean and similar climatic regions had therefore to seek alternative genes to improve plant performance. Pedigree analysis of semi-dwarf wheats from this region shows that dwarfing effects can be traced via Italian varieties (developed by Italian breeders like Strampelli and Orlandi in early 1930s) to old Japanese variety Akakomughi. *Rht8* acts as a weak allele which reduces plant height by replacing the normal strong height promoting allele with a weaker one, leading to wheat height reduction by approximately 7-8cm in England and former Yugoslavia, with no significant pleiotropic effect on other agronomic characters except for a slight increase in spikelet fertility (Worland et al., 1998a; Korzun et al., 1998).

For many decades, study of the presence and genetic effects of Rht8 was hampered by difficulties to recognise it in genotypes. In 1998, a tight linkage was found between the wheat microsatellite marker *WMS 261* (marker *Xgwm261*) 0.6 cM distal to dwarfing gene *Rht8* on the short arm of chromosome 2D (Korzun et al, 1998). Researches conducted over last 9 years revealed the presence of 16 allelic variants at the *Xgwm 261* locus with 165, 174, 180, 192, 194, 196, 197, 198, 200, 201, 202, 204, 205, 207, 210 and 215 base pairs. Nearly 90% of the wheat genotypes (varieties) of worldwide origin carried one of the alleles with either 165bp, 174bp or 192bp (Korzun et al. 1998; Worland et al. 2001; Ahmad and Sorrells, 2002; Schmidt et al, 2004). Until recently, varietal screening failed to detect cultivars with *Rht8* outside the southern European and the former SSSR breeding programs. But recent molecular evaluation of Chinese, Japanese and U.S wheats has revealed the presence of Rht8 gene in 64% of Chinese, 68% of Japanese and 8% of U.S. accessions (Worland et al. 2001; Bai et al. 2004).

Materials and methods

A diverse array of 269 wheat varieties and advanced lines listed in Table 1 were chosen.

Table 1: The presence of allelic variants at *Rht8* dwarfing gene locus in varieties and advanced lines from Serbia

192 bp (Rht 8)			**174 bp**	**165 bp**
Alfa	NS 0.32	NS 69/93	Balkan	Delta
Anastasija	NS 0.58	NS 69/96	Dragana	Dugoklasa
Arija	NS 0.683	NS 69/97	Evropa	Kraljevica
Astra	NS 0.694	NS 69/98	Evropa 90	L-1/91
Bajka	NS 0.733	NS 7/93	Francuska	L-154/89
Bečejka	NS 1/92	NS 71/92	Italija	L-183/90
Beogradanka	NS 1/93	NS 73/97	Kratka	Nevesinjka
Biserka	NS 1/98	NS 732	Novosadska 100	NS 10/94
Bujna	NS 112/92	NS 736	Novosadska 6001	NS 119/95
Balada	NS 114/98	NS 75/01	Novosadska 6389	NS 32/99
Cipovka	NS 116/95	NS 77/95	NS 109/96	NS 39/93
Danica	NS 118/01	NS 79/00	NS 156/98	NS 39/97
Dejana	NS 12/77	NS 79/90	NS 158/01	NS 54-52
Dična	NS 124/95	NS 82/00	NS 18/00 alb	NS 56/90
Diva	NS 125/98	NS 90/96	NS 20/00	NS 74/95
Dina	NS 13/93	NS 900	NS 34/91	NS 76/95
Draga	NS 133/96	NS 92/97	NS 36/91	NS 83/92
Duga	NS 135/90	NS 96/97	NS 36/94	NSJP 471
Fortuna	NS 14-33	NS 974/1	NS 36/98	Venera
Helena	NS 152/98	NS2-2874/2	NS 37/90	
Indija 89	NS 152/01	NS2-3218F	NS 40/96	**~ 200 bp**
Ivanka	NS 163/98	NS2-3827/1	NS 42/96	
Jarebica	NS 17/93	NS2-4523/3	NS 44/95	NS 20/96
Jarka	NS 171/96	NS2-4558	NS 46/98	NS 23/94
Jugoslavija	NS 173/98	NS3-2503	NS 56/97	NS 260/02
KG 100	NS 18/93	NS3-2062/1	NS 625	Jefimija
KG 56	NS 197/98	NSP 16	NS 66/92	Kantata
Kolubara	NS 22/92	NSP 187	NS 67/01	L-152/89
Kompas	NS 22/93	NSP 192	NS 68/01	NS 602
Košava	NS 25/93	NSP 52	NS 76/01	NS 85/97
Kosovka	NS 261/02	NSP 54	NS 8/95	NS2-4629/1
Košuta	NS 29/94	NSR 2	NS 81/01	NSP 40
Kraljevica	NS 3/00	NSR 5	NS 9/93	Sonata
Kremna	NS 3/90	Panonija	NS 90/92	Maksima
L-165/94	NS 30/00	Partizanka	NS 97/95	
L-1A/91	NS 30/95	Partizanka niska	NSP 199	
L-63/89	NS 33/90	PKB Krupna	NSP 88	
L-64/89	NS 35/00	Pobeda	Oda	
L-69/92	NS 38/00	Prima	Pesma	
L-74/92	NS 38/93	Proteinka	Sofija	
Lasta	NS 322	Prva		
Lepenica	NS 4/93	Raduša		
Lira	NS 40/00	Rana niska		
Ljiljana	NS 40/94	Rapsodija		
Majevica	NS 42/00	Renesansa		
Matica	NS 45/00	Rodna		
Milena	NS 46/90	Rusija		
Milica	NS 46/96	Sara		
Mina	NS 46/98	Sava		
Nizija	NS 48/93	Simfonija		
Novosadska Crvena	NS 5/92	Simonida		
Novosadska 5804	NS 51-11	Slavija		
Novosadska 6002	NS 54/01	Sloga		
Novosadska 6238	NS 55-25	Sonja		
Novosadska 6439	NS 55-30	Sreća		
Novosadska 6864	NS 559	Sremica		
Nova Jadranka	NS 56/91	Sremka		
NS 0.1079	NS 56-11	Stamena		
NS 0.1080	NS 57/00	Stepa		
NS 0.1081	NS 57/92	Sutjeska		
NS 0.1082	NS 58-97	Takovčanka		
NS 0.1084	NS 59/91	Tera		
NS 0.1085	NS 60/01	Tiha		
NS 0.1202	NS 603	Viktorija		
	NS 63-24	Vila		
	NS 64/91	Zlatka		
	NS 68/97	Zvezda		

The seed stocks were obtained from Institute of Field and Vegetable Crops germplasm collection. The method used for determining the allelic variant at the *Xgwm261* microsatellite locus are fully described by Korzun et al. (1998).

Results and discussion

Microsatellite analysis of 269 domestic wheat cultivars has revealed that 198 genotypes carry 192bp allelic variant at the *Xgwm261* locus, followed by 40 genotypes with 174bp, 19 with 165 fragment and 12 with fragments of different sizes which could not be joined to either one of the three most frequent alleles (Table 1).

The previously determined alleles (Worland *et al*., 1998a, 1998b; Ahmad and Sorrells, 2002; Pestsova and Röder, 2002) have been confirmed for the following subset of genotypes from this research: 192bp fragment - Biserka, Duga, Dugoklasa, Jarka, Jugoslavija, Kolubara, Košava, NSR 2, Partizanka, Partizanka niska, Raduša, Rana niska, Sava and Sremica; 174bp fragment – Balkan ; 165bp fragment - Dugoklasa.

The presence of the 192bp allele as diagnostic of *Rht8* has been confirmed to be quite common in the wheat genotypes from Serbia (198 genotypes), while the presence of 174bp, 165bp and fragments around 200bp have also been detected but at a much lower rate compared to 192bp allele (Table 1). From these results it can be concluded that breeders from Serbia are looking for certain allelic variability at *Rht8* locus avoiding «adaptive uniformity» of the elite germplasm. This is understandable, since in countries like Serbia, which are geographically and/or climatically diverse, a range of environmental effects are present in light of global climate change, so wheat breeders have to maintain broader germplasm variability in order to make their varieties better adapted to such environment.

Since the 192bp allele corresponds to a height-reducing phenotype of *Rht8* and 174bp allele correlates with a neutral phenotype (Worland *et al*., 1998b), it is clear, at least for the 40 genotypes from this group, that wheat breeders from Serbia tend to increase plant height. The preference for taller plants may be caused by drier growing conditions, a shorter growing season or other environmental factors as stated by Ahmad and Sorrells (2002). Additional decrease in plant height by selecting for the 192bp fragment (and, if the linkage is not broken, for *Ppd-D1* gene) together with *Rht-B1b, Rht-D1b* or other dwarfing genes could become a disadvantage since it may produce a phenotype too short and too early to achieve adequate yield in Serbia wheat growing areas.

Out of the 269 examined wheat genotypes, 19 carry the 165bp allelic variant at *Xgwm261* locus. Majority of the genotypes from this group are either spring or winter wheats of which at least one parent was from Mexico, Australia or China (Table 1). It is well known that excelent results have been obtained in wheat breeding programmes worlwide by incorporation of «spring» alleles into «winter» wheat background resulting in increased yield potential. In this respect, the winter genotypes from this group deserves additional atention from Serbian wheat breeders.

The molecular screening has shown the presence of certain selection presure for specific fragments, but also suggesting there is a recent trend in wheat breeding programmes in Serbia of introduction of novel *Xgwm261* locus alleles (beside 3 main ones) into elite germplasm and varieties. Recently registered varieties from Novi Sad have either the 192bp fragment (Cipovka, Simfonija, Balada, Arija, Rapsodija, Helena, Diva, Vila, Astra), 174bp fragment (Sofija, Dragana, Italija, Francuska, Oda) or fragments around 200bp (Sonata, Kantata, Jefimija). In addition, two very promising advanced lines (NS 260/02 and NS 2-4629/1) and some excellent advanced lines frequently used as parents in hybridization (NS 20/96, NS 23/94, NS 85/97 and L152/89) also carry allele around 200bp in size. From these data it can be concluded that phenotypic selection for improved varieties in Serbia recently implies

promotion of novel fragments at *Xgwm261* locus. The reasons for this are not yet clear, but we presume the research which is on going now will revealed the proper answers.

Finally, in the majority of the papers published in last 30 years in general and 9 years in particular, it was suggested that besides plant height *Rht8* gene is effecting no other agronomic trait except slight increase in spikelet fertility (Korzun et al. 1998), so we can presume the positive effect on many agronomicaly important traits in Serbian wheat varieties and advanced lines is actualy obtained by *Ppd-D1* gene. Since, *Ppd-D1* gene is reducing wheat height as a direct pleiotropic effect of earlier flowering (shorter life cycle) it seems that different agronomic performance of the germplasm with different allelic variants at *Rht8* locus in this research could be rather joint effect of *Ppd-D1* and *Rht8* than the effect of *Rht8* gene alone. Previous findings presumed that in majority of the Serbian and Southern European wheats the linkage between *Rht8* and *Ppd-D1* has been preserved but findings of novel fragments from this research are very strong indication that the linkage has been broken.

Unfortunately, in this moment, we are unable to evaluate on molecular basis 2DS wheat chromosome segment which carry either *Rht8* and/or *Ppd-D1* gene, and than look for possible association of particular alleles to phenotypic performance. But in regard to present interest of wheat scientiests on *Ppd* genes issues it is very likely that in very near future we will be able to disect the particular segment harbouring both *Rht8* and *Ppd-D1* gene and to gain additional knowledge in this respect.

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Identification of GA-insensitive dwarfing genes in barley (*Hordeum vulgare* L.) cultivars grown in Poland

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Introduction

In Poland barley sown area in 2006 reached 1 220 thousands hectares, what amounts to 14.56% of total cereals sown area. In comparison with 2005, it was increase by 9.7%. Harvests of barley achieved 3 161 thousands tons and mean yield 28.55 dt per ha. The most important role play cultivars with spring growth habit. In 2005, harvests of these cultivars gained 2 663 thousands tons, while harvests of winter cultivars gained 498 thousands tons. Spring barley cultivars are the components of cereal mixes, which significance in Poland is important, because their sown area reached 1 544 thousands hectares. Not big importance of winter barley cultivars is the result of less resistance to frost, hence distribution of these cultivars is not steady and is concentrated in west and south regions of Poland, mainly.

Barley is a cereal with broad utilization. In Poland barley seed is mainly used for feed production. For feeding every cultivar can be used and the main choice criterion is height of the grain yield. Feeding value of barley seed is higher than for rye and oat. Barley straw also has better quality in comparison with other cereals, because of higher protein content. The second important direction of barley using is its utilization in food and brewing industry, first of all for melt production. For breweries special brewing barley cultivars, mainly with spring growth habit, are cultivated. These cultivars are characteristic for better technological value. Otherwise barley seed is used for consumer purposes in the form of cereals and germs. Barley seed is also used as addition to pastas and nutrients. Moreover barley is a raw material for pearl barley porridge and bran production.

The increase of cereals' yield has been accompanied by decreases in straw length. Although plant height is known to be a quantitative character, major genes for reduced plant height have been identified in all main cereals (Börner et al. 1999). Major dwarfing genes can be divided into GA-sensitive and GA-insensitive types, depending on their responsiveness to exogenously applied gibberellic acid (Börner et al. 1999, Gottwald 2004). In barley breeding dwarfing genes are also used. Many modern barley cultivars carry GA-sensitive dwarfing gene *sdw1* (*denso*), which was located by Barua et al. (1993) and by Laurie et al. (1993) on the long arm of chromosome 3H. Another dwarfing gene - *Gpert*, which is present in European spring barley cultivars, is localised on chromosome 5H (Thomas et al. 1984). Falk (1994) described dominant gibberellic acid insensitive dwarfing gene *Dwf2* which cause a very short growth habit in barley mutant H930-36. Dwarfism induced by *Dwf2* gene is similar to that exhibited by wheat carrying *Rht-B1c* (Falk 1994, Ivandic et al. 1999). Plants are both very short, both have short, dark green leaves and neither responds to exogenously applied GA_3 (Falk 1994). In further study Ivandic et al. (1999) localised this gene on short arm of 4H barley chromosome. Moreover they showed that chromosomal localization of *Dwf2* gene is homoeologous to *Rht-B1* and *Rht-D1* loci in wheat (Ivandic et al 1999). Börner and Korzun (1996) identified two recessive dwarfng genes on barley chromosome 2H that differ in their response to gibberellic acid (GA_3). One of these, *gai* (*Rht-H1*, *GA-ins*) the GA_3-insensitive gene, was mapped in the centromeric region of 2H. Second of these genes - *gal* (*GA-less*), GA_3-sensitive, was located on long arm of chromosome 2H (Börner et al. 1999).

The aim of this study was to determine the reaction of 41 Polish barley cultivars to the treatment with gibberellic acid (GA_3) and identification GA-insensitive dwarfing genes on this basis.

Material and methods

The screening included 41 barley cultivars from Polish register, among which 8 have winter growth habit and 33 have spring growth habit. After initiation of germination in 25°C for 12 hours, they were placed in 4°C for 72 hours in order to synchronise germination. After that kernels were transmitted to plastic boxes filled with wet perlite. Seedlings were watered every day with gibberellic acid solution, at 50 $\mu g \cdot g^{-1}$ concentration (150 ml on 120 seedligs). Boxes were placed in a vegetation chamber under controlled conditions: 20°C (day), 14°C (night) and artifical illumination. After 18 days, seedling and coleoptile length were measured. The test was carried out in two replications. In one replication were 10 seedlings from each cultivar. As a control barley mutant Hv287 kindly supplied by Andreas Börner (IPK Gatersleben) and containing the *gai* gene was used. In parallel the same experiments with the same Polish barley cultivars using methods as descibed above were performed, but seedlings were watered every day with distilled water. Obtained results were tested, using ANOVA procedure.

Results and discussion

Out of the 41 barley cultivars tested 8 were found to be GA_3-insensitive. Their coleoptiles length didn't differ significant from control form with *gai* gene (Table 1). Among these cultivars Bażant, Bursztyn, Gil, Horus, Lomerit have winter growth habit and Atol, Rastik, Rodos have spring growth habit. That result means that these 8 cultivars may have either *gai* gene, or other unknown GA_3-insensitive dwarfing genes with similar effects.

Mean coleoptiles length for GA_3-sensitive cultivars averaged from 7.96 cm for Binal to 16.29 cm for Orthega, and mean seedlings length were from 22.36 cm for Poldek to 47.74 cm for Blask. Mean coleoptiles length for cultivars with GA_3-insensitive dwarfing genes reached from 5.05 cm for Gil to 6.86 cm for Atol. In the water treatment coleoptile lengths of these cultivars were slightly shorted. Mean coleoptile length after water treatment of cultivars with GA_3-insensitive dwarfing genes ranged from 4.50 cm for cv. Horus to 6.15 cm for Atol. Mean coleoptile length after GA treatment in comparison to water treatment in cultivars: Bażant, Gil, Horus, Lomerit, Atol, Rastik and Rodos did not differ significant. In cultivar Bursztyn, coleoptile length after GA treatment (6.46) were significant higher than after water treatment (4.85), but in comparison, mean coleoptile length after GA treatment in Bursztyn to mean coleoptile length after GA treatment in Hv287 did not differ significant. Mean seedlings lengths for these cultivars were from 18.10 cm for Bażant to 28.06 cm for Atol. In our experiment barley mutant Hv287 achieved mean coleoptile length 5.55 cm and mean seedling length 23.88 cm.

In their examination Börner et al. (1999) used barley mutant Hv287 which has *gai* gene. The mean shoot length for this form after GA treatment was 40 mm. This mutant was crossed with tall, 2-rowed variety Betzes with mean shoot length after GA treatment 130 mm. In F_2 progeny after gibberellic acid application, obtained plants with shoot length from 45 to 155 mm. The same mutant was crossed with tall, 6-rowed variety Monte Cristo (mean shoot length after GA treatment – 150 mm), too. In F_2 obtained plants with shoot length after gibberellic acid test from 35 to 150 mm.

Table 1: Means of coleoptiles and seedlings length after and without GA_3 treatment for barley cultivars

Cultivar	After GA_3 treatment		After water treatment	
	Coleptile length [cm]	Seedling length [cm]	Coleptile length [cm]	Seedling length [cm]
Bażant	**6.20**	**18.10**	**5.20**	**16.34**
Bombay	8.83	23.40	5.28	17.75
Bursztyn	**6.46**	**24.16**	**4.85**	**15.18**
Corbie	8.47	32.00	5.58	18.34
Gil	**5.05**	**20.80**	**4.78**	**15.73**
Horus	**5.73**	**22.08**	**4.50**	**15.20**
Lomerit	**5.89**	**23.25**	**5.30**	**17.57**
Tiffany	8.10	30.20	5.15	18.30
Annabell	10.33	40.55	5.55	18.15
Antek	8.47	36.23	6.16	20.14
Atol	**6.86**	**28.06**	**6.15**	**17.85**
Barke	8.22	32.96	6.68	21.58
Binal	7.96	28.34	5.82	17.82
Blask	8.02	47.74	5.82	20.33
Boss	11.46	39.06	5.12	16.65
Bryl	9.35	43.32	6.48	21.07
Edgar	8.40	41.20	5.55	15.50
Granal	8.85	33.13	5.58	18.00
Jersey	9.93	34.19	5.43	14.38
Johan	7.97	27.83	6.03	17.08
Lot	8.27	30.13	6.36	17.70
Madonna	10.03	35.63	6.07	18.75
Nadek	8.33	33.82	5.80	18.85
Nagrad	9.63	31.41	6.00	19.38
Orthega	16.29	31.79	6.07	19.70
Philadelphia	13.25	26.33	5.65	19.23
Poldek	10.33	22.36	5.62	17.93
Prosa	10.82	24.12	6.10	16.82
Rabel	11.53	25.14	6.05	19.63
Rasbet	10.49	27.55	6.08	19.54
Rastik	**6.11**	**23.22**	**5.72**	**21.40**
Rataj	10.10	22.83	5.78	18.20
Refren	9.23	22.57	4.85	16.90
Rodion	11.82	24.44	5.88	18.64
Rodos	**6.64**	**26.71**	**5.38**	**18.55**
Ryton	10.60	27.62	6.32	21.62
Scarlett	10.12	26.04	5.70	20.46
Start	11.18	26.47	5.92	18.64
Stratus	9.13	24.73	5.85	19.38
Tolar	10.86	25.82	5.90	19.70
Widawa	9.54	23.33	5.74	16.54
Hv287 (*gai*)	**5.55**	**23.88**	**5.27**	**22.83**

The barley GA_3-insensitive dwarfing mutant 93/B694 (*Dwf2*), was crossed with the GA_3-insensitive dwarfing mutant Hv287 (*gai*). One single F_1 plant of this combination was used to produce 128 F_2 seeds. All F_2 seeds of the cross 93/B694×Hv287 were studied together with their parents by use of a GA_3 seedling test. The mean shoot length for mutant 93/B694 was 25 mm and for mutant Hv287 - 35 mm. In F_2 progeny obtained plants with shoot length from 20 to 190 mm (Ivandic et al. 1999).

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A wheat CMS system based on *Hordeum chilense* cytoplasm

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Cytoplasmic male sterility (CMS) in wheat is obtained by substituting the wheat nucleus into the cytoplasm of alien species by the backcross method (Kihara 1951). Over 20 different cytoplasms from *Aegilops*, *Haynaldia*, *Secale* and *Triticum* promote male sterility in wheat (Pickett 1993).

The cytoplasm donor species is often a source of fertility restoring genes (*Rf*). Fertility restoration is usually a complex genetic trait. *Rf* genes, or their modifiers, have been located on 17 of the 21 wheat chromosomes (Sage 1976). The restoration is further complicated by differences among females for ease of restoration and by the effect of the environment on fertility restoration (Wilson 1984). Pickett (1993), after reviewing the history of hybrid wheat concluded that CMS derived from *Triticum timopheevii* (Zhuk.) Zhuk. appeared to present fewer problems. Nevertheless, other authors claimed that the alloplasmic wheat in *T. timopheevii* cytoplasm present some negative traits such as seed wrinkling or ear sprouting, which do not favour commercial hybrid wheat (Chen 2003). Thus, it seems that there is room for the search of additional sources of CMS.

The potential of the genus *Hordeum* has not been fully explored with this objective. *Hordeum vulgare* L. alloplasmic wheat has been obtained after backcrossing to wheat the hybrid *H. vulgare* × *T. aestivum*. In addition to the common problems of alloplasmic forms, this combination presents chromosome instability and pistilloidy, which make useless this cytoplasm as a source of CMS for wheat. Occasionally reversion to fertility by paternal

transmission of cytoplasm has been observed in these alloplasmic (Aksyonova *et al.* 2005) and also in *Hordeum* × *Secale* hybrids (Soliman *et al.* 1987) which additionally restrain the utility of this source of cytoplasm for CMS wheat. None of those limitations have been detected till date on alloplasmic wheat in *Hordeum chilense* Roem. et Schult. cytoplasm.

Hordeum chilense is a diploid wild barley, native to Chile and Argentina, included in the section *Anisolepis* Nevski of the the Triticeae tribe. This species explores contrasting environments; the sea side or up to 2100 m altitude, prairies or disturbed habitat like road sides, humid versus xeric habits, resulting in a contrasted polymorphisms both at morphological or molecular levels.

Three ecotypes (I, II and III) are recognised, based on morphology and molecular markers (Vaz Patto *et al.* 2001). Groups I and III are more distinct being II intermediate.

Fig. 1: *Hordeum chilense* spikes of group I (left) and group III (right) accessions

The spike morphology of ideotypes of groups I and III are presented in figure 1. Wider and flat spikes, more erect culms and a shorter uppermost internode until flag leaf, distinguish III from I group.

The main interest of *H. chilense* for breeding is its high crossability with other members of the Triticeae tribe, *Aegilops*, *Agropyrum*, *Dasypirum*, *Secale*, *Triticum* and ×*Triticosecale* (Bothmer and Jacobsen, 1986; Martín *et al.* 1998). Fertile amphiploids named Tritordeums (×*Tritordeum* Ascherson et Graebner) were obtained after chromosome doubling of hybrids between *H. chilense* and tetraploid and hexaploid wheat. Chromosome addition and substitution lines of *H. chilense* in bread wheat have been developed (Miller et al. 1981; Miller et al. 1985).

In recognition of the number of interesting traits present in *H. chilense* germplasm, over 250 octoploids and hexaploids tritordeums have been generated using different accessions of the species (both barley and wheat) in order to increase the genetic variability available for breeding (Martín et al. 1998). Backcrossing the amphiploids to the wheat parent, alloplasmic wheats on *H. chilense* cytoplasm are produced in two or three generations. Alloplasmic bread wheat on *H. chilense* accessions from group I (H1, H8, H57), group II (H74, H303) and group III (H7, H46, H56) have been obtained. The wheat parental lines used in this work were cultivars from different origins, mainly CIMMYT and traditional Spanish wheat accessions, although Chinese Spring and *T. sphaerococcum* were also included. The alloplasmic in cytoplasm H7, H46 and H56 are fertile while the others, belonging to groups I and II, are male sterile. Chinese Spring was the only common wheat parental used, on both H7 and H1 cytoplasm, resulting on fertile or male sterile alloplasmic respectively. Although the data are insufficient, this result supports the hypothesis that *H. chilense* group III deserves de rank of subspecies based on cluster analysis using morphology and AFLP markers as proposed for Vaz Patto *et al.* (2001).

The morphology of male sterile alloplamic wheat is quasi normal; only a reduction in height, delay on flowering and greenish colour differentiates the alloplasmic from the euplasmic line in every combination bread wheat - *H. chilense* cytoplasm obtained. Pistilloidy or grain

shriwelling, frequent on alloplasmic wheat in *H. vulgare* cytoplasm, are not present in this case.

Restoration of fertility

To check if restoration by other known restorer genes works on this system, hybrids between alloplasmic wheat and 26 restorer lines from other systems were obtained. Hybrids were grown during two successive years under open field or greenhouse conditions. No restoration whatsoever was observed.

In the process of obtaining the alloplasmic wheat some pollen fertility was observed on plants with left behind *H. chilense* chromosomes. With the aim of identifying the chromosome restoring pollen fertility, hybrids of alloplasmic lines (kindly supplied by Steve Reader, JIC, Norwich, UK) with chromosome addition of *H. chilense* on Chinese Spring (CS) were obtained. Except for chromosome $3H^{ch}$, every *H. chilense* chromosome was tested on CS on cytoplasm of *H. chilense* accession H1 (msH1CS). Either whole chromosome or ditelosomic (when available) lines were used. Only the hybrid alloplasmic CS × ditelo addition $6H^{ch}S$ showed some pollen fertility. The hybrid was backcrossed to CS ditelo $6H^{ch}S$ and alloplasmic CS ditelo $6H^{ch}S$ was selected on the progeny. This plant was fully fertile and maintained fertility along years and under different environments.

Chromosome group 6 effect on fertility restoration

The possibilities of using *H. chilense* cytoplasm for hybrid seed production are nil if based on restoration by aneuploidy. Therefore we started to analyze the fertility of chromosome combinations involving chromosome homoeologous group 6 with the goal of obtaining an euploid restorer with an introgression of *H. chilense* .

The substitution line of chromosome 6A by ditelo $6H^{ch}S$, available from the JIC, Norwich, UK, was also used on backcrossing msH1CS. Nevertheless, the alloplasmic nuli 6A ditelo $6H^{ch}S$ was male sterile, as well as mono 6A mono telosomic $6H^{ch}S$.

As we said previously monosomic addition of $6H^{ch}$ on msH1CS is malesterile. Pollinating this form with CS nullisomic 6D a doble monosomic 6D, $6H^{ch}$ was obtained. This form is self-fertile, which suggests that a balance between doses of 6D and $6H^{ch}$ are controlling restoration of pollen fertility. Nevertheless, in the progeny of such a plant the chromosome substitution 6D by $6H^{ch}$was obtained and this plant is also self-fertile. Backcrossing msH1CS by the substitution $6D(6H^{ch})$ the msH1CS double monosomic 6D $6H^{ch}$ is easily obtained and in the progeny of this combination the translocation $6H^{ch}S/6DL$ should be obtained hopefully and therefore an euploid restorer.

Stability of male sterility

A CMS system to be useful for commercial hybrid production requires complete and stable male sterility and complete restoration of fertility to the F_1 hybrid in a number of environments and years. As we said previously reversion to fertility by paternal transmission of *Hordeum* cytoplasm has been observed in wheat in *H. vulgare* cytoplasm (Aksyonova *et al.* 2005). Consensus chloroplast SSRs developed by (Chung and Staub, 2003) were used to confirm that alloplasmic lines with restored fertility possess the *H. chilense* cytoplasm.

Cross pollination studies

Three years of trials were carried over the growing seasons 2004-2005, 2005-2006, 2006-2007 on the IAS-CSIC farm at Córdoba, Spain. Each experiment consisted on alternative rows forming concentric circles of fertile vs. male sterile lines. The distances between alternative fertile and male sterile lines increases from 0 m, in the central point in which a mixture of fertile and male steriles were sown, to 2.5 m, with increment o 0.5 m. A randomised set of male sterile and fertile plants were scored for anthesis time. Each ear from presumed male sterile plants was looked at individually and scored for seed setting. The male sterile population comprise e mixture of every seed obtained from backcrossing the alloplasmic line msH1 CS plus the hybrid of this line with the 26 restorers with different wheat cultivars. The pollinators are also a mixture of wheat cultivars usually grown in the area.

There appeared to be no correlation between seed setting and position in the circle which suggest absence of wind influence on pollen flow in Córdoba. The distance between male sterile and fertile plants does correlate with seed setting. After 1.5 m there is a decrease of seed setting.

There are differences among females for ease of restoration in this experiment in every year. After the 2004-2005 season, two populations have been maintained in the experiment. On circles, seeds coming from plants with high or low seed setting have been sown alternatively in different sub-sectors. A disruptive selection is being carried out with the aim of developing material to unravel the genetic bases of this trait and mainly selecting for easiness of out pollination.

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Symptom expression and chromosomal location of leaf rust resistance from *Aegilops markgrafii* introgressed into hexaploid wheat background

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Wheat leaf rust caused by the plant pathogenic fungus *Puccinia recondita* Rob. ex Desm. occurs in all wheat growing areas resulting in annually yield losses of 5 to 10% (Manninger 1992, Long et al. 1998). The gene pool for resistance within the genus *Triticum* is exhausted, but wild relatives of wheat carry genes, which could be transferred by wide crosses into wheat background. Most accessions of the genus *Aegilops* show resistance against powdery mildew, leaf rust and eyespot (Börner et al. 2004). Also *Aegilops markgrafii* (Greuter) Hammer comprises resistance against leaf rust, yellow rust, powdery mildew (Frauenstein and Hammer 1985, Valkoun et al. 1985) and hessian fly (El Bouhssini et al. 1998).

wheat - *Aegilops* introgression lines express traits of both parents. However, often also undesirable characters of the wild parent are transferred to the high-yielding wheat cultivar and vice versa the wheat background has an influence on resistance expression. In the present study both effects were analysed in wheat introgression lines carrying alien chromatin of *Ae. markgrafii*. Furthermore, the localisation of the new resistance gene will be described.

Materials and methods

From the initial cross *Triticum aestivum* L. (2n=6x=42) x *Ae. markgrafii* (2n=2x=14) 31 euploid leaf rust resistant introgression lines were developed, one showing a wheat-like growth habit. The selection process was based on consecutive tests with a race mixture of the pathogen at seedling stage. The intensity of the disease was assessed according to a 0 (= no visible symptoms) to 4 (= fully developed pustules) scale. In parallel chromosome numbers were counted and metaphase I pairing was analysed to select stable euploid lines.

The host-pathogen-interaction was assessed by fluorescence, scanning electron and transmission electron microscopy.

To detect alien chromatin introgressed into wheat background microsatellites were applied. The localisation of leaf rust resistance based on an F_2 population originating from a cross between the resistant introgression line showing the wheat-like growth habit and the susceptible hexaploid cultivar ‘Borenos’. Seedling tests to analyse the resistance/ susceptibility were carried out as described above. The QGENE program (Nelson, 1997) was used for QTL (Quantitative Trait Loci) calculation. Microsatellites were applied to localise the leaf rust resistance.

Results and discussion

Ae. markgrafii influences spike and whole plant morphology of the leaf rust resistant introgression lines. Plant height was reduced in all lines and spikes exhibited a loose structure, except for the line with wheat-like growth habit which showed the same phenotype as the susceptible wheat parent (Fig. 1).

Wide crosses require much breeding work because undesirable traits are also introduced from the wild parent. The transfer of *Pseudocercosporella herpotrichoides* resistance from *Ae. ventricosa* Tausch to marketable cultivars required ~25 years (Börner et al. 2002). To overcome such disadvantages the use of donor material with higher ploidy level as shown by the transfer of powdery mildew resistance from tetraploid wheats (Schuster and Blüthner 1992) or the use of species with homologous genomes, e.g. *Ae. tauschii* (Miranda et al. 2006) is advisable.

Fig. 1: Spike morphology of wheat cultivar 'Alcedo', *Ae. markgrafii* and euploid introgression lines (from left to right)

Fig. 2: Symptom expression after leaf rust infection of wheat cultivar 'Alcedo', *Ae. markgrafii* and euploid introgression lines (from left to right)

Concerning plant morphology the impact of the wild parent was obvious. But as to leaf rust expression also a definite influence of the wheat background was obvious. Leaves of the resistant *Ae. markgrafii* accession had no macroscopically visible symptoms, whereas the introgression lines showed clearly detectable chlorotic and necrotic areas on the leaf surface visible with the naked eye (Fig. 2).

Investigations by fluorescence and transmission electron microscope revealed a strong hypersensitive reaction for *Ae. markgrafii*. In general the collapse of one to three cells was sufficient to stop the growth of the fungus successfully (Fig. 3a). A similar mechanism is active in the introgression lines, but the reaction had not the same intensity. The collapse of larger cell areas was necessary to arrest the pathogen (Fig. 3b). According to Heath (1995) the speed to stop the pathogen growth successfully depends on the speed at which recognition events, controlled by avirulence and resistance genes occur. The wheat background may have an influence on these recognition events resulting in larger necrotic areas visible on introgression line leaves.

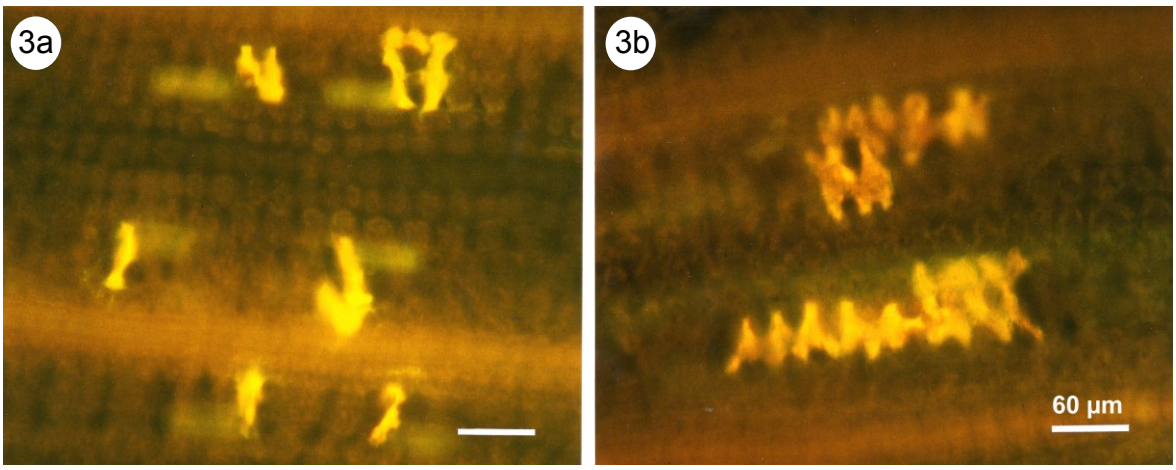

Fig. 3: Fluorescence microscopy images of collapsed mesophyll cells (showing autofluorescence) after the attack of six (a) and two (b) different leaf rust spores on *Ae. markgrafii* and wheat- *Ae. markgrafii* - introgression line leaves

In addition to the impact on hypersensitivity the wheat background also affects the fungus before entering the stoma. On leaf surfaces of the susceptible wheat parent 'Alcedo' and of the introgression lines a wax layer is visible with the naked eye in contrast to *Ae. markgrafii*. However, by Environmental Scanning Electron Microscopy (ESEM) also on *Ae. markgrafii* leaves wax particles (but different in structure and less dense) resulting in a dark green plant colour, were proven (Fig. 4). This type of wax structure did not affect pathogen development, whereas the ability of the fungus in both stoma localisation and appressoria formation was remarkably reduced on 'Alcedo' and the introgression lines. Besides the differences in wax structure the chemical wax composition could also have an influence on the failure of the fungus to detect stomata and to develop appressoria.

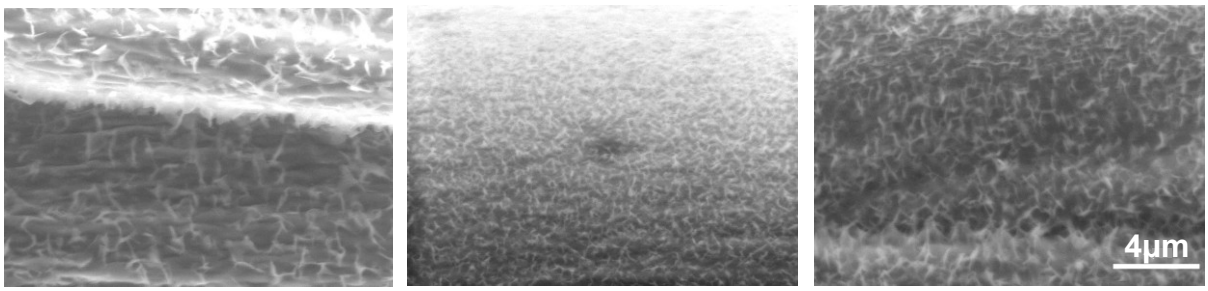

Fig. 4: Leaf surface wax layer of *Ae. markgrafii*, hexaploid wheat cultivar 'Alcedo' and an euploid introgression line (from left to right)

To develop infection structures leaf rust requires thigmotropic and chemical impulses (Allen, 1991). Multiple topographical signals like a special arrangement of ridges and grooves are important for the induction of infection-structure differentiation (Read et al. 1997). Reduced appressoria formation on leaves of the introgression lines may be caused by a failure of topographical signals as a result of the wax layer presence. Also Rubiales et al. (2001) describe that mutants with increased wax layer clearly reduce the differentiation of infection structures of fungal pathogens.

The leaf rust resistance of *Ae. markgrafii* was localised on chromosome T2CS.4CL (according to the wheat homoeology; Schubert, 2001). Therefore, a set of 117 wheat SSR (Single Sequence Repeats) markers developed for wheat chromosome arms 2S and 4L were used to detect polymorphisms between the parental lines. Another set of 226 microsatellites distributed over the whole wheat genome were applied to detect additional polymorphisms.

71% and 63.43% polymorphisms were found for the parental lines, respectively. The polymorphic markers were used to test the introgression lines. Nine markers were suitable to identify *Ae. markgrafii* chromatin on five different wheat chromosomes (Fig. 5).

From a total of 140 F_2 plants resulting from a cross of the euploid resistant introgression line with wheat-like growth habit and the susceptible wheat cultivar 'Borenos' 19 plants reacted susceptible at seedling stage. The highly significant segregation distortion observed only for SSR loci located on chromosome arm 2AS, whereas the segregation of the other SSR loci marking *Ae. markgrafii* chromatin on the other chromosomes correspond with the expected 3:1 ratio, was the reason to attempt a QTL (Quantitative Trait Loci) approach. A significant QTL (LOD score 5.14) on chromosome arm 2AS (Fig. 5) was found and designated QLr.ipk-2A (Iqbal et al. 2007).

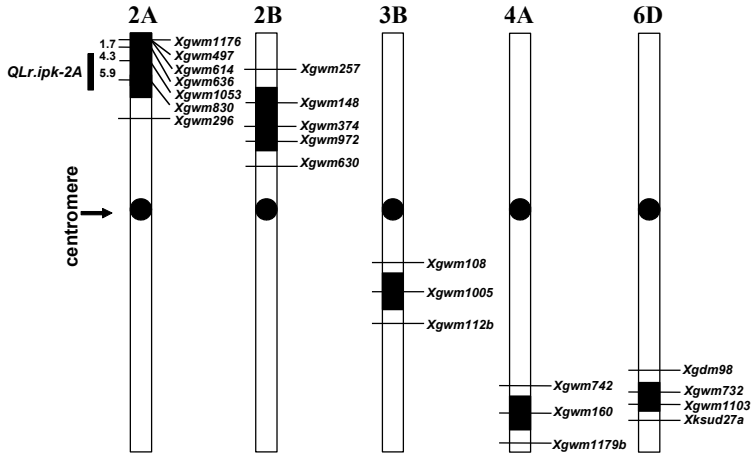

Fig. 5: Localisation of alien chromatin in wheat - *Ae. markgrafii* - introgression lines, marked as black rectangles including corresponding and flanking markers. Underlined markers on chromosome 2A are applied for linkage analysis (genetic distances in cM)

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Posters

Wheat precise genetic stocks development by using *Zea* system

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Rapid progress of haploid induction methods in the last years has been mainly determined by great potential of derived doubled haploid lines (DHLs) for use in practical breeding and fundamental research. One of these methods known as Zea system offers in common wheat some advantages compared to others: is relatively genotypic independent, all regenerants are haploid green plants and derived DHLs showed normal segregation patterns.

Besides the application in practical wheat breeding, the Zea system could be now successfully used for the development of precise wheat genetic stocks and for mediated alien gene transfers, too. The knowledge gathered from investigation of genetic stocks will certainly increase our understanding on wheat genetics and will contribute to farther wheat improvement. In this paper two of these newly developed wheat genetic stocks will be shortly present.

I. 7B-Recombinant substitution lines

Genetic analysis of the Romanian winter wheat breeding line F26-70 using disomic substitution lines Favorit/F26-70 showed that four chromosomes 4B, 4D, 5B and 5D are significantly involved in controlling high protein content per grain (2). The positive influence of 7B chromosome on protein content, bread making characteristics and earliness with three days compared to recipient genotype Favorit was also quoted.

For more detailed genetic analysis we have developed 7B – recombinant substitution lines (RSLs): 46 RSLs by classical procedure and 27 RSLs by using *Zea* system.

Results of two years field experiments with 46 RSLs showed a good correspondence between protein content and earliness of heading (Fig. 1). There are also reports about the correlation between protein content accumulation and earliness (6). If earliness could arrise from the effect of earliness “per se” gene or it is determined by a Vrn gene action, remains to be established. The effect of 7B chromosome on grain protein concentration was also reported (5) and it was suggested that a major gene/ genes are present on the short arm (4). This gene would be important for nitrogen translocation into the grains (1).

However, it is premature to assume that chromosome 7B of F26-70 could carry genetic factor(s) for grain protein content. The difference could be explained by the action of other factors which affect different pathways of plant development: maturity, ear development, number and size of the grain, nitrogen uptake, kinetics of protein accumulation, etc.

Recombinant substitution lines for chromosome 7B could generate new information about specific gene locations on 7B by using marker mediated approach, now in progress at IPK-Gatersleben.

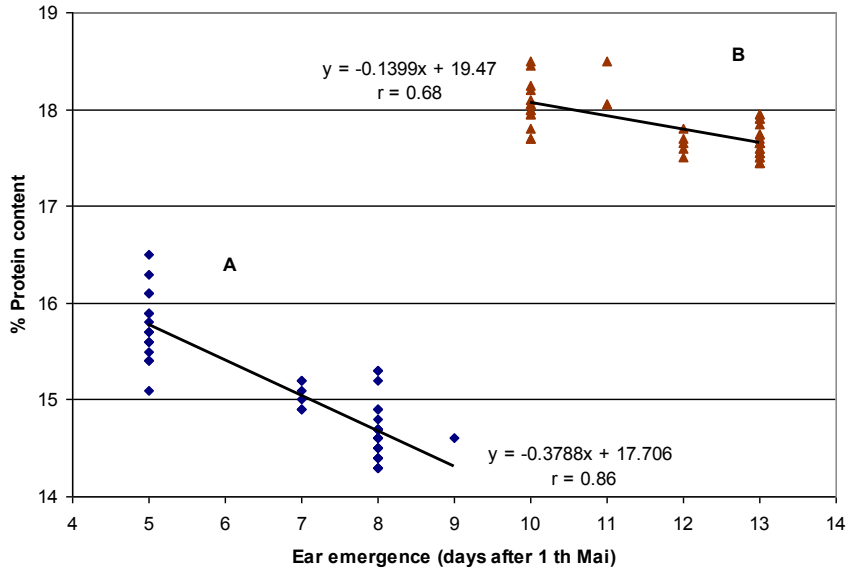

Fig. 1: Protein content (%) and ear emergence (no. of days after 1th May) in 46 recombinat substitution lines for chromosome 7B in 2004 (A) and 2005 (B)

II. DH – lines population F 132/G 603-86

Grain size is phenotypically the most stable yield component in wheat and it is correlated with flour yield.

A wheat line G 603-86 derived from the cross Cologna x F 6-75 was selected for their large grains with a grain weight over 60 mg while the most known cultivars have grain weights between 35- 45 mg. The high grain weight of G 603-86 is associated with increased grain length of about 9 mm as compared with 6 to 7 mm in most cultivars.

Genetic control of grain characters is known to be complex. Genetic analysis carryied out on F3 disomics – extracted cytologically from F2 monosomic populations showed that large grains of G 603-86 appers to be the effect of genes located in many chromosomes (3). Grain weight was significantly increased by the presence of chromosomes 6D and 4A, but significantly decreased by chromosomes 5B and 5D of G603-86 parent. Grain length was positively influenced by chromosomes 4A, 4B and 1B and negatively influenced by chromosomes 7B, 5B and 1A. As for grain width, chromosomes 1A and 1B had a significant positive effect and 5D and 4B were associated with significantly lower grain width. For farther genetic analysis we developed DHLs population (87 DHLs) from a cross between two contrasting genotypes regarding several grain characteristics, ear emergence, plant height etc (Table 1).

Table 1: Some differentiated traits between DHLs parents F 132 and G 603-86

Traits	**F 132**	**G 603-86**
Ear emergence (days after 10th May	5	22
Plant height (cm)	75	92.5
TKW (g)	39.76	62.6
Spike length (mm)	13.6 ± 0.22	18.8 ± 0.25
No. spikelets/spike	22.9 ± 0.31	20.4 ± 0.37
No. seeds/spike	88.8 ± 2.58	51.6 ± 0.95
Grain length (mm)	6.6 ± 0.04	9.39 ± 0.06
Grain width (mm)	3.96 ± 0.03	3.8 ± 0.04

In a preliminary field screening a large distribution of several analysed traits was observed. All 87 DHLs are now phonotypically evaluated in a replicated field design.

Another two newly mapping populations namely: DHLs Izvor/ Jiana (69 DHLs) and Martonvasar 9/ DH 1-30 F132 (151 DHLs) are now phonotypically analyzed for the pollen expression of osmotic regulation (this newsletter) and respectively for intergeneric crossability by pyramiding the recessive alleles kr1 and kr2.

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Microsatellite mapping of genes for coloration of different wheat plant organs on homoeologous groups 1 and 7 chromosomes

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Glume coloration is an important taxonomic discriminator in wheat. It is also used for the varieties' homogeneity/distinctness determination and it is known to be associated with the crop's adaptability in the regions with high light intensity, short growing season and low temperatures during the vegetative period (Khlestkina et al. 2006, Börner et al. 2005). Anthocyanin pigmentation of wheat anthers, auricles, coleoptiles, straw, grains or leaves is known. Anthocyanins are secondary metabolites playing an important role in UV protection of plant tissues (Khlestkina et al. 2002). In wheat, major genes are described for the coloration of glumes (*Rg1, Rg2, Rg3, Bg*), for anthocyanin pigmentation of coleoptiles (*Rc-A1, Rc-B1, Rc-D1*), anthers (*Pan1, Pan2*), auricles (*Ra1, Ra2, Ra3*), straw (*Pc1, Pc2*) and grains (*Pp1, Pp2*) (McIntosh et al. 2003). In the present study we have exploited microsatellite markers (Röder et al. 1998 and unpublished data) for the precise comparative mapping of genes determining black (*Bg*), red (*Rg1*, *Rg3*) and smokey-grey glume colors and hairiness of glumes (*Hg*) on homoeologous group 1 chromosomes, and genes for anthocyanin pigmentation of anthers (*Pan1*), straw (*Pc2*), and leaves (*Pl*) on chromosome 7D of hexaploid wheat. Furthermore we report the distribution of alleles at microsatellite loci linked to some of these genes within sets of hexaploid wheats originating from Russia, Albania, Nepal and India.

Materials and methods

Five crosses between hexaploid wheat (*Triticum aestivum* L.) cultivars, lines and accessions were used to create mapping populations, two crosses were made for allelism test (Table 1). The sets of wheat (*T. aestivum*) cultivars and accessions from Russia (Table 2), Albania, Nepal and India (Table 3) were used for phenotyping and microsatellite-based genotyping. Microsatellite analysis was performed as described by Röder et al. (1998). Linkage maps were constructed with MAPMAKER 2.0 (Lander et al. 1987).

Table 1: Crosses and population sizes

Cross No	Used for mapping (1-5) or allelism test (6-7) of genes	Cross	Number of F_2 plants/F_3 families	% SSRs polymorphic	Number of SSRs mapped
(1)	Rg3, Hg	'Zhnitsa' x 'TRI 542'	48 F_3 families	67 % (1A)	10 (1A)
(2)	*Bg, Hg*	'i:S29BgHg' x 'TRI 546'	97 F_2 plants	67 % (1A)	10 (1A)
(3)	*Rg1*	'Federation' x 'TRI 546'	105 F_3 families	76 % (1B)	16 (1B)
(4)	The smokey-grey glume color gene, *Pc2, Pan1, Pl*	'Golubka' x 'Novosibirskaya 67'	74 F_3 families	60 % (1D) 62 % (7D)	9 (1D) 8 (7D)
(5)	The smokey-grey glume color gene	'Golubka' x 'L301'	44 F_2 plants	60 % (1D)	3 (1D)
(6)	*Rg3* and *Bg*	'Zhnitsa' x 'i:S29BgHg'	154 F_3 families	-	-
(7)	The smokey-grey glume color gene and gene *Rg2*	'Golubka' x 'Synthetic'	93 F_3 families	-	-

Results and discussion

A total of 35 microsatellite loci were mapped on the homoeologous group 1 chromosomes (Table 1, Figure 1). The genes *Bg*, *Rg1*, *Rg3* and the smokey-grey glume color gene map between the markers *Xgwm1223* and *Xgwm0033* at the distal ends of the short arms of the homoeologous group 1 chromosomes (Figure 1). From the results obtained we concluded to have mapped a homoeologous series of loci, proposed to be designated *Rg-A1*, *Rg-B1* and *Rg-D1*, with *Rg-A1a*, *Rg-B1a* and *Rg-D1a* representing the non-colored alleles. Genes *Rg3* and *Bg* were considered to be different alleles at the locus *Rg-A1*. Both *Rg3* and *Bg* were found to be closely linked to the major glume pubescence gene *Hg*, also mapped in the present study. The *T. aestivum* smokey-grey glume gene and *Rg2* (1D), mapped previously in synthetic wheat *Triticum durum* (AABB) x *Aegilops tauschii* (DD), were proposed to be different alleles at the locus *Rg-D1*. Allelism test confirmed that the genes *Rg3* and *Bg* are different alleles of the locus *Rg-A1* and that the smokey-grey glume gene and *Rg2* are different alleles of the locus *Rg-D1*. Following the new designation, the red and black alleles at *Rg-A1* become *Rg-A1b* and *Rg-A1c,* respectively, and *Rg2* and the smokey-grey glume genes become *Rg-D1b* and *Rg-D1c*, respectively. Based on the comparative mapping performed in the present study for hexaploid wheat and by Dubcovsky and Dvorak (1995), Van Deynze et al. (1995), Blanco et al. (1998), Salina et al. (2006), Börner et al. (2002) for diploid and tetraploid wheats and *Aegilops*, it can be concluded that the homoeology of *T.aestivum Rg*-genes extends to the other *Triticeae* species.

A total of 8 microsatellite loci were mapped on the chromosome 7D (Table 1). The major gene loci *Pl* (purple leaf), *Pc2* (purple culm), and *Pan1* (purple anthers) were mapped in a region, about 15 cM distal from the centromere on chromosome 7DS.

Table 2: Glume color and microsatellite marker data for a set of Russian spring wheat cultivars and the parents of the mapping populations (black rectangle – "present", white – "absent", grey – "likely present", * - genes controlling glume color of these cultivars were determined by Efremova et al. 1998)

Cultivar or accession	White glume	Red glume	Black glume	Smokey-grey glume	*Rg-A1b (Rg3)*	GWM0136-264bp (1A)	GWM1223-170bp (1A)	*Rg-B1b (Rg1)*	TAGLGAP- 250bp (1B)	GWM0033-120bp (1B)	*Rg-D1c* (smokey-grey)	GWM1223-165bp (1D)	GWM0337-185bp (1D)
TRI 546	■												
TRI542	■												
FEDERATION		■						■		■			
ZHNITSA*		■			■	■	■						
I:S29BGHG			■										
SYNTHETIC		■											
OPATA 85	■												
GOLUBKA				■							■	■	■
ISKRA*		■			■	■							
TCEZIUM 111		■		■				■	■		▒		■
OMSKAYA 9	■												
OMSKAYA 11	■												■
NOVOSIBIRSKAYA 89	■												
KANTEGIRSKAYA 89	■												
SIBAKOVSKAYA 3	■												
TCELINNAYA 60	■												
SELENGINSKAYA*		■						■					
MILTURUM 2078*		■			■			■					
KRASNOYARSKAYA 1103*		■						■	■				
SKALA	■												
TARSKAYA 2		■						▒	■				
PYROTHRIX 28*		■					■	■	■				
STRELA*		■			■	■	■	■					
ALENKAYA*		■						■	■				
BALAGANKA *		■						■	■				
GDS-11		■						▒	■				
SIBIRKA 1818*		■						■	■				
IRTYSHANKA 10	■												
SIBIRYACHKA 8	■												
NIVA*		■					■	■					
DUVANKA 501*		■			■	■	■	■					
KROHINSKAYA		■						▒	■				
POBEDA*		■						■	■				
TCEZIUM 94		■		■				▒	■		▒		
NARYMSKAYA 3		■						▒	■				
NOVOSIBIRSKAYA 22	■												
NARYMSKAYA 246		■						▒	■				
SIBIRYACHKA 4	■												
LYUTESTCENS 62	■												
AKMOLINKA 1		■						▒	■				
MILTURUM 553*		■			■	■	■	■					
ALBIDUM 43	■												
SARATOVSKAYA 29	■												
NOVOSIBIRSKAYA 67	■												
IRKUTSKAYA 49	■											■	■
LYUTESTCENS 116	■											■	■
ALBIDUM 3700	■												■
SARATOVSKAYA 36	■												
SARATOVSKAYA 39	■												
ALTAISKAYA 50	■												
ALTAISKAYA 60	■												
ALTAISKAYA 98	■												
ALTAISKII PROSTOR	■												

In the same region the gene *Rc-D1* for anthocyanin pigmentation of coleoptile was mapped previously (Khlestkina et al. 2002). We supposed that a single ancestor regulatory gene for anthocyanin biosynthesis could exist on the short arm of chromosome 7, different copies of which appeared in the same region and became tissue-specific.

The microsatellite markers linked to the *Rg* genes were used to analyse a set of Russian spring wheats to find association between particular microsatellite alleles and phenotype (Table 2). Most of the markers of chromosomes 1A, 1B and 1D did not amplify specific alleles for *Rg*-genes. However, an association was found between *Rg-A1b* and *Xgwm0136*_264bp allele and between *Rg-B1b* and *Xtaglgap*_250bp. *Rg-B1c* (*Bg*) is associated with another allele - *Xgwm0136*_300bp. For red glume coloration *Rg-B1b* is more common in wheat, while *Rg-A1b* is very rare and was found only in a few Russian wheat cultivars (Efremova et al. 1998). Therefore further application of gene specific markers was done for *Xtaglgap* only (Table 3). The results obtained suggest that besides 250 bp, alleles of 241, 244 and 247 bp of *Xtaglgap* locus may be spesific for *Rg-B1b* in different wheat collections. The amplification of specific alleles suggests that red glume color in Albanian and Nepal wheat accessions is controled by *Rg-B1b* gene, while in Indian collection besides *Rg-B1b* other *Rg*-genes may be found (Table 3). Landjeva et al. (2006) observed a correspondence between 244 bp allele of marker MW1B002 (another name of *Xtaglgap*) and red glume color gene *Rg-B1b* in Bulgarian wheat cultivars.

Table 3: Genotyping wheat accessions in *Rg-B1b*-specific microsatellite locus *Xtaglgap* (1B)

Wheat collections sights and year	**Collection size (% of the red glumed accessions)**	***Rg-B1b*-specific Xtaglgap allele (bp)**	**% of the red glumed varieties having specific allele**	**Reference to microsatellite genotyping of the collection**
Russia (1920-2000)	44 (45% *Rg-B1b*)	250	71%	Khlestkina et al. 2004a
Albania 1941	18 (33%)	244	100%	Khlestkina et al. 2004b
Nepal 1937	18 (11%)	247	100%	Khlestkina et al. 2004b
Nepal 1971	18 (50%)	241	89%	Khlestkina et al. 2004b
India 1937, 1976	34 (35%)	241 or 244	67%	Khlestkina et al. 2004b

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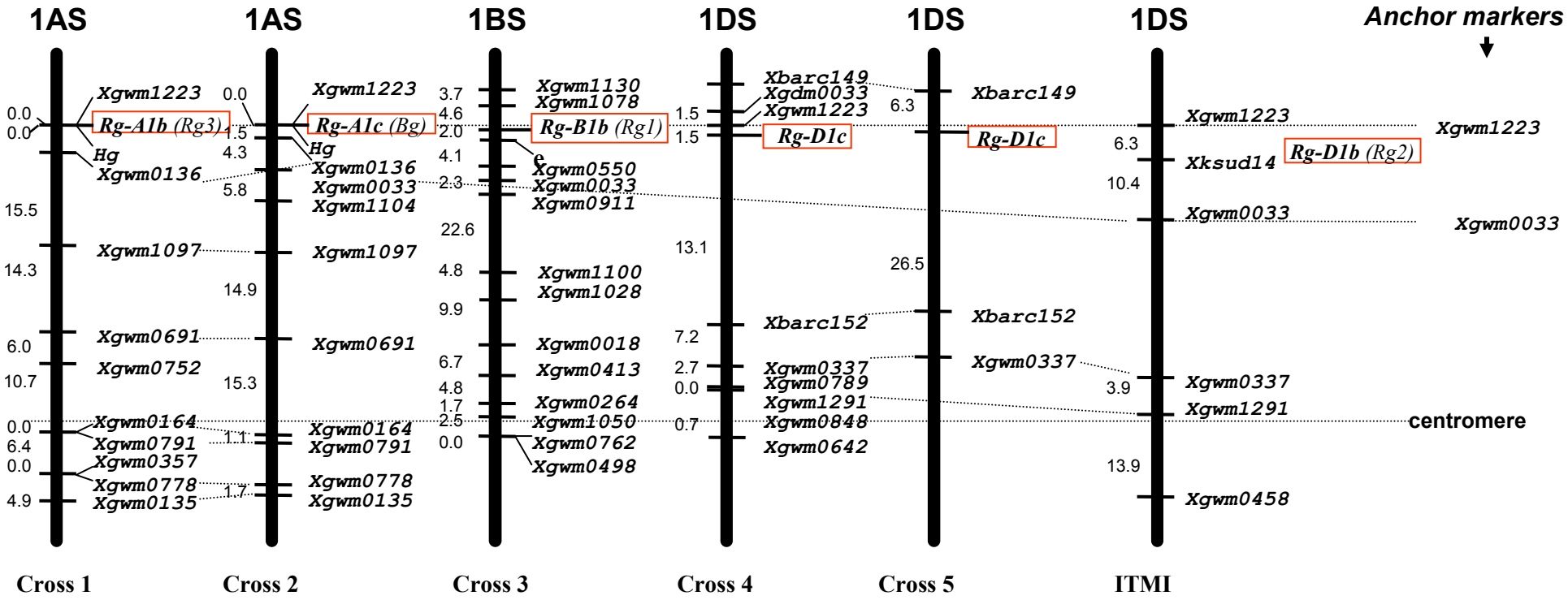

Fig. 1: Comparative mapping of the genes for glume coloration and pubescence in hexaploid wheat. Chromosome designations are indicated above. Genetic distances are given in centimorgans (cM). Map of 1DS chromosome in ITMI population was obtained from Börner et al. (2002), and Röder et al. (1998).

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Cleaved amplified polymorphic sequence (CAPS) screening of the thermostable alleles of *β*-amylase gene in Egyptian barley

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Barley is the fourth largest of the cereal crops after wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), and maize (*Zea mays* L.); it is important cereal crop for the majority of world's populations. Worldwide, barley is important, being the most suitable raw material for the production of beer, whiskey and some kinds of food. In Egypt, barley is primarily produced and used as animal feed. It is also consumed as malt, as food and Fayrouz and Birelle (non-alcoholic malt beverage). Strong enzymatic activities in the germinating barley grain, together with protein and starch content, are crucial for high extraction values in the resulting malt and, therefore, barley malting quality.

In the grain of malting barley, *β*-amylase is one of the most important hydrolytic enzymes. During malting, not only *β*-amylase activity but also its thermostability plays an important role. There are two barley (*Hordeum vulgare* L.) *β*-amylase genes encoding important starch degrading enzymes. The endosperm-specific *β*-amylase 1 (*Bmy1*), the more abundant isozyme in cereal seeds, has been thoroughly characterized. The lesser abundant *β*-amylase 2 (*Bmy2*) has not been biochemically characterized from any cereal seeds.

The *Bmy1* gene encoding *β*-amylase that is abundant in the starchy endosperm of ungerminated barley seeds was isolated and characterized (Thacker et al., 1992; Yoshigi et al., 1995). Four natural allelic forms of seed specific barley *β*-amylase gene *Bmy1* (Sd1, Sd2H, Sd2L, Sd3) exhibit different thermostability and kinetic properties (Ma et al., 2001; Paris et al., 2002).

The four forms of *β*-amylase exhibit different rates of thermal inactivation in barley extracts, with low, intermediate and high levels of thermostability respectively. Thermal inactivation analysis of the three enzymes revealed T_{50} temperatures of 56.8ºC for the Sd2L enzyme, 58.5ºC for the Sd1 enzyme, and 60.8ºC for the Sd3 *β*-amylase from wild barley. This

variation was shown to persist after the CAPS assay. Analysis of the relationship between *β*-amylase thermostability and fermentability in 42 commercial malt samples indicates that increased thermostability results in more efficient starch degradation (Eglinton et al., 1998).

The *Bmy1* gene has been characterized (Tacker et al. 1992, Yogishi et al. 1995) and localized on the long arm of chromosome 4H (Kreis et al. 1987). The *Bmy1* gene is expressed during grain filling (Nielsen et al., 1983; Kreis et al., 1988; Netsvetaev, 1992).

Paris et al. (2002) developed two single nucleotide polymorphism (SNP) markers and a cleaved amplified polymorphic sequence (CAPS) assay enabling the identification of the four *β*-amylase alleles which are based on two SNPs in positions 495 and 698 of the cDNA. They proposed to use these markers for marker-assisted selection in barley breeding programmes. In this research, The goals of our investigation were (i) to genotype the C698--T polymorphism in Egyptian commercial barley varieties; (ii) to follow the destiny of *β*-amylase alleles with higher thermostability in these varieties and (iii) to analyse the presence of different *β*-amylase alleles in 35 barley accessions originating from Egypt.

Materials and methods

Barley cultivars: A complete list of barley (*Hordeum vulgare*) varieties tested in this study is shown in table 1. The seeds of the barley accessions were obtained from the Agriculture Research Center (ARC), Giza, Egypt; USDA GenBank, United States and Genebank, Leibniz-Institut für Pflanzengenetik und Kulturpflanzenforschung (IPK), Gatersleben, Germany. These accessions were grown in the field of Genetic Engineering and Biotechnology Research Institute (GEBRI), Menoufia University, Sadat City**,** Egypt. Harvested seeds were used for *β*-amylase analysis.

Genomic DNA isolation: Leaves from 14-day-old plants were cut as tissue samples for DNA extraction from the greenhouse. An equal amount of tissue was taken from each single plant. Usually, 5-10 plants per accession were pooled in order to detect possible heterogeneities within an accession. Genomic DNA was extracted according to the previously described procedures (Plaschke et al. 1995) and used as a template for the PCR assays. Quality and quantity were checked electrophoretically and spectrophotometrically.

PCR amplification and genotyping: PCR procedures were as described by Röder et al. (1995; 1998). Briefly, each polymerase chain reaction (PCR) was performed in a volume of 25 µl of PCR buffer (0.01 M Tris, 0.05 M KCl, 1.5 mM MgCl2, 0.01% gelatine) and contained approximately 100 ng of genomic DNA, 0.2 mM of dCTP, dGTP, dTTP, dATP, 0.4 lM of each primer and 1 U of *Taq* polymerase. Amplification for the CAPS was performed according to the following program conditions. After 3 min at 94 ºC, 45 cycles were performed with 1 min at 94 ºC, 1 min at 60 ºC, 2 min at 72 ºC and a final extension step of 10 min at 72 ºC.

CAPS assay: A CAPS assay was used to reveal the C698--T polymorphism. The method is based on the existence of an *MspI* restriction site, C698/CGG, and was performed according to Paris et al. (2002). The *Bmy1*P fragment was amplified using the primers *Bmy1*PF 5'-GCA CGA TAA TAT ATA CCA TTG-CY5 and *Bmy1*PR 5'-TTG TTG GAG TAC CAT GCA AG. The primers *Bmy1*PF and *Bmy1*PR were used to amplify a fragment of 270 bp or 277 bp. The products were size fractionated and analyzed. Varieties yielding a 171 bp fragment after restriction were scored as possessing superior *Bmy1*-Sd2H or *Bmy1*-Sd3 alleles. In all investigated barley varieties two product sizes 277 bp or 270 bp were observed after PCR amplification. The 270 bp amplification product is due to a 7 bp deletion upstream of the *MspI* restriction site (Sjakste and Röder, 2004). In the present experiments only accessions yielding the 270 bp amplification product harboured the restriction site of *MspI*. Therefore,

the presence of the alleles *Bmy*-Sd2H or *Bmy*-Sd3 could be scored by simple analysis of the fragments after PCR without restriction reaction.

Statistical Analysis: Gene diversity (H) at *Bmy1* locus was calculated by the gene diversity index of Nei (1973): $H = 1 - \Sigma\ pi^2$, in which *pi* is the frequency of the *i*th allele of the locus. Anderson et al. (1993) referred to gene diversity as the polymorphic information content (PIC).

Table 1: Accession number/cultivar name, seed source, year of release, pedigree and seed source of the Egyptian barley materials analysed

Accession number/cultivar name/seed source	Year of release	Pedigree	cSNP698	*Bmy1* P S/L	Fragments bp	Phenotype
HOR 19704 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 17683 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 16107 [1]	No Data	Selected landrace	C+T	S+L	171-270-277	Heterozygous
HOR 16102 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 14411 [1]	No Data	Selected landrace	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
HOR 16097 [1]	No Data	Selected landrace	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
HOR 8255 [1]	No Data	Selected landrace	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
HOR 8658 [1]	No Data	Selected landrace	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
HOR 8659 [1]	No Data	Selected landrace	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
HOR 8806 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 819 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 1938 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 2252 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 3711 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 7419 [1]	No Data	Selected landrace	C+T	S+L	171-270-277	Heterozygous
HOR 8212 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 19027 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 19308 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
HOR 19720 [1]	No Data	Selected landrace	C+T	L	277	Heterozygous
HOR 20117 [1]	No Data	Selected landrace	C	S	270	*Bmy1*-Sd2H or *Bmy1*-Sd3
Saharawy [2]	1955	Baladi 16/Atsel	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Bonus [2]	1956	introduction	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 117 [2]	1958	Baladi 16/Palestine 10	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 118 [2]	1963	introduction	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 119 [2]	1973	Gem/Baladi 16	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 121 [2]	1977	No Data	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 123 [2]	1988	Giza 117/FAO 86	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 124 [2]	1988	Giza 117/Bahteem 52//Giza 118/FAO 86	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 125 [3]	1995	No Data	C+T	S+L	171-270-277	Heterozygous
Giza 126 [3]	1995	Baladi Bahteem/SD 729-Por 12762-BC	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 127 [3]	No Data	No Data	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Giza 128 [3]	No Data	No Data	C+T	S+L	171-270-277	Heterozygous
Club Mariout [3]	No Data	Selected landrace	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Line 105 [3]	No Data	Selected landrace	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1
Baladi [1]	1930	Selected landrace	T	L	277	*Bmy1*-Sd2L or *Bmy1*-Sd1

[1] IPK, Gatersleben, Germany
[2] ARC, Giza, Egypt
[3] USDA GeneBank, US

Results and discussion

Insertional/deletion polymorphism: As shown in Table 2, a wide variation in *β*-amylase was found in the 35 accessions of Egyptian barley. The amplification with the *Bmy1*-P primers which are located in intron III and exon IV of the *Bmy1* gene revealed the existence of two alleles of 277-bp (long allele or L) and 270-bp (short allele or S) in length in the tested accessions (Table 2). The amplification products of 19 accessions with long alleles (HOR 14411, HOR 16097, HOR 8255, HOR 8658, HOR 8659, HOR 19720, Saharawy, Bonus, Giza 117, Giza 118, Giza 119, Giza 121, Giza 123, Giza 124, Giza 126, Giza 127, Club Mariout, Line 105, Baladi) and 12 varieties with short alleles (HOR 19704, HOR 17683, HOR 16102, HOR 8806, HOR 819, HOR 1938, HOR 2252, HOR 3711, HOR 8212, HOR 19027, HOR 19308, HOR 20117) were sequenced to reveal the source of this polymorphism. The 7-bp difference between the L and S alleles resulted from two insertion/deletion events of 1-bp at A2177, and of a 6-bp sequence TCGTAC after C2186 in the 3′ region of intron III. The affected sequence is characterized by the repeated motif TACT. The same insertion/deletion events can be identified from the alignment of the previously published sequences of 'Adorra' and the Finnish landrace line HA52 (Erkkil et al. 1998) containing the L-allele and the Japanese variety 'Haruna Nijo' (Yoshigi et al., 1995) and *H. spontaneum* strain PI 296897 (Erkkila and Ahokas, 2001) both presenting the S-allele. All analyzed L-alleles were identical to each other as well as all analyzed S-alleles in the intron III region of *Bmy1* gene. Except two insertion/deletion events of 1-bp at A2177, and 6-bp sequence TCGTAC after C2186 the sequences of the amplified *Bmy1*P intron region were almost identical between accessions containing S- and L-alleles and in comparison to the corresponding region of the previously published *Bmy1* gene of the accession Haruna Nijo (GenBank D49999, gij762857j).

CAPS assay: From 35 Egyptain barley accessions tested in the CAPS assay the *Msp*I site was found only among 12 accessions which all amplified the 270-bp *Bmy*1P alleles or S alleles (Table 2). All four accessions that showed heterogeneity in the S/L alleles (HOR 16107, HOR 7419, Giza 125, Giza 128) revealed also heterogeneity in the C698-T polymorphism according to the CAPS assay (Table 2). The *Msp*I site determining the *Bmy1*-Sd2H and/or Sd3 allele was present in homozygous stage in the most old Egyptian varieties.

The PIC value of the CAPs assay polymorphism of all accessions studied was 0.57. The most frequent alleles were 171 bp, 270 bp and 277 bp with 9.30%, 37.20 and 53.49%, respectively as shwon in figure 1.

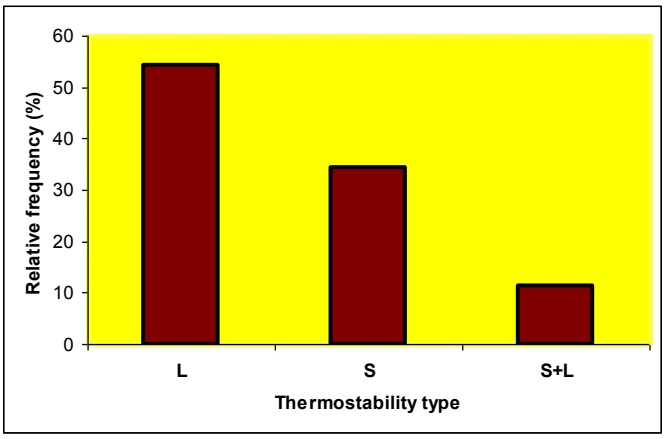

Fig. 1: Frequency distribution of *β*-amylase thermostability types in tested Egyptian barley

Diversity Index of β-Amylase genotype: Figure 1 shows the genetic diversity of *β*-amylase genotype. The total diversity index of investigated accessions was 0.57. The polymorphic information content (PIC) value of the CAPs assay polymorphism of all accessions studied was 0.57. The most frequent alleles were 171 bp, 270 bp and 277 bp with 9.30%, 37.20 and 53.49%, respectively.

We detected a low level of C alleles present in Egyptian barley. Mostly old, wild or varieties derived from wild accessions carried this mutation. While originally present in some pedigrees C698 was not introduced in most new commercial varieties. The impossibility of testing for malting quality during the early stages of the breeding process may be one reason of this situation. By using marker assisted selection (MAS) the thermostable *β*-amylase alleles from old varieties may be re-introduced into modern Egyptian varieties. From this point of view, our data clearly illustrate the importance of introducing marker assisted selection in the Egyptian barley breeding process.

Acknowledgments

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The diversity of the trait "leaf hairiness" among the bread wheat accessions and their hybrids

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Outgrowths of the epidermis of plant organs, called trichomes are common in many plant species. They form the layer of hairiness on leaf blade surface which decrease the air movement next to the leaf and protects the leaf surface from the intensive solar irradiation and extensive water evaporation. Leaf hairiness in wild and cultivated cereals is known to play an important role in adaptation of plants under droughty environment. This character is widely spread among the best old and modern commercial cultivars of common wheat in droughty environment of Siberia, Kazakhstan and Volga region. In addition, a role of hairiness in the control of insect pests has also been supposed. One example is the cereal leaf beetle (*Oulema melanopus* L.) resistance in wheat, which was shown to be initially attributed to trichome density (Gallun et al. 1973).

The inheritance of this complex quantitative trait is poorly studied and the diversity of the trait in wheat has not been described yet. Maystrenko (1976) using F_2 monosomic analysis found the gene *Hl1* for leaf hairiness to be located in 4B chromosome in common wheat cultivars. This gene has a quantitative performance reducing the thickness of leaf hairiness. Another gene was discovered in 7BS chromosome of common wheat (Taketa et al. 2002) and in the same chromosome of the line with introgression from *Aegilops speltoides* Tausch. (Pshenichnikova et al. 2007). Both genes were mapped (Dobrovolskaya et al. 2007). The diversity of leaf hairiness among wild and cultivated cereals described by botanists (Krupnov, Tsapaikin, 1990) supposes the existence of other genes determining this trait. Their combinations in genotypes give the wide range of manifestation of the trait.

In this work, the different types of leaf hairiness with known and unknown genetic control found among bread wheat cultivars and lines are presented as well as the phenotypes segregating in the crosses between some of them.

Materials and methods

Genetic material: bread wheat cultivars Rodina, Saratovskaya 29 (S29), Janetzkis Probat (JP), Hong-mang-mai, bread wheat line 102/00[i] with introgression from *Aegilops speltoides* Tausch. (Lapochkina, 2001), bread wheat line 821 with introgression from *Triticum timopheevii* Zhuk. (the line was kindly donated by E. Budashkina, originator). The surface of the young flag leaves were firstly examined and described by touching with fingers. After that the leaf folds were examined using microscope "Axioscop" 2" Plus at 5 × magnification. Input of digital information was carried out using the high-resolution color CCD-camera AxioCam HRc (ZEISS, Germany with AxioVision softare package). Images were additionally processed using Adobe Photoshop.

Results and discussion

Bread wheat cultivar S29 obtained in droughty steppe Volga region has a thick leaf hairiness strongly expressed since early stages of development under any conditions (Fig.1). It may be supposed that S29 carries several genes responsible for this trait. One of them having a quantitative expression was localized and mapped in 4B chromosome (Maystrenko, 1976;

Dobrovolskaya et al. 2007). Introducing of 4B chromosome from the donor JP with another type of hairiness into S29 genotype caused a reducing of leaf hairiness (Fig.1). After crossing of S29 with cultivar Rodina lacking leaf hairiness two types of hairiness were identified in F_2 generation (Fig 2), one with different density levels and another with very short trichomes rarely spread over the leaf surface. The latter genotypes were firstly described as glabrous and only after microscope analysis the new type of hairiness was detected. The segregation ratio between these two phenotypes was approximately 15:1 (χ^2=0,61; P>0,50). The inheritance of this trait will be continued in F_3 generation.

Another type of leaf hairiness was found in two bread wheat lines with introgressions from *Aegilops speltoides* and *Triticum timophe*evii (Fig 2). It consists of two layers of short and long trichomes rather rarely dispersed on the leaf surface. In the cross between them many types of leaf hairiness were observed which points on segregation in F_2 of several non-homoeologous genes. Two contrast phenotypes are presented on Fig.3, with hairiness more expressed than in parental cultivars and with extremely rare hairiness of both types. It should be noted that the gene for the long hairs in the line 102/00^i was localised in 7B chromosome (Pshenichnikova et al. 2007; Dobrovolskaya et al. 2007) Segregation for this character may testify for the presence of one more gene for leaf hairiness in the line 821 introgressed from *T. timopheevii*. This idea is supported by the preliminary results of genetic analysis of the trait in the cross between this line and bread wheat cultivar Hong-mang-mai which carries the same gene in 7B chromosome as the line 102/00^i (Dobrovolskaya et al. 2007). In this case, again the various leaf hairiness phenotypes segregated with the different expression of trichome density. Additionally, in this cross the F_2 plants were found with contrasting hairiness phenotypes on the upper and lower sides of the leaf blade (Fig 4, b and c). Further segregation of the trait in F_3 generation will be continued.

Taking into account a complexity of the trait both cytogenetic and molecular methods should be attracted to understand its genetic control.

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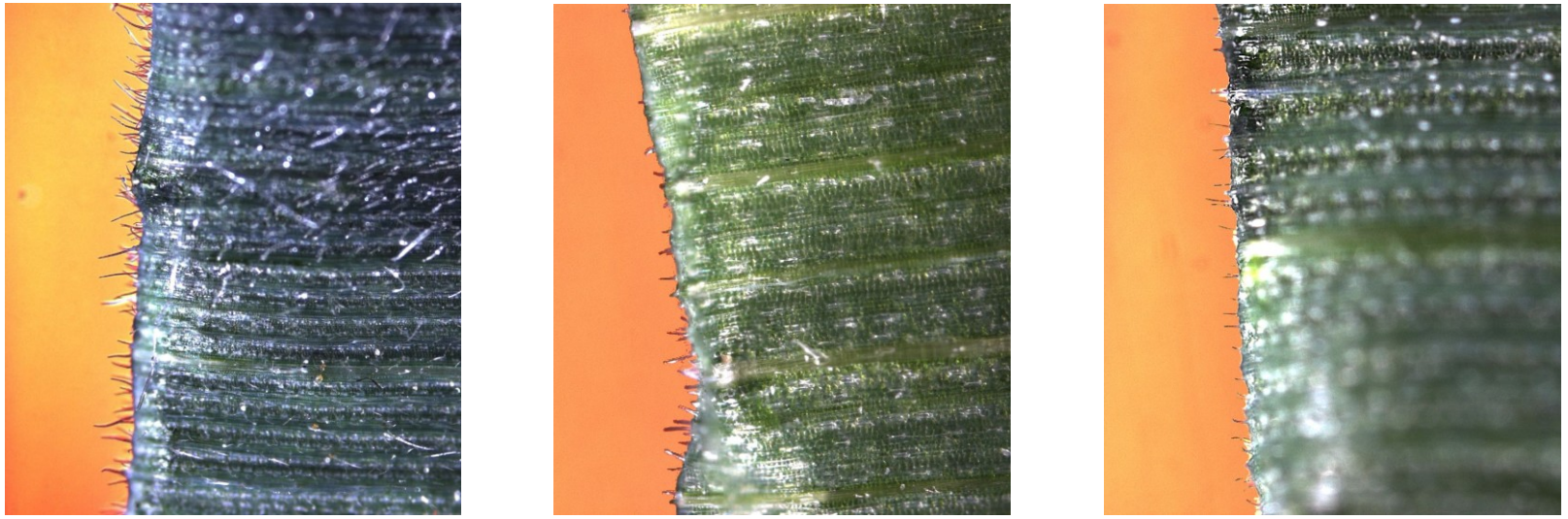

Fig. 1: Leaf hairiness (left to right) of bread wheat cultivars Saratovskaya 29 (S29), Janetzkis Probat (JP) and substitution line S29/JP 4B.

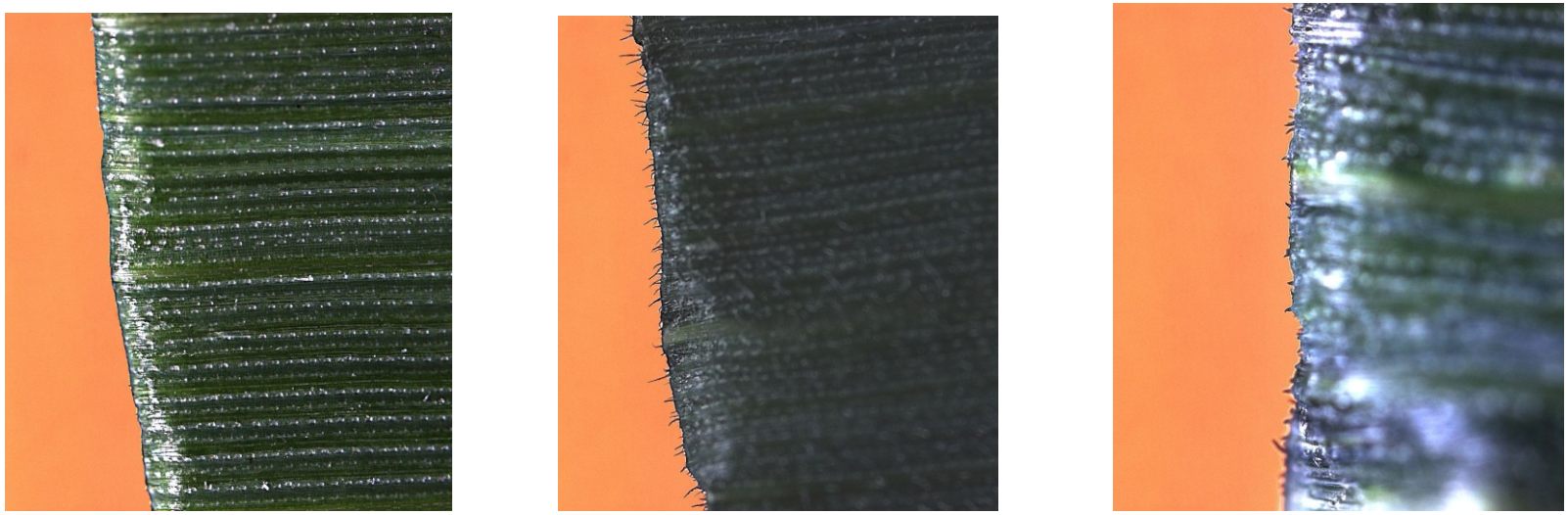

Fig. 2: Leaf hairiness (left to right) of bread wheat cultivar Rodina and two F_2 segregates from the cross Rodina × S29.

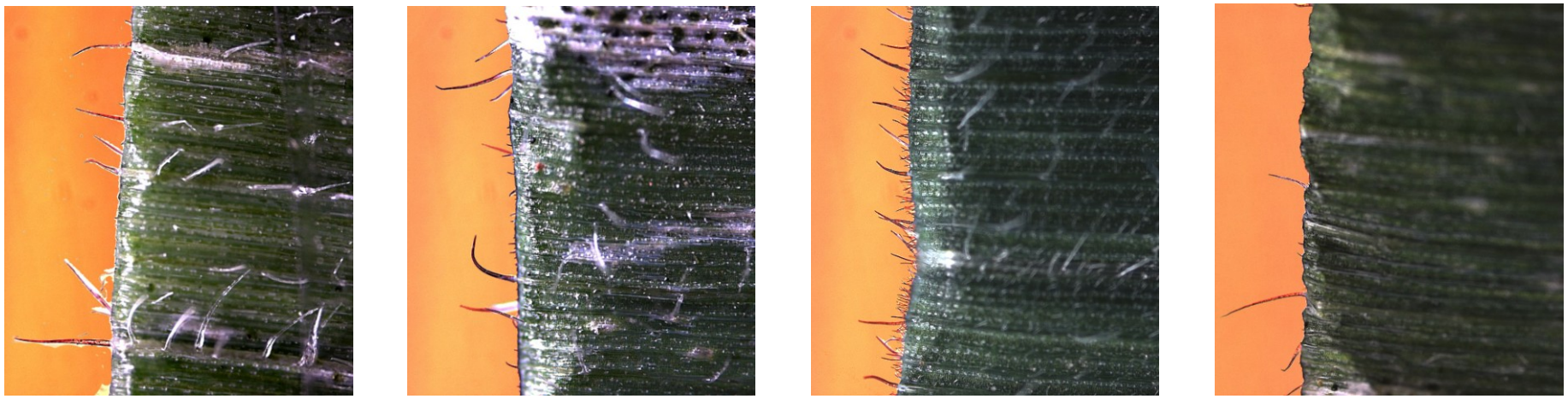

Fig. 3: Leaf hairiness (from left to right) of accessions 821, 102/00[i] and two F_2 segregates of the cross between them.

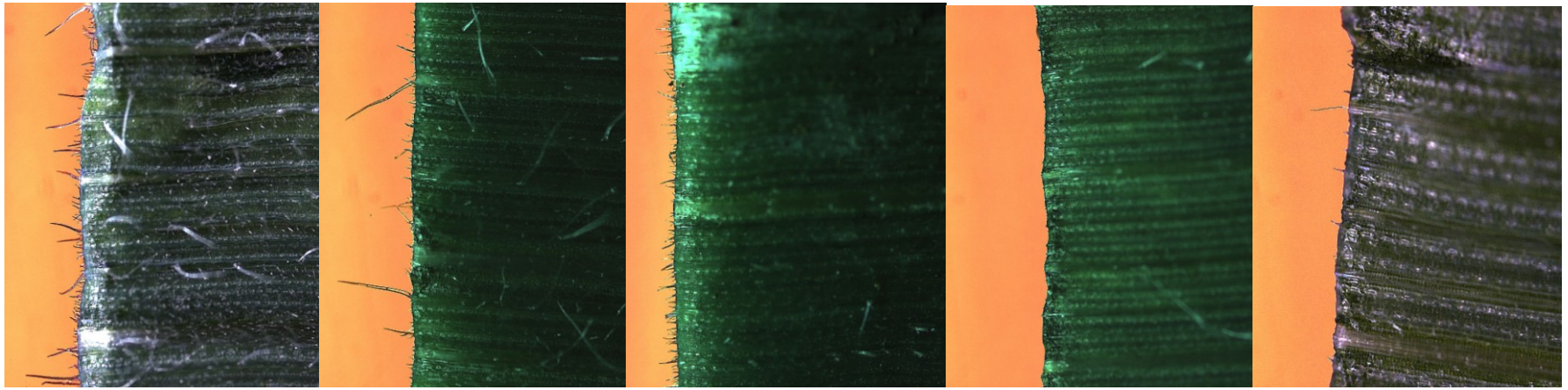

Fig. 4: Leaf hairiness (from left to right) of bread wheat cultivar Hong-mang-mai (a) and some F_2 segregates from the cross Hong-mang-mai × 821.

Chromosome substitutions lead to free-threshing habit in tritordeum

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Hexaploid tritordeums are the amphiploids derived from the cross between the wild barley *Hordeum chilense* Roem. et Schulz and durum wheat. At present, a breeding program is being carried out at the Institute for Sustainable Agriculture (IAS-CSIC) with dual purpose, to obtain plant materials useful for wheat breeding and to develop a new crop.

The grain and flour of tritordeums have comparable properties to that of bread wheat (Alvarez et al. 1995), but they have higher carotenoid content (Ballesteros et al. 2003, Atienza et al. 2007a) so grain production for food and feed would be its main use. The much higher content of lutein compared to durum wheat (Atienza et al. 2007a) may confer potential to produce functional foods from tritordeum grain.

The non-free threshing habit of this amphiploid limits its possibilities to become a new crop. Therefore, the free-threshing habit has been a main objective since the beginning of the tritordeum breeding program. To date, the free-threshing habit has not bean achieved *via* mutagenesis not by spontaneous mutation. All the tough plants that have been detected during the years have proved to be aneuploids with either 40 or 41 chromosomes.

During the last decade we developed a new crossing program between tritordeum and common wheat in order to develop D/(H^{ch}) chromosome substitution lines. In this work we report the development and characterization of free-threshing lines through chromosome substitution.

Cytological and molecular characterization

Three tritordeum accessions (HT374, HT376 and HT382) were selected. All three lines were derived after 6-7 selfing generations from crosses (tritordeum×bread wheat)×tritordeum and thus they were expected to be substitution lines involving D and H^{ch} chromosomes. These lines were characterized using cytological, GISH (Genomic In Situ Hybridization) and molecular methods. For the molecular characterization we used both chromosome-specific SSRs markers (Röder et al. 1998) and barley-ESTs markers developed by (Nasuda et al. 2005) and assigned to *H. chilense* chromosomes by Hagras et al. (2005) using *H. chilense* – wheat addition lines.

Different chromosome substitutions lead to free-threshing habit in tritordeum

The results revealed that HT382 is a double chromosome substitution line between *H. chilense* and the wheat D genome involving chromosomes 1D/($1H^{ch}$) and 2D/($2H^{ch}$) while HT374 and HT376 each have the pair of $5H^{ch}$ chromosome substituted by wheat 5D chromosomes.

The *H. chilense* genome is collinear to other Triticeae genomes, including *H. vulgare* and bread wheat (Hernández et al. 2001). This collinearity has been further demonstrated by the candidate gene approach (Atienza et al. 2007b) and by physical mapping of barley ESTs (Hagras et al. 2005). This collinearity suggests that the genetic systems controlling the free-threshing habit in this species may be similar to those reported in wheat.

The free-threshing lines reported in this work may suppose a substantial advance in the tritordeum breeding program.

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Pollen grain expression of osmotic adjustment in Romanian winter wheat

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Water stress is one of the most important yield limiting factors. Therefore, in Romania, improvement of drought resistance is one of the main objectives of breeding programs.

Of the various adaptation traits, osmoregulation is of particular significance because it is cellular process that is only activated when water stress occurs (Hellebust 1976). Osmotic adjustment is an adaptative response to water stress and involves an increase in the solute content of cells, leading to maintenance of turgor.

Morgan (1991, 1995) demonstrated that the differences in osmoregulation in wheat are conditioned by alternative alleles at a single locus (*OrOr* / *oror*) on the short arm of chromosome 7A, with high response being recessive. The different osmoregulation capacity of wheat genotypes are indicated by different response of pollen grains (morphological modification) in a stressing concentration of polyethylene glycol 6000 (PEG) following the application of a low concentration of potassium chloride (KCl).

Materials and methods

In this study we tested the osmoregulation capacity of 13 common wheat cultivars (11 from Romania and 2 from France) and 69 doubled- haploid lines obtained from a cross between a cultivar with high osmoregulation capacity (Izvor) and a cultivar with intermediary osmoregulation capacity (Jiana).

Pollen grains are usually approximately spherical/ ellipsoidal in shape. A stressing concentration of PEG (40%- 55%, depending by genotype) induce a maximum shrinkage of pollen grains assume a more conical shape, often with concavities.

In general 30% was the lowest concentration that could be tolerate without bursting, so we propose the PEG solution by 30% concentration like a non-stressing control solution.

Addition of KCl 10 mM in a stressing PEG solution induce a diminution of osmotic potential of glycol solution. The genotypes with alleles conditioning low osmoregulation show little response, whereas genotypes with alleles conditioning high osmoregulation show substantial response.

Pollen grains were stressed by immersing them in PEG solution with different concentration between 30% to 55%.

The pollen was sampled from anthers near the point of dehiscing. After a little agitation to release the pollen grains, the anthers section was removed and the solution covered with a cover slip. Slides were incubated at 20° C for 2 days. Microscopic observations were made using a magnification of 10X and 40X.

Results

Using PEG solution by different concentration, 30%, 40%, 45%, 50% and 55% we could differentiate 3 classes of osmoregulation capacity for the common wheat cultivars tested. The French cultivars tested (Renan and Bersee) and 2 Romanian cultivars, F4 and Doina showed grains shrinkage by immersing in a PEG solution by 40%, 7 Romanian cultivars (Alex, Ardeal, Boema, Delabrad, Dropia, Gloria, Iancu) present grains shrinkage on 45% PEG and only 2 cultivars, Izvor and Faur, present a pollen bursting to a 55% PEG solution.

The size differences at 55% with and without KCl was used for the identification of DH lines (obtained from a cross between the cultivar Izvor with high osmoregulative capacity and the cultivar Jiana with intermediary osmoregulative capacity) carrying the *or* allele.

The 69 DH lines was characterized from osmotic adjustment point of view in a sufficient water supply conditions. The percent of the lines with low osmoregulative capacity is few percentage more that what we were expecting, but the deviation is not significant adequate χ^2 test (probability bugger then 6% that the deviation to be accidentally) (Fig. 1).

The effect of water stress on yield increases produced by the selection for the osmoregulation gene "*or*" was examined using the 69 DH lines (Jiana x Izvor). The osmoregulation differences capacity (or gene) selected by pollen test were not relevant for yield (Fig. 2).

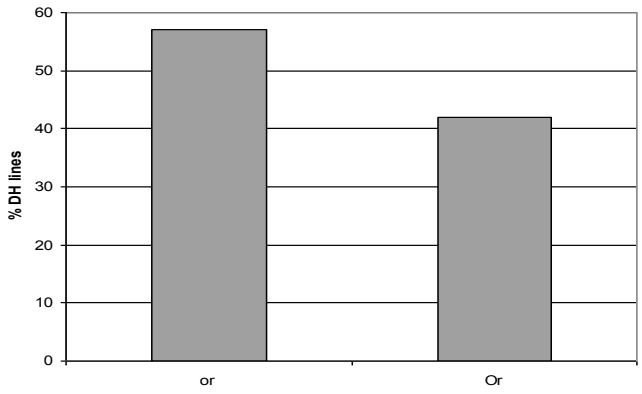

Fig. 1: The classification of DH lines regarding or gene presence estimated with pollen grain test

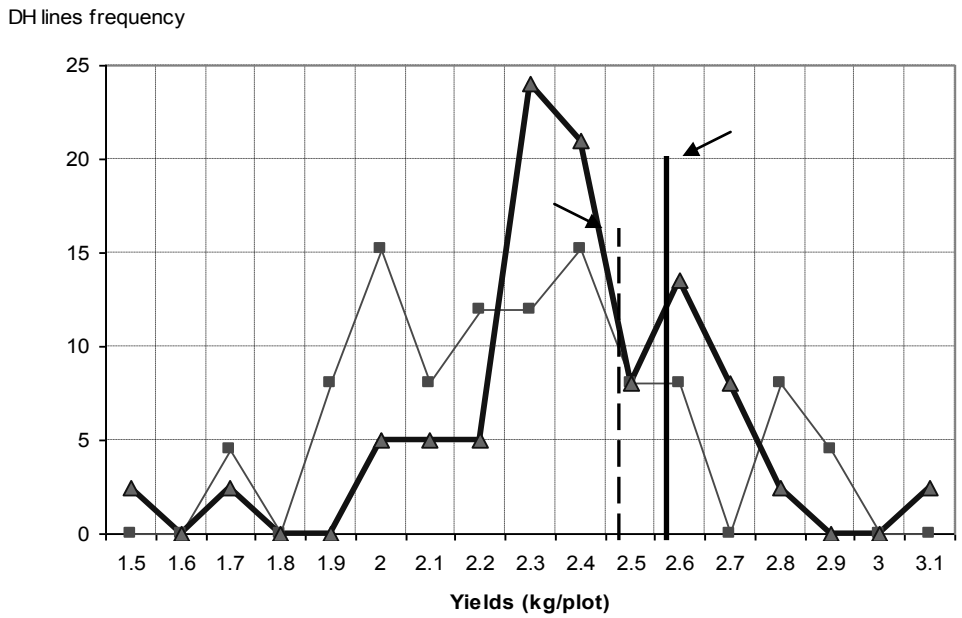

Fig. 2: Distribution of DH lines for yield plot in accordance with or gene presence

Conclusions

- The Romanian cultivars were differentiated in 3 classes of osmoregulative capacity
- Helped by method of pollen grain expression of osmotic adjustment we selected genotypes with high osmoregulative capacity that are implicated in Romanian breeding programs.

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Genetic collection of common wheat lines carrying adaptive morphological traits

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The collection of common wheat (*Triticum aestivum* L.) lines, created on basis of form Genotroph-1 (G-1) at the Institute of Biology and Biotechnology of Plants by Dr. of biologic sciences Elizabeth D. Bogdanova, is offered for use in adaptive breeding. Genotroph-1 is produced in the result of epigenetic variability induced with natural nicotinic acid at the cultivar of spring common wheat Kazakhstanskaya-126 (K-126). Offered collection lines are carrying definite adaptive morphological mark traits, such as:

- thick, rough pubescence of leaves, promoting to increase of dry resistance and protecting from leaf-gnawing pests;

- wax bloom at the different parts of plant influences light reflecting ability, temperature, water schedule photosynthesis, heat- and dry resistance;

- heightened level of anthocyanins synthesis in the tissues increasing both the covered smut resistance (*Tilletia caries*) and temperature negative impact;

- changing of leaves form and their spatial orientation for protecting from intensive insolation and re-heating and for prevention of great water loosing and promoting to long leaves functioning;

- high productive tilling capacity which is necessary for breeding of cultivars grown in conditions of ridge technology.

For increasing of wheat yield in Kazakhstan arid conditions it is necessary creating of cultivars which are resistant to biotic and abiotic environment factors. Our original genetic collection contains about 200 lines carrying definite morphological mark traits controlled with a small number of genes.

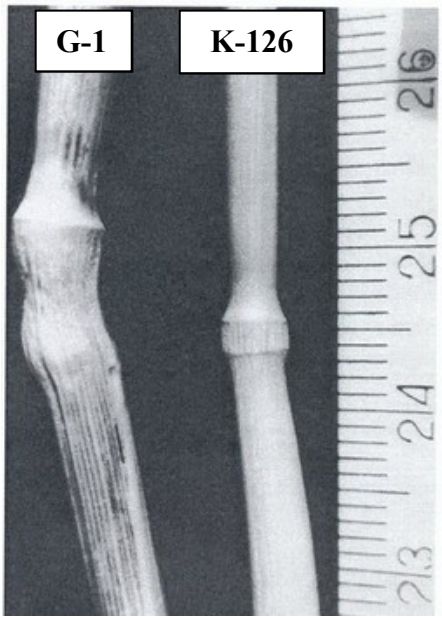
These lines were created on the base of Genotroph-1 induced with natural nicotinic acid. We have discovered that the treatment of seeds and vegetative plants of common wheat (*Triticum aestivum* L.) by nicotinic acid induces heritable epigenetics varieties.

Genotroph-1 induced by nicotinic acid has following morphological traits

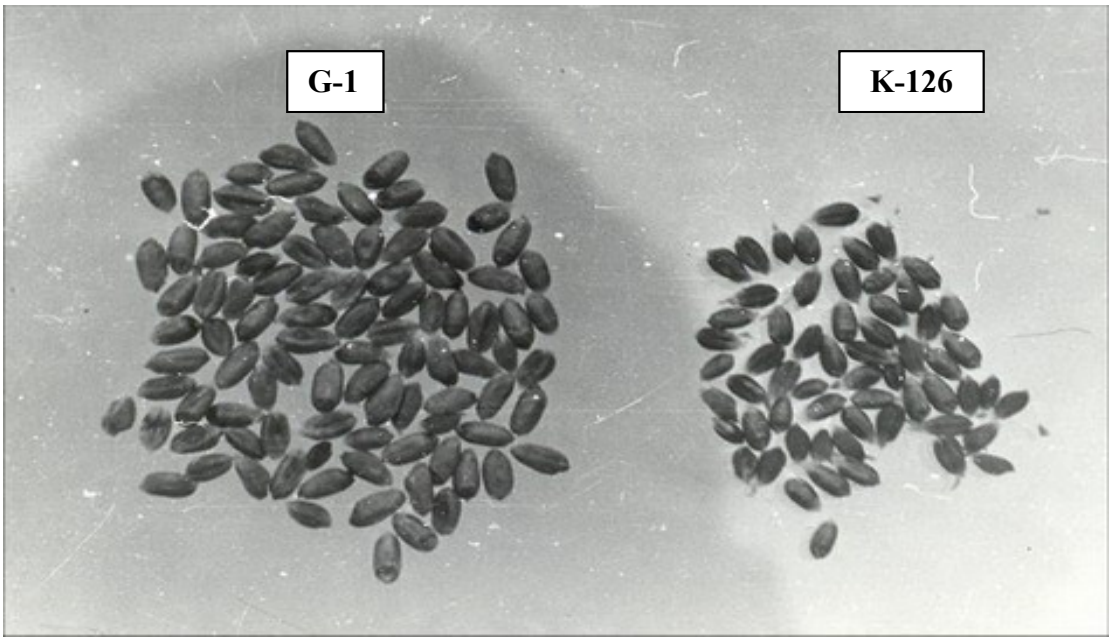
- higher and thick stem;
- over-growing and elongated knots;
- long spike;
- large grain;
- large and intensive pubescent blue-grey and green leaves;
- anthocyan dyeing of coleoptiles, anthers, leaves back side during tilling capacity phase and stem in the moment of ripeness.

Long spike

Over-growing and elongated knots, thick stem



Large grain



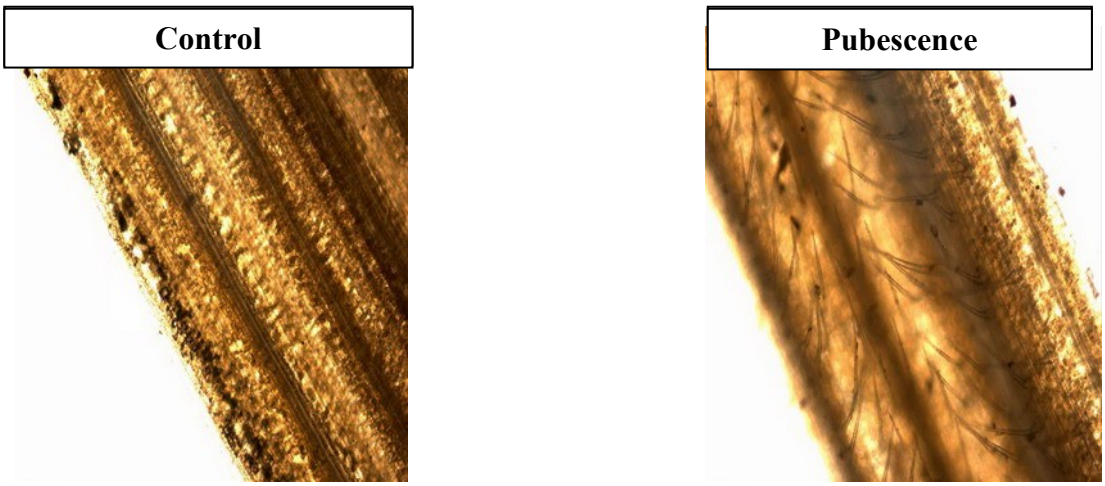
Genotroph-1 in comparing with initial form Kazakhstanskaya-126 is characterized with:

- high life ability;
- larger resistance to abiotic and biotic stresses;
- changed spectrum and activity of endogens growth regulators;
- high level of nicotinic acid in grain.

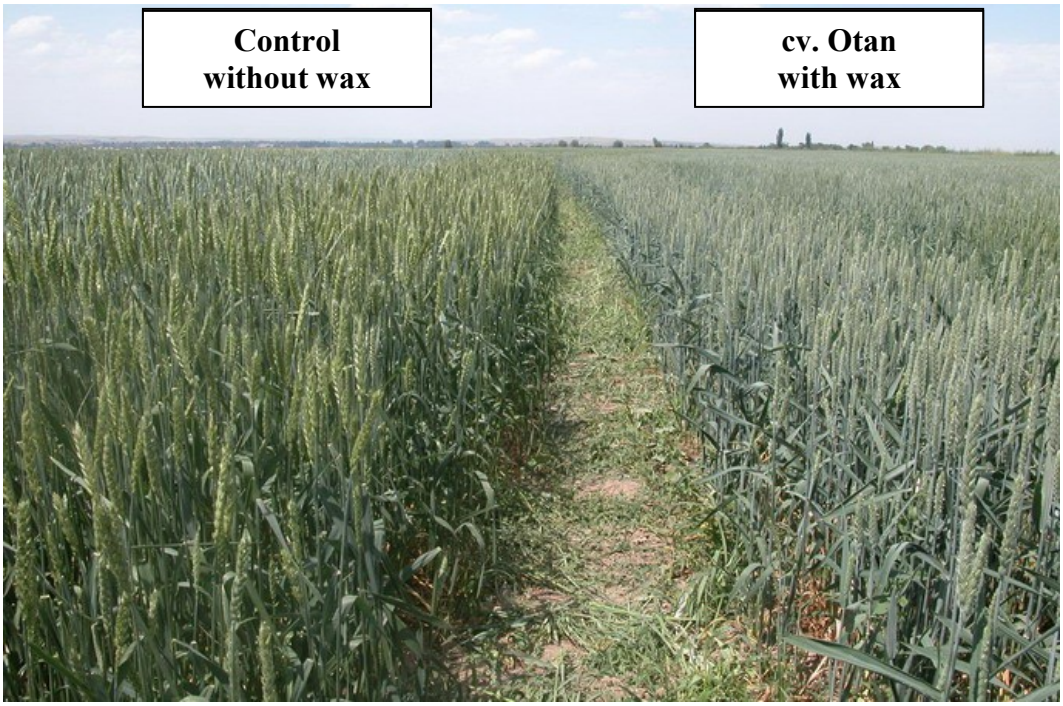
Initial cultivar K-126 contains 3.8±0.3 mkg per g of nicotinic acid in compare with Genotroph-1 which contains 10.5±0.6 mkg per g of nicotinic acid.

Lines from our collection carry these traits:

Thick rough pubescence of leaves provides dry resistance and pest protecting



Wax on the different parts of wheat plants provides high light reflecting ability, heat and dry resistance



Changing of plant architectonic, leaves form and their space orientation provides long functioning of leaves, protecting from the intensive insolation and water losing

Rolling leaf

Lowering flag leaf

Gostianum-88 | **F_1** | **Omskaya-9 (control – unlowering flag leaf)**

cv. Otan-1
with wax and rolling leaf

High productive tilling capacity is necessary for breeding of cultivars which are planted for ridge technology

Table 1: Elements of productivity of wheat dwarf-lines

Lines	Productive tilling capacity	Length, sm		Number of grain in the spike	Mass of 1000 grains, g
		stem's	spike's		
Dwarf-106	10.4±0.18	40.2±1.30	14.3±0.20	52.2±1.85	32.42
Erythrospermum-239	9.3±0.22	50.3±1.52	15.6±0.30	76.4±1.72	34.93
Milturum-301/85	12.3±0.20	52.2±1.40	15.8±0.25	72.3±1.78	38.40
Graecum-244	9.6±0.23	42.8±1.32	14.2±0.15	73.4±1.25	33.20
Lutescens-248	11.2±0.28	48.4±1.25	14.9±0.20	63.7±1.64	36.32
Pseudograecum -251	9.6±0.14	50.3±1.30	13.5±0.18	62.0±1.60	32.26
Erythrospermum-254	11.0±0.25	51.6±0.40	15.2±0.20	74.6±1.84	35.40
Bezostaya-1	6.2±0.23	98.4±1.50	9.0±0.18	43.0±1.90	45.20

Thus our original genetic collection of common wheat lines carrying definite morphological raits which are controlled by a small number of genes is offered for using in adaptive breeding.

Adaptation of leaf and stomata apparatus of bread wheat to drought by the intervarietal substitution of chromosomes

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Stomata apparatus of leaves plays the most important role in biomass production and water regulation processes and directly involved into adaptation of plant to drought. In this work the influence of intervarietal chromosome substitution in wheat on stomata density and leaves mass as well as its variability under water shortage was studied.

Materials and methods

Genetic material: Intervarietal substitution lines Chinese Spring/Synthetic involving chromosomes 4B, 4D, 5A and 5B of Synthetic (2n=42; *Triticum dicoccoides* x *Aegilops tauschii*, AABBDD) and recipient cultivar Chinese Spring (CS) were used. The seeds for investigation were kindly provided by Dr. Andreas Börner, IPK, Gatersleben, Germany

Experimental design: Plants were grown under the field conditions in East Siberia not far from Irkutsk. Two groups of plants of every genotype, control and experimental, were sown in soil watered on 80%. No more water supplies were given until tillering. After tillering the control variant was watered abundantly while the experimental variant received 1/10 of water supply. Both variants were protected from natural rainfalls. The prints were taken from the surface of the middle part of upper and lower sides of the leaf blade. The results of measuring of stomata density for three levels of canopy are presented beginning down from the flag leaf as well as weight of leaf dry mass for these leaves.

Results and discussion

In normal conditions only the line with 5A substitution from the Synthetic showed a constant exceeding in weight of dry leaf mass comparing to the recipient CS. Other lines have a lower meaning of this parameter except the flag leaf in the line CS/Synthetic 5B. Under water shortage all the lines demonstrated a substantial decrease of the leaf mass. Again the line with 5A substitution showed the surpass comparing to the other lines.

Under normal watering no significant differences were detected between the lines and the recipient on stomata density on both sides of the leaf (Table1). But the lines showed a various reaction on water deficit. Two lines with substitutions for 4D and 5B chromosomes increased stomata density on the upper side of flag and boot leaves in the first case and on the upper side of all three leaves. The effect was more expressed in 5B substitution line. The line with 4B substitution showed a decrease of this parameter on the lower side of the leaves (with exception of flag leaf). This data corresponds to the earlier obtained results. Davydov (2001) showed the important role of 4D chromosome in determining this character in monosomic lines of Chinese Spring and the 5th homoeological group was found to be responsible for this character in substitution lines Saratovskaya 29/Janetzkis Probat (Davydov et al. 2006).

Table 1: Number of stomata per square mm of leaf blade in the substitution lines and the recipient cultivar under different environment

Upper side

	Flag leaf		Boot leaf		Pre-boot leaf	
	normal	drought	normal	drought	normal	drought
Chinese Spring	65,0 ± 2,9	59,9 ± 5,0	56,3 ± 6,8	85,0 ± 5,6	51,9 ± 6,8	61,0 ± 3,3
CS/Synthetic 4B	61,1 ± 5,5	76,3 ± 7,6	49,3 ± 6,0	48,8 ± 4,4	41,0 ± 4,1	71,6 ± 9,3
CS/Synthetic 4D	65,1 ± 7,0	96,2 ± 6,3**	61,2 ± 6,4	118,7 ± 10,5***	42,7 ± 3,3	73,9 ± 4,3
CS/Synthetic 5A	67,1 ± 6,2	80,9 ± 3,2	48,6 ± 3,6	99,7 ± 4,6	47,9 ± 4,3	62,2 ± 7,1
CS/Synthetic 5B	56,7 ± 5,8	122,0 ± 2,8***	47,9 ± 5,6	104,9 ± 3,7***	44,2 ± 3,4	101,9 ± 14,3***

Lower side

	Flag leaf		Boot leaf		Pre-boot leaf	
	normal	drought	normal	drought	normal	drought
Chinese Spring	35,6 ± 6,4	42,0 ± 3,5	39,4 ± 4,3	31,9 ± 8,5	40,6 ± 5,3	28,6 ± 5,7
CS/Synthetic 4B	32,7 ± 7,4	34,1 ± 8,6	35,1 ± 5,0	14,7 ± 5,5***	31,0 ± 5,1	17,9 ± 3,3***
CS/Synthetic 4D	43,8 ± 1,7	43,8 ± 6,2	41,5 ± 6,1	32,9 ± 10,4	32,2 ± 6,3	32,1 ± 6,5
CS/Synthetic 5A	40,0 ± 5,6	40,3 ± 5,0	40,8 ± 3,7	34,6 ± 3,0	36,0 ± 2,9	25,0 ± 3,9
CS/Synthetic 5B	40,9 ± 6,0	27,6 ± 14,6	35,8 ± 4,5	32,7 ± 5,4	30,5 ± 3,6	44,6 ± 9,0

, * - $P<0,01$; $P<0,001$

The complex characters with polygenic inheritance were studied in this investigation. Nevertheless, the chromosome substitution allows identifying the "critical" chromosome, carrying the genes responsible for adaptation processes. To this moment the introgression lines have been obtained for chromosomes of CS/Synthetic substitution set involved in this study (Pestsova et al. 2006; A. Börner, personal communication). Earlier, using this material several QTLs were discovered responsible for a number of quantitative traits determining plant productivity. This makes possible to map as a QTLs the position of the genetic factors associated with adaptability to drought. It is known that the high stomata density characteristic of the species from droughty environment. D-genome of Synthetic was introduced from *Aegilops tauschii* and B-genome from *T. dicoccoides*. It may be supposed that the potential for physiological adaptation to abiotic stresses was introduced into substitution lines from these wild bread wheat relatives. It is interesting to note that our investigation implies the possibility of participation of different factors in genetic control of stomata formation on upper and lower sides of one leaf blade which may promote the understanding of the developmental processes in ontogenesis.

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Study of multiple allelism of the *Vrn-1* locus in common wheat

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Vernalization requirement in common wheat is mainly controlled by three orthologous *Vrn-1* genes located on chromosomes 5A, 5B and 5D (Law et al. 1976, Snape at al. 2001). The *Vrn-A1, Vrn-B1* and *Vrn-D1* genes are dominant for spring growth habit and epistatic to the alleles for winter growth habit (Pugsley 1971). Genotypes with the *Vrn-A1* and/or *Vrn-B1* dominant alleles are the most frequent in spring wheat cultivars throughout the world, including Russia. The old wheat cultivars are typically late. The recently observed trend in commercial cultivars is their increased earliness accompanied by the loss of the weak vernalization sensitivity-determining allele of *Vrn-A1*.

Multiple allelism for *Vrn* genes was demonstrated by the means of substitution lines for group 5 chromosomes, (Snape et al. 1976, Law et al. 1997, Roberts and McDonald 1984, Maystrenko 1992). Using substitution lines of Saratovskaya 29 (S29) and Diamant (Dm), where chromosome 5A was replaced with its homologues from the winter cultivars Mironovskaya 808 (M808), Skorospelka 35 and Odesskaya 51 the presence of *Vrn-B1a* and *Vrn-B1b* alleles established at the *Vrn-B1* locus on chromosome 5B was demonstrated (Maystrenko 1992). Wheat cultivar S29 and Dm both have the *Vrn* genotype (*Vrn-B1, Vrn-B1, vrn-D1*) and do not differ significantly in ear emergence time. In Dm and S29 substitution

lines for chromosome 5A dominant *Vrn-A1* allele was replaced by its homologue from the winter wheat cultivars. Thus, the substitution lines contain only the single dominant allele at *Vrn-B1* on chromosome 5B. However, the substitution lines derived from these varieties and having the *vrn-A1, Vrn-B1, vrn-D1* genotype differ in ear emergence time by 10 – 12 days. Based on the obtained data, it was concluded that there are at least two alleles at the *Vrn-B1* locus - *Vrn-B1a* and *Vrn-B1b*. The expression of the *Vrn-B1a* allele is stronger than that of *Vrn-B1b.* However, the genetic analysis of alleles of the *Vrn-B1* locus was performed in the genetic environment of the spring varieties S29 and Dm as recipients in the absence of the main gene *Vrn-A1*.

To enable the experimental clarification of multiple allelism at *Vrn-B1* locus, substitution and near-isogenic lines were required as novel genetic models. Two substitution lines, Dm/M808 5A and S29/M808 5A, were used as donors of different *Vrn-B1* alleles. At first stage we developed two lines, in which chromosome 5B of the winter cultivar Sava was replaced by its homologue in the spring varieties S29 (*Vrn-B1a*) or Dm (*Vrn-B1b*). Sava's monosomic line kindly provided by Tony Worland was used as recipient. The recipient variety Sava developed in Yugoslavia is insensitive to photoperiod. The substitution lines were obtained by a classical scheme using disomic plants derived from self-pollination of monosomic plants as the parental forms. The final backcross was BC_7. In each $BC_{1\text{-}7}F_2$ generation, 10 cytologically tested disomic plants were selected and their ear emergence time was measured. At second stage, we developed two near-isogenic lines from the winter cultivar Bezostaya 1. Near-isogenic lines carry different alleles of the dominant gene *Vrn-B1* of S29 (*Vrn-B1a*) or Dm (*Vrn-B1b).* The recipient variety Bezostaya 1 was developed in Russia and is characterized by early sensitivity to photoperiod. F_1 plants and their successive backcross generations were grown in a greenhouse without vernalization and under selection for short ear emergence time. The parental forms used for the development of the near-isogenic lines were plants with spring growth habit. Homozygous plants were selected in progenies of $BC_8F_{2\text{-}4}$**.** In every generation 20 plants were sowing and heading time determining.

Data for heading time of substitution lines Sava/S29 5B and Sava/Dm 5B $BC_{5\text{-}7}F_2$ under greenhouse condition are presented in Fig. 1. It was shown that in Sava/S29 5B line carrying allele *Vrn-B1a* ear appeared to 45 days whereas in Sava/Dm 5B with *Vrn-B1b* allele to 55 days. The data received on the disomic $BC_{5\text{-}7}$ F_2 progeny suggested that the substitution lines Sava/S29 5B and Sava/Dm 5B differ in ear emergence time in 7 – 12 days.

Fig.2 demonstrated the data for heading times of near-isogenic lines in the greenhouse. Comparison of the ear emergence times of Bezostaya 1 (*Vrn-B1a)* and Bezostaya 1 (*Vrn-B1b)* in two experimental conditions revealed significant 19-day difference between these two lines. The mean ear emergence time for the Bezostaya 1-derived near-isogenic lines carrying the weak *Vrn-B1b* allele, is 78 days, comparing with *Vrn-B1a* line which has 59 days. The difference for this trait between Sava's substitution lines and Bezostaya 1 near-isogenic lines, probably results from photoperiod sensitivity of cultivar-recipients. For further investigation of photoperiod sensitivity it is necessary to performed experiments under controllable conditions.

Conclusion

Excitement over the multiple allelism of the *Vrn* genes is because of the genes determining the length of the vegetative period have a pleiotropic effect on many agronomically important traits. Based on the evidence received by us, ear emergence time is polymorphic in wheats at least because of the presence of the different alleles of the dominant *Vrn* genes. It is hypothesized that neither old nor modern Russian varieties possess the weak *Vrn-A1* gene allele, probably due to climatic conditions and cropping practices (Goncharov 2002). *Vrn-A1a*

allele was present in more than half of the spring varieties released in the United States and Argentina between 1970 and 2000 (Yan et al. 2004). It has also been established that three out of five assayed early varieties from CIMMYT carry this allele (Yaqui 54, Sonora 64 and Siete Cerros 66). Spring growth habit is a trait that is controlled in as few as 2% of the modern Russian varieties by one dominant *Vrn-B1* gene and in 64 %, by this gene in cooperation with other *Vrn* genes (Goncharov 2002). Because the *Vrn-A1* gene has an epistatic effect on the other dominant *Vrn* genes (Pugsley 1971), its presence, wherever this is the case, hampers the study of the phenotypic manifestation of the *Vrn-B1* gene. The genetic models that we developed - the substitution lines and the near-isogenic lines - carry two alleles, *Vrn-B1a* and *Vrn-B1b,* replaced with recessive alleles from winter wheats at the *vrn-1* loci. Our observation provides further experimental support to the results obtained by O.I. Maystrenko on multiple allelism at the *Vrn-B1* locus. Our data evidenced that cultivar S29, Pyrothrix 28, Janetzkis Probat and Omskaya 9 carry allele *Vrn-B1a*. Except Dm, old Siberian cultivar Milturum 321 and its offspring Milturum 553 also contain allele *Vrn-B1b*. It is expected that the use of different alleles of the dominant *Vrn* genes will improve chances to study and control the length of the vegetative period, which is an important point in plant breeding practice.

Acknowledgments

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EST-based analysis of salt stress-induced gene expression in Turkish bread and durum wheat cultivars

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Expressed sequence tags analysis is an effective method in discovering novel genes and investigating gene expression (Adams et al., 1991) in different plant organs and tissues in response to environmental stresses. In our previous work, 136 seedling and 268 root contig tags were selected and assembled from salt stressed *Triticum aestivum* EST database (http://wheat.pw.usda.gov/cgi-bin/westsql/est_lib.cgi). Now, all these contig tags have been analyzed using BLASTX algorithm for functional annotation. Based on these results, 23 different functional categories were identified in root and seedling tissues. Under each category, the genes (expressed in wheat under high salinity) with high sequence similarity to the contig query were selected and tabulated. According to BLASTX results, 9 contig tags with high sequence hit (300 or up) to the corresponding genes were assigned and specific sense-antisense primers were designed to investigate expressional differences in bread and durum wheat genotypes. Turkish bread wheat (*Triticum aestivum* cvs. Alpu01, ES14) and durum wheat (*Triticum durum* cvs. Ç1252, Meram) genotypes were subjected to gradual salt stress over 3 days until a final concentration of 150 mM NaCl was achieved. Leaf and root samples were harvested at 8h and 27h following stress treatment for RT-PCR analysis. Preliminary RT-PCR results indicated that salt tolerant bread and durum cultivars showed expression profiles in salt-treated seedling tissues at the end of 8h and 27h stress application in leaf specific primer combinations. Detailed quantitative expression analysis by Real-Time PCR analysis are currently under investigation. These results will be valuable to understand the mechanism of salt stress tolerance in wheat cultivars.

Assembly of contigs and primer synthesis

In our previous work, 136 seedling and 268 root contig tags were selected and assembled from salt stressed *Triticum aestivum* EST database (http://wheat.pW.usda.gov/cgi-bin/westsql/est_lib.cgi). All these contig tags have been analyzed using BLASTX algorithm for functional annotation (Table 1) (Altschul et al., 1990) and 23 different functional categories were identified in root and seedling tissues. According to BLASTX results, 9 contig tags with high sequence hit (300 or up) were assigned and specific sense-antisense primers were designed to investigate expressional differences under salt stress by RT-PCR.

RT-PCR analysis showed that the expression of glutamine synthetase isoform GSR2 (AAR84348) identified with Contig4 sequences and phosphoethanolamine methyltransferase (AAL40895) identified with Contig7 sequences were amplified efficiently only in salt treated seeding samples at the end of 8 and 27 hours of stress application (Figure 1). But no expression differences were detected with root specific primers.

Table 1: Major functional categories

Major functional categories	Leaf		Root		Total		Major functional categories	Leaf		Root		Total	
	n	%	n	%	n	%		n	%	n	%	n	%
Protein degradation			1	1,12	1	0,83	Transport faciliation	2	6,45	5	5,61	7	5,83
Signal Transduction	1	3,2	2	2,47	3	2,5	Cellular nitrogen metabolism			1	1,12	1	0,83
Energy metabolism			4	4,49	4	3,33	Post-transcriptional regulation			2	2,47	2	1,66
Translation	2	6,45	8	8,98	10	8,33	Plant development	1	3,2	6	6,74	7	5,83
Protein folding			1	1,12	1	0,83	Endosperm developing			1	1,12	1	0,83
Transcriciption			3	3,37	3	2,5	Metabolism	4	12,90	9	10,11	13	10,83
Detoxification	3	9.67	4	4.49	7	5.83	Protein synthesis			2	2.47	2	1.66
Transferases			1	1.12	1	0.83	Kinases	1	3.2	1	1.12	2	1.66
Cell cycle	1	3,2	5	5,61	6	5	Oxidative stress	2	6,45	3	3,37	5	4,16
Cell division and growth	4	12.9	4	4.49	8	6.66	Photosynthesis	2	6.45			2	1.66
Lignin biosynthesis			1	1.12	1	0.83	Light regulated	1	3.2			1	0.83
Cell resque and defence	7	22,58	15	16,85	22	18,33	No hits			10	11,23		
Total	31	100	89	100	120	100							

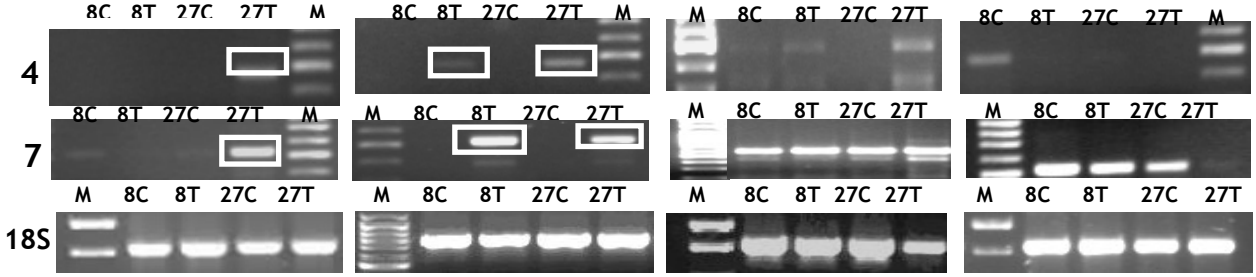

Fig. 1: RT-PCR analysis

Real-time PCR

Real-time PCR analysis (Ma et al., 2006) was performed with ABI 7300 Sequence Detection System by using SYBR® Green I. Data were analyzed using the SDS 2.0 software (Applied Biosystems). All amplification plots were analyzed with an Rn threshold of 0.2 to obtain Ct (threshold cycle) value. Relative gene expression of treated and untreated samples was normalized to the GAPDH gene as endogenous control. Figure 2 shows the fold difference in gene expression ($2^{-(\Delta\Delta CT)}$) between salt-treated and untreated samples was given in $\log_2$ scale. Treated sampes of Alpu and Meram showed high expression with Contig4 and 7 relative to the control samples.

Conclusion

The genetic information obtained from data mining of ESTs can make huge contribution to the characterization of genes in response to salinity stress, and is strongly expected to aid our understanding of the molecular mechanism of salinity stress tolerance of durum and bread wheat.

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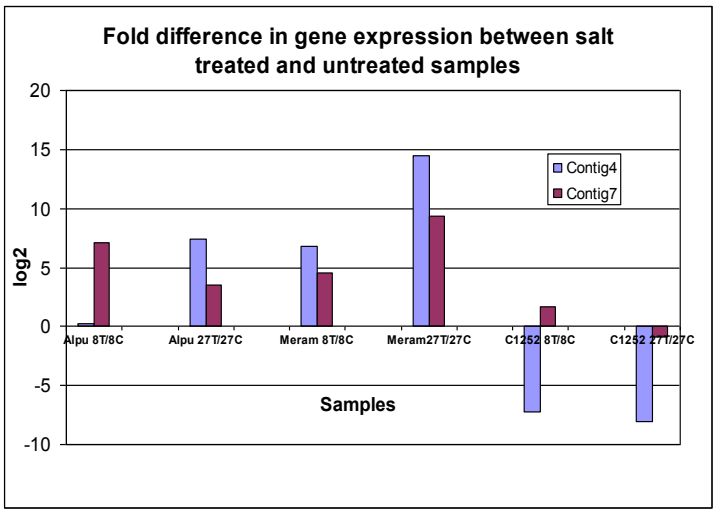

Fig. 2: Fold difference in gene expression

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Chromosome substitution as the possibility of regulation of bread wheat agrochemistry status

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At present, polygenic control of agrochemistry indices of wheat plant is found. Chromosomes of all three genomes of hexaploid wheat take part in the control of uptake, translocation, metabolism and utilization of mineral nutrition elements (Kiss et al., 1983; Manyowa, Miller, 1991; Gamzikova, 1994 and others). In this report the effect of intraspecific and intergeneric chromosome substitution on bread spring wheat agrochemistry status is considered.

Nitrogen

Study of two sets of substitution lines of bread wheat, obtained at the Institute of Cytology and Genetics, SB RAS (Maystrenko, 1987), allowed to find effects of individual chromosomes on such indices as uptake and consumption of soil and fertilizer nitrogen, nitrogen concentration in tissues, protein content and plant productivity. In the first set of lines chromosome 5A of bread spring wheat recipient cultivar Saratovskaya 29 (S29) was

substituted for the homologous from ten winter wheat donor cultivars. In the second set seven S29 chromosomes were replaced by homologous chromosomes of the spring cultivar Janetzkis Probat (JP). Field studies of many years demonstrated that genes of chromosome 5A from winter donors had the maximum effect in the genetic environment of the spring cultivar S29. Being more productive even under the conditions of scarce nitrogen supply (yield exceeding over the recipient by 21-86%), the most of S29/winter wheat 5A substitution lines as well demonstrated a significant advantage in various schemes of nitrogen fertilization input (table 1).

Table 1: Grain productivity of recipient cultivar Saratovskaya 29 and S29/winter wheat 5A substitution lines on nitrogen nutrition levels, g / plant

Genotype	N_0	N_{60}	N_{120}
S29	1.46	1.76	1.64
S29/ Avrora 5A	1.90*	2.44*	1.97*
S29/ Albidum 11 5A	1.84*	2.25*	2.15*
S29/ Iljichovka 5A	2.05*	2.62*	2.39*
S29/ Kavkaz 5A	1.77*	1.75	1.46
S29/ Lutescens 230 5A	2.14*	3.06*	2.55*
S29/ Mironovskaya 808 5A	2.06*	2.56*	2.96*
S29/ Mironovskaya 808 (improved) 5A	2.09*	2.20*	2.25*
S29/ Mironovskaya Ubileinaya 5A	2.71*	2.79*	2.40*
S29/ Ulianovka 5A	1.84*	2.29*	2.01*
S29/ Trible Dirk 5A	1.96*	2.54*	2.55*

* significant at $P<0.05$

The best substitution lines exceed the initial cultivar in fertilizer nitrogen uptake (35-39 vs 19 mg per plant in the recipient cultivar). Moreover, the percentage of fertilizer nitrogen in overall consumption by these lines is also higher (36-39 vs 30% in S29), and the index of fertilizer nitrogen utilization increase twofold.

According to the experiment results the contribution of 5A chromosome, introduced into the recipient S29 from the intensive spring cultivar JP, is less significant. The advantage of the substitution line S29/JP 5A over the recipient manifests itself only at high nitrogen fertilizer application and comprises 15-17% for grain weight per plant (table 2).

The same nutrition background revealed a significant positive effect of the donor chromosomes 1D and 7D (14 and 24%, respectively). Under the same conditions, three lines displayed significant negative deviations from the recipient cultivar, caused by the substitution of 4A, 6D, and 7B chromosomes. The line S29/JP 3A had the same productivity as the recipient cultivar. None of the investigated lines reached the level of the donor cultivar for grain weight under applied nutrition backgrounds. This provides another piece of evidence for participating of loci of numerous chromosomes in nitrogen nutrition of wheat.

Table 2: Grain productivity of parental cultivars and Saratovskaya 29/ Janetzkis Probat substitution lines on nitrogen nutrition levels, g / plant

Genotype	N_0	N_{60}	N_{120}
Saratovskaya 29	1.86	2.47	2.43
Janetzkis Probat	2.20*	3.15*	3.74*
S29/ JP 1D	1.80	2.37	2.78*
S29/ JP 3A	1.52	2.21	2.19
S29/ JP 4A	1.52	2.39	1.88*
S29/ JP 5A	1.90	2.46	2.84*
S29/ JP 6D	1.52	1.29*	1.44*
S29/ JP 7B	1.60	2.08*	2.03*
S29/ JP 7D	1.56	2.69	3.01*

* significant at $P<0.05$

Potassium

The results of investigation of substitution lines S29/JP under the elevated potassium nutrition background demonstrate that intervarietal chromosomes substitution leads to the alteration of productivity process rates and potassium use efficiency (Gamzikova, Mitrakova, 2006). Chromosomes 1A, 4B, 4D, 5A, and 5B demonstrate the highest effects, providing the increase of grain yield per plant under the different potassium nutrition background from 10 to 62% (figure 1). Positive effects of this chromosomes are realized through the different yield components: the number of grains (1A, 5A, 5B chromosomes), 1000 grain weight (1A, 4B, 4D), and the productivity tillering (1A, 5B). At the same time the alterations of the indices of plant potassium consumption, such as percentage of potassium in the straw and potassium use efficiency are found. The change of chromosome effect on the productivity depending on the growth conditions was observed in the experiments. Under the favorable conditions the functioning of chromosome 1D led to the increase of grain yield to 21-25%, while under the moisture deficit and high air temperature the line S29/JP 1D decreased productivity to 12-17% at all potassium nutrition backgrounds.

The influence of intergeneric chromosomes substitution on the adaptability of wheat plant under the elevated potassium levels was studied using the wheat/rye substitution lines. In these lines 5A chromosome of spring cultivars was substituted for 5R chromosome of spring rye cultivar Onokhoiskaya (Efremova et al., 1996). The recipients were bread spring wheat cultivars Saratovskaya 29 (S29), Omskaya 9 (Om9), Janetzkis Probat (JP), Hybrid 21 (H21) and nearly isogenic line ANK 18 (A18). Three types of 5A/5R substitution effects were determined: indifference, increase and decrease of the character. In the control (without potassium) in the most cases the rye chromosome transfer has led to significant advantages in productivity of substitution line over the recipient (table 3). The grain yield exceeding per plant were 27.1-89.0% and maximum exceeding were observed in line Om9 5R(5A).Under the elevation of potassium nutrition level the grain yield exceeding fluctuated from 30 to 51%. The effect of the rye chromosome in the genotype of the line ANK 18 consisted in decrease of productivity of the substitution line (from 15 to 16%). The reason may be in the inhibition of is productivity rates by the increased potassium level, and (or) in the disturbance of the ratio of available macroelements in soil. The observed alterations in grain productivity of investigated lines relatively to the initial cultivars were determined by the effect of 5A

chromosome substitution on such process as the fertility and grain formation. Mainly the 1000 grain weight determined the advantages of substitution lines developed for the cultivars S29 and H21 from 27.4 to 51.7%. In lines Om9 5R(5A) and JP 5R(5A) the rye chromosome ensured the positive effect on two yield components (number and 1000 grain weight), providing the highest advantages of this lines over the parental cultivars in grain productivity (49.1-89.1%)(figure 2).

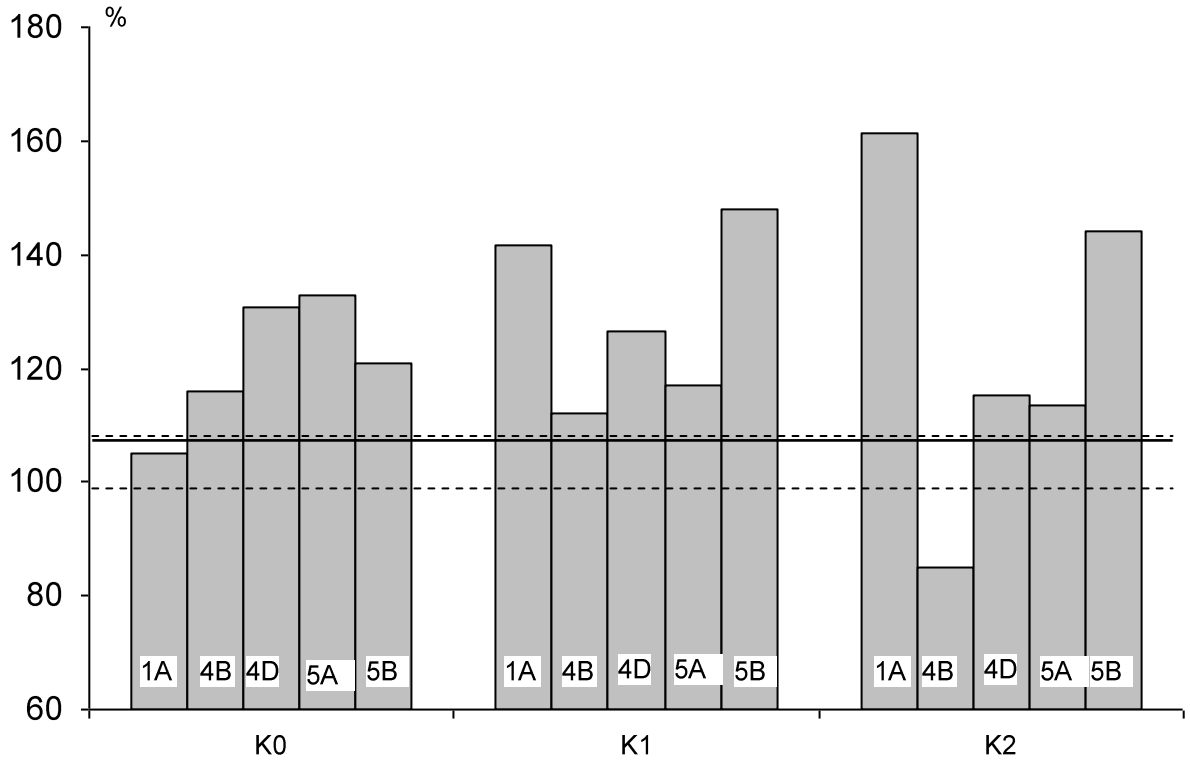
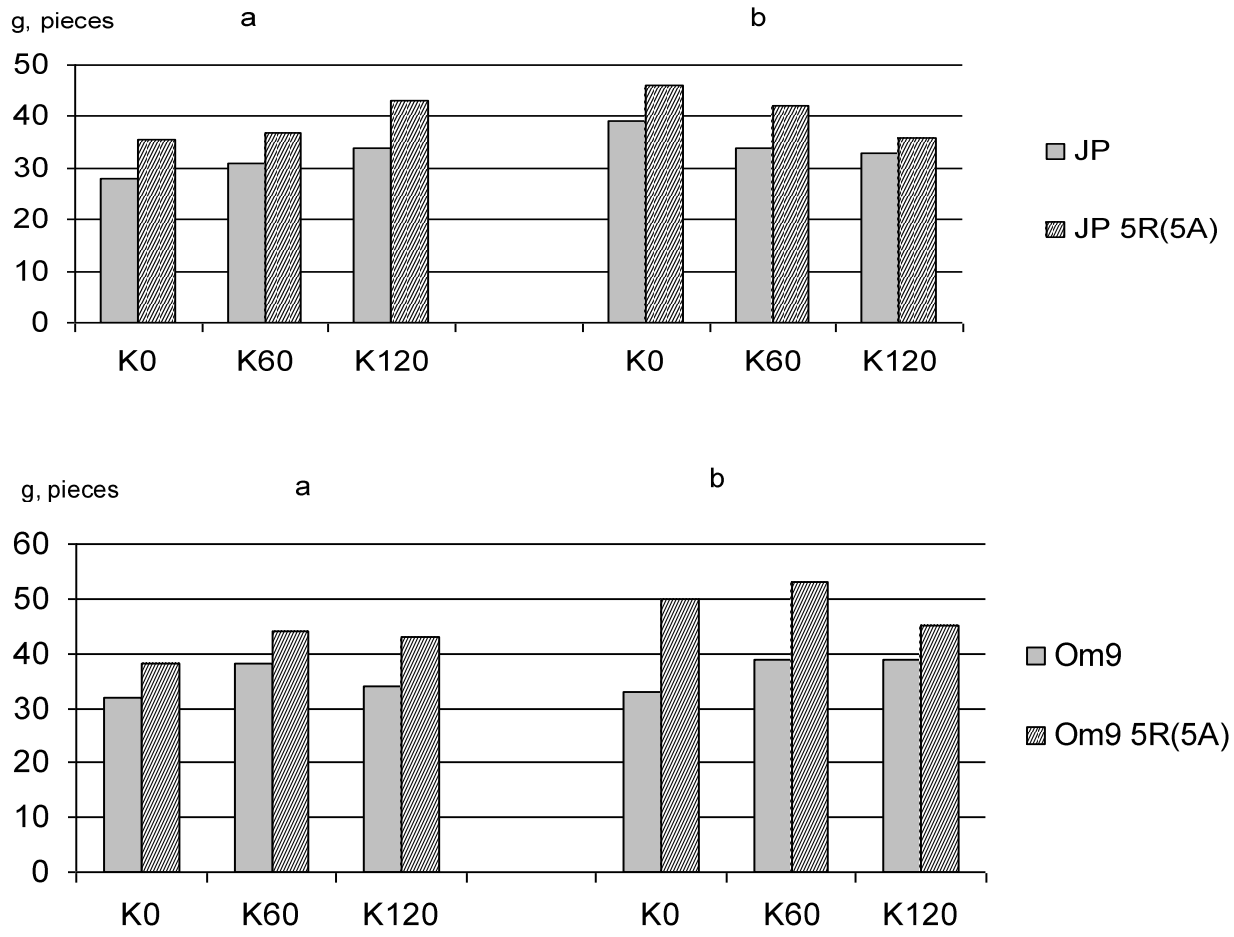


Fig. 1: Chromosomes that demonstrate the highest effect on grain productivity on potassium nutrition levels, % relatively recipient cultivar Saratovskaya 29

Table 3: Grain productivity of parental cultivars and wheat/rye substitution lines on potassium nutrition levels, g / plant

Genotype	K_0	K_{60}	K_{120}
Saratovskaya 29	1.13	1.12	1.32
Saratovskaya 29 5R(5A)	1.44*	1.46*	1.45
Omskaya 9	1.10	1.66	1.43
Omskaya 9 5R(5A)	2.08**	2.51**	2.03**
Janetzkis Probat	1.23	1.12	1.22
Janetzkis Probat 5R(5A)	1.74*	1.67*	1.65*
ANK 18	1.18	1.32	1.36
ANK 18 5R(5A)	1.50*	1.11	1.15
Hybrid 21	0.98	0.95	0.89
Hybrid 21 5R(5A)	1.00	1.43*	1.35*

* significant at P<0.05
** significant at P<0.01



a – 1000 grain weight, g; b – number of grains, pieces

Fig. 2: Influence of potassium nutrition level on the yield components of parental cultivars and substitution lines

It is necessary to say that high potassium background limits the increasing of grain number in ear, but not the 1000 grain weight. The effect of chromosome substitution 5A/5R on the number of productivity stems of wheat plant was not revealed.

Transfer of 5R chromosome practically had no influence on grain macroelement status of investigated cultivars. The exclusion was the increase of grain protein content under the high potassium background (3.16% vs 2.73%) in substitution line of cultivar JP. From the other side, a decrease of potassium concentration independently from the nutrition level was observed. In particular, it is suggests that the inhibition of potassium translocation flow from vegetative organs to grain takes place. Thus, the present investigation suggests that chromosome 5R change the constituents of potassium status of common spring wheat, increasing not only its requirements in potassium but the efficiency of element utilization for creation of specific yield.

According the obtained results the intergeneric chromosome substitution (under the choice of the components of donor-recipient system) may be more resultive than the intraspecific one.

The above experiments prove that the contribution of concrete donor chromosome is determined by the donor chromosome, its interaction with gene pool of a recipient, and the severity of environmental factors.

Conclusions

The practical application of chromosome substitution method has discovered the large possibilities in the creation of agrochemically effective wheat genotypes. They rationally use the mineral elements from fertilizers and soil, including those poor available compounds for plants. The results give hope that recipient and donor cultivars can be chosen so that their combinations would result in the genotype possessing a set of commercially important characters, including those responsible for uptake and utilization of mineral nutrients in production process. Of great importance is the use of recipient cultivars best adapted to the local complex of biotic, abiotic, and edaphic environmental factors.

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Allelic variation at the *Xgwm261* locus in Polish hexaploid wheat (*Triticum aestivum* L.) cultivars

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Dwarfing or reduced height genes (*Rht*) have been associated with large increases in the yield potential of wheat and have been a key component of the Green Revolution. Dwarfing genes were introduced in wheat breeding programs since begin of 20th century. Most current wheat cultivars contain GA-insensitive *Rht-B1b* or *Rht-D1b* genes, which were transferred from the Japanese cultivar 'Norin 10' (Gale, Youssefian 1985, Flintham et al. 1997). Some Polish common wheat cultivars as 'Elena', 'Parada', 'Broma', 'Henika', 'Polna', 'Santa' and 'Sigma' carried these genes (Kowalczyk 1997; Kowalczyk et al. 1999). The GA-responsive dwarfing gene *Rht8* and *Rht9* were introduced from the Japanese landrace 'Akakomugi' into Southern European cultivars developed by N. Strampelli (Gale, Youssefian 1985, Lorenzetti 2000). After World War II the *Rht8* gene was introduced to Polish wheat breeding program. The *Rht8* gene was identified in Polish old cultivars: 'Aria', 'Grana', 'Luna'. (Kowalczyk 2006).

In order to identify gene corresponding to a specific dwarfing genotype, it is important to be able to reliably identify and locate it on the genetic map. Microsatellites showed high level of polymorphism in hexaploid bread wheat (Röder et al. 1995, Ma et al. 1996, Röder et al. 1998). Genetic analysis has revealed that a wheat microsatellite marker *Xgwm 261* on the short arm of chromosome 2D cosegregated with *Rht8* gene, with a genetic distance of 0.6 cM between them (Korzun et al. 1998). Analyses of polymorphism at locus *Xgwm 261* revealed

that the alleles of 192 bp was linked with reduction in plant height (Korzun et al. 1998). Many cultivars carried the 165 bp and 174 bp alleles in this locus (Worland et al. 1998; 2001; Chebotar et al. 2001; Ahmad, Sorrells 2002). Moreover, a number of 17 rare alleles in this locus were identified (Worland et al. 1998; 2001; Chebotar et al. 2001; Ahmad, Sorrells 2002; Schmidt et al. 2004; Ellis et al. 2005, Liu et al 2005). The aim of this research was analysis of allelic variation in *Xgwm 261* locus and identification of *Rht8* genes in new Polish common wheat cultivars.

Material and methods

28 Polish common wheat cultivars which were bred in many Polish Plant Breeding Stations and were registered after 2001 year were used for this investigation. Genomic DNA was extracted from 10 days etiolated seedlings with a Milligan's (1992) extraction procedure. The primer sequences of wheat microsatellite *Xgwm 261* for analysis of allelic variation and identification of *Rht8* gene were: 5'-CTCCCTGTACGCCTAAGGC-3' and 5'-CTCGCGCTACTAGCCATTG-3'. Conditions for the PCR were as follows: 1× buffer PCR (10×: 100 mM Tris-HCl pH 8,8 in 25°C; 500 mM KCl, 0,8% Nonidet P40); 1,5 mM $MgCl_2$; 200 μM of each dNTP; 250 nM of each primer; 1 U DNA *Taq* polymerase (Fermentas); 60 ng of genomic DNA. Thermocycling condition were: initial denaturation at 95°C for 3 minutes; followed by 45 cycles of 94°C for 1 minute, 55°C for 1 minute, 72°C for 2 minutes, and final extension step of 10 minutes at 72°C. PCR was performed in T1 Biometra thermocycler. The PCR products were separated by electrophoresis on a denaturing polyacrylamide gel using a Bio-Rad Sequi-Gen system, and visualized by silver staining following a protocol from Chalhoub et al. (1997).

Results and discussion

Investigated Polish common wheat cultivars showed allelic variation in *Xgwm 261* locus on the basis of microsatellite analysis. The highest alleles frequency was observed for a 192 bp fragment. This fragment linked with *Rht8* dwarfing gene was observed in 6 spring modern cultivars. There were: 'Bombona', 'Griwa', 'Hewilla', 'Histra', 'Kosma', 'Napola'. Among winter wheat cultivars only two: 'Legenda' and 'Naridana' had 192 bp allele. Ganaeva et al. (2005) used microsatellite markers for identification of *Rht8* gene in Bulgarian wheat cultivars and showed that the 192 bp allele was identified in 84% of studied modern cultivars, of which 58% carried *Rht8* gene alone. Liu et al. (2005) showed that many Chinese wheat cultivars carried the allele of 192 bp linked with *Rht8* gene. The 165 bp DNA fragment size correlated with an increase in plant height was observed in 7 winter wheat cultivars. There were: '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 DNA fragment size with 180 bp was observed in 'Ostka Strzelecka' and 198 bp in 6 cultivars: 'Bogatka', 'Nutka', 'Parabola', 'Smuga', 'Sukces', 'Tonacja'. Worland et al. (1998) used SSR markers for analysis of allelic variation in *Xgwm 261* locus in 800 wheat cultivars from different countries. The Authors showed that 90% of cultivars had 165 bp, 174 bp or 192 bp alleles. Cultivars from Southern Europe carried very often the 192 bp band linked with *Rht8* gene. Many cultivars from CIMMYT carried 165 bp allele. Ahmad and Sorrels (2002) analyzed allelic variation in *Xgwm 261* locus in 71 cultivars from different country. In these cultivars the most popular was 174 bp allele, because this allele was present in 37 of them. Authors showed that 19 tested cultivars had 165 bp allele. Only four cultivars had 192 bp allele linked with *Rht8* gene.

Table 1: Classification of wheat varieties for allelic variations of microsltellite *Xgwm 261*

Cultivar		Fragment size (bp)				
		165	174	180	192	198
Batuta	W		+			
Bogatka	W					+
Bombona	S				+	
Finezja	W	+				
Fregata	W	+				
Griwa	S				+	
Hewilla	S				+	
Histra	S				+	
Izyda	W		+			
Kobiera	W		+			
Kosma	S				+	
Legenda	W				+	
Muza	W	+				
Nadobna	W		+			
Napola	S				+	
Naridana	W				+	
Nutka	W					+
Ostka Strzelecka	W			+		
Parabola	W					+
Radunia	W	+				
Rywalka	W	+				
Satyna	W		+			
Sława	W	+				
Smuga	W					+
Sukces	W					+
Tonacja	W					+
Turnia	W	+				
Żura	S		+			

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Use of DNA markers for identification of *Vrn-H2* genes in Polish barley (*Hordeum vulgare* L.) cultivars

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In Poland, barley sown area in 2006 reached 1 220 thousands hectares, what amounts to 14.56% of total cereals sown area. Harvests of barley achieved then 3 161 thousands tons and mean yield 28.55 dt per ha. The main ways of barley utilisation in Poland are brewing and feeding industry and production of cereals.

Vernalization requirement in barley is mainly controlled by three loci: *Vrn-H1*, *Vrn-H2* and *Vrn-H3* (Dubcovsky et al. 2005, Von Zitzewitz et al. 2005). 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).

Dubcovsky et al. (2005) characterized precisely allelic variation at the *Vrn-H2* locus in barley and designed STS primers for this locus (VRN-Ha-F and VRN-Ha-R). These primers amplify a 208-bp fragment in varieties carrying the dominant *Vrn-H2* allele and do not produce any amplification product in varieties carrying recessive *vrn-H2* allele.

The aim of our experiment was to describe dominant or recessive character of *Vrn-H2* locus in 24 Polish barley cultivars.

Material and methods

24 Polish barley cultivars were used for this investigation. Within these cultivars 20 have spring growth habit and 4 have winter growth habit (Table 1).

Table 1: Growth habit and presence of 208-bp DNA fragment for tested Polish barley cultivars (S = spring, W = winter)

Cultivar	Growth habit	DNA fragment size 208 bp
Atol	S	-
Binal	S	-
Blask	S	-
Boss	S	+
Bryl	S	+
Edgar	S	+
Granal	S	-
Lot	S	-
Nadek	S	-
Nagrad	S	-
Poldek	S	-
Rabel	S	+
Rasbet	S	-
Rastik	S	+
Rataj	S	-
Refren	S	+
Rodion	S	-
Rodos	S	+
Ryton	S	-
Start	S	-
Bażant	W	+
Bursztyn	W	+
Gil	W	+
Horus	W	+

Kernels were placed on water moistened filter paper on Petri dishes in 24°C for 7 days. After this time genomic DNA was isolated from coleoptiles according to Milligans protocol (Milligan 1992). PCR reaction contained: 1× reaction buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 250 nM each primer, 0.4 U *Taq* polymerase and 50 ng genomic DNA in total volume of 20 µl. Primers and the PCR conditions used were according as described Dubcovsky et al. (2005). Primers sequences were: VRN-Ha-F (5'-GCCTCTTCTTCTTCCTCGAC-3') and VRN-Ha-R (5'-ACTGGTACTCGTGCAGTGGG-3'). The temperature conditions for PCR reaction were: 94°C for 5 minutes, 38 cycles at: 94°C for 30 seconds, 55°C for 30 seconds, 72°C for 30 seconds; and the final extension step at 72°C for 7 minutes. The reaction products were separated on a 1,5% agarose gel and sized using the GeneRuler™ 100bp DNA Ladder Plus (Fermentas). The experiment was performed in two replications.

Results and discussion

Primers designed by Dubcovsky et al. (2005) amplified a 208-bp fragment in 7 cultivars with spring growth habit, and in 4 cultivars with winter growth habit (Table 1). Presence of 208-bp DNA fragment showed dominant character of *Vrn-H2* allele in winter cultivars: Bażant, Bursztyn, Horus, Gil and in spring cultivars: Boss, Bryl, Edgar, Rabel, Rastik, Refren, Rodos. The lack of this fragment confirmed presence of recessive *vrn-H2* allele in spring cultivars: Atol, Binal, Blask, Granal, Lot, Nadek, Nagrad, Poldek, Rasbet, Rataj, Rodion, Ryton and Start. Recessive *vrn-H2* allele did not occur in tested winter cultivars.

Dubcovsky et al. (2005) used this primers set to characterize *Vrn-H2* locus in 12 barley forms from different parts of the world. They carried out presence of 208-bp band in 6 winter varieties with *Vrn-H2* allele. This product did not exist for 6 spring varieties carrying *vrn-H2* allele.

Von Zitzewitz et al. (2005) characterized the *Vrn-H2* locus in the 11 genotypes by multiplex amplification of a conserved region of the *Vrn-H2* candidate genes *ZCCT-H*, as well as the tightly linked *Vrn-H2* locus-proximal *HvSnf2* gene. The *ZCCT-H* are present in each 5 winter accessions, but deleted from 2 facultative and 4 spring accessions. *HvSnf2* locus amplified from all varieties, what confirmed deletion of *ZCCT-H* genes and excluded PCR amplification failure.

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Use of molecular markers for identification of *Vrn-B1* genes in Polish common wheat cultivars

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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* (McIntosh et al. 2003). Dominant alleles of these genes are inhibitors of vernalization requirement. The dominant allele of *Vrn-A1* completly inhibits vernalization requirement, dominant *Vrn-B1* and *Vrn-D1* inhibit it partially (Košner and Pánková 1998). In winter varieties all three loci occur in recessive (*vrn*) form, but these loci could differ among winter wheat varieties (Košner and Pánková 1998). Yan et al. (2004) analysed the *Vrn-1* promoters in wheat. They found that dominant *Vrn-A1* allele could occur in 3 different forms. Two of these forms differ from the winter allele: the *Vrn-A1a* allele has a duplication including the promoter region and the *Vrn-A1b* allele has two mutations in the host direct duplications region and 20-bp deletion in the 5' UTR. The *Vrn-A1c* allele and all the dominant *Vrn-B1* and *Vrn-D1* alleles didn't differ from respective recessive alleles in promoter regions (Yan et al. 2004). Fu et al. (2005) found that varieties with dominant *Vrn-A1* allele have large deletion within the first intron of this gene. A 2,8-kb segment within this deletion is conserved for different recessive alleles and probably includes regulatory elements impotant for the vernalisation requirement. Similar deletions were revealed for *Vrn-B1* and *Vrn-D1* alleles (Fu et al. 2005). Moreover they designed primers sets for confirmation or exclusion of this deletion for each wheat genome (Fu et al. 2005).

The aim of our study was to describe dominant or recessive character of *Vrn-B1* alleles in 20 Polish wheat cultivars, using primers detected deletion within the first intron of this gene, designed by Fu et al. (2005).

Material and methods

20 Polish wheat cultivars were used for this investigation. Within these cultivars 10 have spring growth habit and 10 have winter growth habit (Table 1). Kernels were placed on water moistened filter paper on Petri dishes in 24°C for 7 days. After this time genomic DNA was isolated from coleoptiles according to Milligans protocol (Milligan 1992). PCR reaction contained: 1× reaction buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 200 nM each primer, 0.4 U *Taq* polymerase and 50 ng genomic DNA in total volume of 20 µl. Primers and the PCR conditions used were according as described Fu et al. (2005). Primers sequences were: Intr1/B/F (5'-CAAGTGGAACGGTTAGGACA-3') and Intr1/B/R3 (5'-CTCATGCCAAAAATTGAAGATGA-3'). The temperature conditions for PCR reaction were: 94°C for 5 minutes, 38 cycles at: 94°C for 1 minute, 58°C for 1 minute, 72°C for 1 minute and 20 seconds; and the final extension step at 72°C for 10 minutes. The reaction products were separated on a 1,5% agarose gel and sized using the GeneRuler™ 100bp DNA Ladder Plus (Fermentas).

Results and discussion

Primers designed by Fu et al. (2005) amplified a 709-bp fragment in 7 cultivars with spring growth habit, and in 2 with winter growth habit (Table 1). That 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 in winter cultivars Parabola and Radunia, too. 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.

Fu et al. (2005) used primers Intr1/B/F and Intr1/B/R3 to describe occurrence or lack of deletions in the first intron in *Vrn-1* loci of the B genome in 88 common wheat varieties grown in Argentina and California. They stated no deletion in the 37 winter varieties of common wheat analyzed in they study. However, among 51 spring varieties 20 showed deletion in this intron in B genome.

Reddy et al. (2006) used primers developed by Fu et al. (2005), which were specific for presence and absence of intron 1 deletion in the *Vrn-B1* gene. They characterized nullitetrasomic lines of *Triticum aestivum* cv. Chinese Spring: N5AT5D, N5BT5D and T5BN5D, Chinese Spring, Triple Dirk B, Triple Dirk D, Triple Dirk F and Norstar winter wheat. They stated presence of 709-bp product in 2 forms: Triple Dirk B and Triple Dirk F.

Table 1: Growth habit and presence of 709-bp DNA fragment for tested Polish wheat cultivars (S = spring, W = winter)

Cultivar	Growth habit	DNA fragment size 709 bp
Bombona	S	-
Bryza	S	-
Hewilla	S	+
Histra	S	+
Kosma	S	+
Monsun	S	+
Rubens	S	-
Triso	S	+
Zebra	S	+
Żura	S	+
Alkazar	W	-
Finezja	W	-
Fregata	W	-
Nutka	W	-
Parabola	W	+
Radunia	W	+
Rywalka	W	-
Satyna	W	-
Smuga	W	-
Tonacja	W	-

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A comparison of ear emergence and some quantitative traits in old and new Polish common wheat cultivars with Mercia *Ppd-A1*, *Ppd-B1* and *Ppd-D1* isogenic lines

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Day length is one of the most important factors affecting the rate of development in wheat (Worland et al., 1993). The genetic control of day length insensitivity is determined by many genes present on different chromosomes. The genes *Ppd-D1* (formerly *Ppd1*), *Ppd-B1* (formerly *Ppd2*) and *Ppd-A1* (formerly *Ppd3*) localized on the homoeologous group 2 chromosomes (2D, 2B and 2A, respectively) have the major influence on this trait (Welsh et al., 1973; Law et al., 1978; Scarth, Law, 1984, Snape et al., 2001). Moreover, genes localized on other chromosomes from homoeologous groups 1 and 6 (Law, 1998), 3D (Miura and Worland, 1994), and 4B (Halloran and Boydell, 1967) also affect the date of heading in response to changes in photoperiod.

In order to evaluate precisely the effects of the genes *Ppd-A1*, *Ppd-B1*, *Ppd-D1*, a series of homozygous recombinant lines were created by the John Innes Centre, England (Worland et al., 1988). These lines were tested in several countries: England (Worland et al., 1988), Yugoslavia (Worland et al., 1988; 1990), Germany (Worland et al., 1991; Börner et al., 1993) and Poland (Miazga et al. 1995; Kowalczyk et al. 2003). In this paper pleiotropic effects of the *Ppd-A1*, *Ppd-B1* and *Ppd-D1* genes on yield and its components were investigated in Poland, using isogenic lines of the wheat cv. Mercia. The results of this investigation were also compared with the performance of old and new Polish wheat cultivars for some quantitative traits.

Materials and methods

Experiments were carried out over three seasons (2001/02, 2002/03, 2003/04) at the Experimental Farm in Czeslawice. The experiment was conducted using a randomized blocks

design. In each year, seed of the cultivars and lines listed in Table 1 were sown into five-row 1m plots using a 2×20 cm spacing in six replications. For each plot, heading time was recorded as the number of days from 1st May until full ear emergence. A random sample of ten leading tillers in each plot was used for the calculation of average plant height, number of grains per ear, 1000 grain weight and spikelet fertility. The results obtained were statistically analyzed individually for each year using ANOVA.

Results and discussion

In all the three seasons, recombinant lines with genes for insensitivity to photoperiod were earlier that all the Polish wheat cultivars. Of the old Polish cultivars the latest were: Konstancja Granum, Konstancja Wierzbieńska and Płocka. The earliest old cultivars were Antonińska Wczesna, Biała Kaszubska and Wyskolitewska Sztywnosłoma. More cultivars which were registered in the early 1980s were earlier than the old cultivars, and similar to the earliest old cultivars. The ear emergence times of new Polish cultivars were similar to the latest cultivars registered in the 1980s. Worland *et al.* (1998) analyzed recombinant lines of Cappelle-Desprez which had insensitive alleles of *Ppd-D1* from Mara and Ciano. The alleles originating from Ciano accelerated heading date the most, though differences were small. Many authors found the same results in experiments performed using recombinant, substitution and monosomic lines, as well as varieties with insensitive alleles at *Ppd-D1* as compared to sensitive alleles (Worland, Law 1986; Petrović, Worland 1988; Worland et al., 1988; Worland et al., 1990; Börner et al., 1993).

Over the three seasons all recombinat lines with photoperiod insensitive alleles were shorter than their control lines (Table 1.) Of the tested Polish wheat cultivars the tallest were old cultivars. Over the last 70 years Polish breeders have bred cultivars with shorter growth habit using reduced height genes, particularly *Rht8* (Kowalczyk 2006). Therefore these cultivars were significantly shorter than old cultivars. Börner et al. (1993) studied Cappelle-Desprez (Mara 2D) recombinant lines in Germany finding that the insensitive *Ppd-D1* allele caused plant height reduction.

Recombinant lines with the *Ppd-B1* insensitivity allele set more kernels in the spike as compared to the control and to the lines with *Ppd-A1* and *Ppd-D1* insensitivity alleles. No significant differences were found between the isogenic lines and their control varieties in the third year of study (Table 1). Old Polish cultivars showed more variation for this trait. These differences were between years, for example in 2002 the cultivar Dańkowska Graniatka set an average of 67,5 kernels but in 2003 only 25,9. Polish wheat cultivars which were registered in the early 1980s and at the end of the 20th century had similar values for this trait to the Mercia lines. Moreover differences between these cultivars were smaller than in the old cultivars. Worland et al. (1998) tested Cappelle-Desprez lines the *Ppd-D1* insensitivity alleles from Mara and Ciano in England and Germany and showed that Cappelle-Desprez (Mara 2D) recombinant lines in both countries set more kernels in the spike. However, in recombinant lines of Cappelle-Desprez (Ciano 2D), they found higher (in England) and lower (in Germany) values of the trait than the controls.

In experiments performed in 2002-2004 with recombinant lines with different *Ppd* alleles and Polish cultivars, it was shown that 1000-kernel weight depended mainly on the season and to a lesser extent on the genotype. All lines and cultivars had 1000 grains weights higher than 40g. Worland *et al.* (1998) showed unfavorable pleiotropic effects of alleles for photoperiod insensitivity originating from Mara and Ciano on values of the trait in England. In Germany, recombinant lines of Cappelle-Desprez (Mara 2D) had lower, and Cappelle-Desprez (Ciano 2D) higher values of the trait than the controls.

Recombinant lines with different *Ppd* alleles had similar spikelet fertility to their control lines in all years. However, there were large differences in this trait between years and cultivars in old Polish cultivars. For example 'Biała Kaszubska' in 2003 had a spikelet fertility of only 1,02, but in that year 'Antonińska Wczesna' had a spikelet fertility of 2,38 (Table 1). The highest value of this trait was in 2002 in 'Dańkowska Graniatka' (3,14). More stable values for spikelet fertility were in Polish wheat cultivars registered in the early 1980s and at the end of the 20th century (Table 1). Worland *et al.* (1998) showed that lines with insensitivity alleles of *Ppd-D1* in England were characterized by a higher spikelet fertility. In Germany, Cappelle-Desprez (Mara 2D) recombinant lines had higher, and Cappelle-Desprez (Ciano 2D) slightly lower values of this trait than the controls.

Table 1: Date of ear emergence and values of some quantitative traits in Polish old and new cultivars in comparison to Mercia *Ppd* isogenic lines (Czesławice, Poland)

Cultivar/ Line	Ear emergence (days from 1st May			Plant height (cm)			Number of grains per ear			Fertility of spikelets			Weight of 1000 grains (cm)		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Cultivars registered in 1959 - 1961 years															
Antonińska Wcz.	27.3	39.0	45.0	115.6	137.5	135.6	43.9	43.1	31.1	2.38	2.38	1.67	54.2	49.8	56.8
Biała Kaszubska	29.5	37.5	43.5	119.8	141.0	142.0	38.7	20.3	41.6	1.97	1.02	1.95	53.5	52.7	52.1
Bogatka	29.3	37.0	43.0	104.0	139.4	137.5	44.5	30.3	33.6	2.60	1.77	1.68	49.3	55.2	56.1
Bożena	32.5	41.0	47.0	115.8	136.8	134.2	52.3	32.9	48.6	2.62	1.76	2.16	48.8	44.2	51.4
Dańkowska Gran.	29.0	38.0	45.0	93.4	134.5	132.8	67.5	25.9	48.5	3.14	1.18	2.27	44.0	42.2	48.2
Konstancja Gran.	37.0	44.0	52.0	122.4	132.6	133.8	45.0	30.1	38.6	2.46	1.87	2.12	50.8	48.4	45.9
Konstancja Wierz.	34.5	43.0	49.0	120.0	151.7	147.3	50.5	39.2	31.4	2.51	2.09	1.47	44.7	46.7	52.6
Leszczyńska Wcz.	29.0	38.0	43.0	109.6	126.0	128.0	54.0	42.8	63.6	2.64	2.30	3.21	48.8	49.4	47.3
Płocka	34.0	43.0	48.0	118.0	142.0	140.0	33.0	32.3	28.8	1.80	1.47	1.36	49.9	45.7	51.0
Wysokolitewska Szt.	29.0	36.0	42.0	115.0	124.2	126.8	46.2	31.9	35.8	2.52	1.67	1.68	43.2	47.2	52.6
Cultivars registered in 1982 - 1984 years															
Asta	29.2	40.5	45.5	98.2	85.0	97.0	54.4	57.2	61.2	2.78	2.96	2.56	44.5	46.3	52.4
Begra	26.4	35.0	40.7	67.4	77.6	97.3	50.4	48.0	41.8	2.79	2.78	2.25	48.5	56.8	50.0
Beta	28.0	37.0	42.0	77.6	67.8	97.6	49.2	63.3	47.3	2.44	3.21	2.17	46.4	47.3	48.9
Emika	25.0	35.5	43.5	77.2	73.4	97.2	41.8	37.2	26.6	2.26	1.98	1.44	51.2	50.4	44.8
Jawa	25.5	34.0	39.0	66.8	65.0	85.0	47.7	51.6	43.3	2.77	3.14	2.22	44.5	46.3	50.7
Liwilla	25.0	34.5	41.5	76.0	87.6	102.5	47.9	38.3	40.4	2.44	2.05	2.11	52.2	55.0	53.1
Panda	30.3	39.0	45.0	82.8	84.0	104.0	39.1	26.6	37.5	2.17	1.44	2.13	41.5	44.8	50.9
Polanka	29.0	37.5	43.3	90.6	105.4	109.5	51.2	55.0	54.4	2.55	2.79	2.63	48.5	45.7	52.4
Rota	27.5	36.0	42.0	81.6	82.6	101.7	37.7	46.9	39.6	2.11	2.76	2.12	43.9	45.9	51.8
Salwa	27.5	35.0	41.0	70.2	85.4	105.8	44.9	39.8	45.8	2.36	2.29	2.18	44.7	52.7	49.8
Cultivars registered in 1997 – 1998 years															
Kaja	26.3	35.5	42.5	81.4	80.4	98.6	46.6	49.4	41.1	2.60	2.93	2.52	49.7	52.5	51.5
Korweta	29.3	38.0	43.0	84.6	72.6	96.8	45.1	51.0	41.0	2.35	2.95	2.25	45.7	41.0	49.3
Mobela	28.5	37.0	43.0	84.8	85.0	97.0	51.2	54.1	34.5	2.62	2.92	2.17	48.1	49.5	54.0
Rysa	29.0	36.5	43.3	85.4	78.4	99.6	40.0	44.0	32.1	2.21	2.37	2.01	45.9	55.1	53.2
Wanda	29.0	37.0	43.0	87.4	83.5	103.7	47.7	48.3	44.3	2.44	2.57	2.38	48.6	48.9	53.8
Recombinant lines Mercia with *Ppd* genes															
Mercia *Ppd-A1*	19.1	27.3	30.4	84.9	85.2	86.8	43.6	44.7	48.3	2.20	2.60	2.77	46.5	50.0	48.2
Mercia *Ppd-B1*	20.7	28.3	31.9	85.6	87.2	87.0	46.3	48.8	49.6	2.25	2.71	2.74	45.5	48.7	49.5
Mercia *Ppd-D1*	17.6	25.5	28.1	84.1	85.3	84.6	42.0	47.8	48.5	2.24	2.77	2.75	47.9	51.4	48.9
Mercia *ppd*	21.8	30.4	33.0	87.4	90.0	89.3	43.8	45.9	48.1	2.20	2.68	2.78	45.6	50.3	49.4
LSD at p=0.05	1.7	1.5	1.9	11.5	8.9	10.5	7.5	8.3	9.1	0.45	0.41	0.52	7.6	8.2	7.1

Conclusions

1. Polish wheat cultivars, both old and new, were later in heading than Mercia lines with *Ppd* insensitivity alleles.
2. Plants of recombinant lines with different *Ppd* alleles were shorter than their control lines. A significant reduction in plant height was shown by Polish cultivars registered in the early 1980s and at the end of the 20st century in comparison to old cultivars.
3. On a basis of three years of study it was found that the number of kernels in the spike as well as spikelet fertility depended mainly on the year rather than on genotype.

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Variability in seedling growth and biochemical response to osmotic stress among Bulgarian bread wheat cultivars

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Seed germination and early seedling growth are vulnerable to stress conditions, in particular drought. The genetic variation in plant responses to drought stress is a prerequisite for creating tolerant genotypes, which is a breeding tool to respond adequately to the climate dynamics. In wheat, the global distribution of the height-reducing (*Rht)* genes poses important questions regarding performance of semi-dwarf cultivars and the impact of *Rht* genes on growth responses under drought.

The objective of this study was to evaluate the genetic variability among Bulgarian bread wheat germplasm with respect to the changes in germinability, seedling growth and some biochemical parameters in response to osmotic stress with emphasis on the effects of various *Rht* genes.

Material and methods

Eighty-seven Bulgarian winter wheat (*Triticum aestivum* L.) cultivars were used, of which 12 were tall (*rht*), 51 were GA-responsive (GA-R; 46 carry *Rht8*) and 24 were GA-insensitive (GA-I; 5 carry *Rht-B1b*, 17 carry a combination of *Rht-B1b* and *Rht8*) (Ganeva et al. 2005, unpublished results). For each cultivar, 2 x 50 seeds were germinated at 22±1^{o}C in the dark. Water deficit was simulated using 15% PEG under two regimes: 3-day-long stress applied on 4-day-old seedlings (T_1) and 7-day-long stress starting from the onset of germination (T_2). Distilled water was used in controls. Germination was recorded on 4th and 7th day. The root, coleoptile and shoot length of 15 seedlings per cultivar per treatment was measured on both the 4th and 7th day. Two independent experiments (replicates) were conducted. For 14 cultivars (7 GA-R; 7 GA-I), the accumulation of proline (Bates et al. 1973) and malondialdehyde (MDA; Cakmak and Horst 1991) in response to stress was determined in roots and shoots of 7-day-old seedlings after 3-day-long treatment with 15% PEG. The increase in proline and MDA content is expressed in % as: (Content after stress – Content in control)/Content after stress × 100. Statistical analysis was performed using STATISTICA StatSoft, Inc., version 7.1.

Results and discussion

Effects of osmotic stress on germination and seedling growth

The analysis of variance indicated considerable amount of genetic variability for all the studied traits. The major effect was attributed to the PEG treatment, followed by the genotype (both background cultivar-specific genes and various semi-dwarfing genes) and their interaction (Table 1).

Comparison of cultivars – carriers of rht, GA-responsive and GA-insensitive Rht genes

The presence of *Rht* genes had substantial effects on all the traits (Fig. 1). In control, the expression of mean performance for all studied traits (except for the coleoptile length on day 7th) was higher for GA-R cultivars. The better performance of GA-R cultivars was maintained under both the short-term (T_1) and the long-term (T_2) stress. The mean performance for all the traits was in the order *Rht8* > *Rht-B1b* + *Rht8* > *Rht-B1b* in control (Fig. 2). In both stress environments (T_1 and T_2) the dominance of *Rht8*-genotypes and the intermediate performance of the double dwarfs were preserved.

Key tolerance-related traits at seedling stage are root characteristics, long coleoptile and early seed vigour (Richards et al. 2000). Root elongation under drought may help plants get deeper water, thus avoiding water deficits near the soil surface. Longer coleoptiles are important for seedling emergence in cases of deep sowing for efficient use of the soil water reserves. A promising alternative for breeding programmes targeting longer coleoptiles is the use of GA-R genes, such as *Rht8* (Ellis et al. 2004). *Rht8* produces a coleoptile longer than the GA-I genotypes (Botwright et al. 2001), and yet achieves the required plant height reduction (Worland et al. 2001). This allele has been broadly used commercially in Southern parts of Europe, Russia, Ukraine, China and Japan (Worland et al. 1998; Chebotar et al. 2001; Ganeva et al. 2005).

Table 1: Univariate tests of significance (F-values) for germination and seedling traits in controls and under two regimes of PEG-induced drought stress in 87 Bulgarian bread wheat cultivars (Group – *rht*, GA-R and GA-I group of cultivars; Allele – *Rht-B1b, Rht8* and *Rht-B1b+Rht8*; d.f. - degrees of freedom; *** - significant at P<0.001)

Trait	Cultivar (C) (d.f. 86)	Treatment (T) (d.f. 2)	C × T (d.f. 172)	Group (G) (d.f. 2)	Treatment (T) (d.f. 2)	G × T (d.f. 4)	Allele (A) (d.f. 2)	Treatment (T) (d.f. 2)	A × T (d.f. 4)
Germination percentage - 4th day	488.0***	6951.0***	82.0***	1883.2***	1844.7***	44.7***	91.9***	1202.7***	5.2***
Germination percentage – 7th day	723.0***	2523.0***	86.0***	3093.0***	799.0***	25.0***	251.0***	212.0***	38.0***
Root length – 4th day	51.6***	84.1***	3.9***	421.5***	9.2***	23.2***	250.4***	19.5***	6.72***
Root length – 7th day	28.9***	4816.4***	5.5***	273.1***	2412.5***	13.7***	123.3***	1435.4***	1.68ns
Coleoptile length – 4th day	53.4***	5712.8***	5.6***	263.9***	2360.5***	12.5***	233.8***	1424.0***	4.77***
Coleoptile length – 7th day	20.6***	1311.5***	6.6***	161.4***	837.1***	11.1***	171.3***	425.9***	6.1***
Shoot length – 7th day	22.3***	34160.1***	7.6***	200.3***	18374.1***	16.5***	228.1***	9545.4***	22.8***

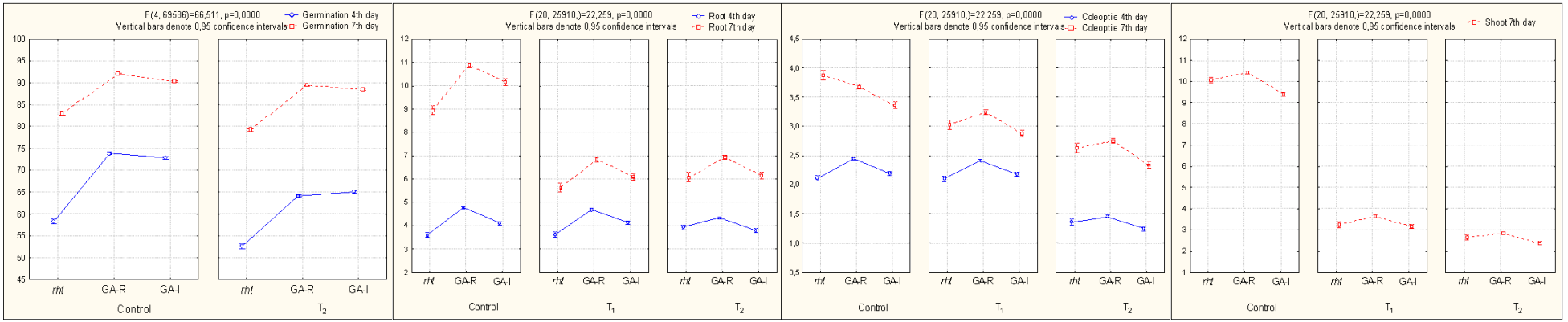

Fig. 1: Germination and seedling traits in controls and under PEG-induced 3-day-long (T_1) and 7-day-long (T_2) drought stress conditions in Bulgarian bread wheat cultivars - carriers of *rht* (12), GA-responsive (GA-R; 51) and GA-insensitive (GA-I; 24) genes.

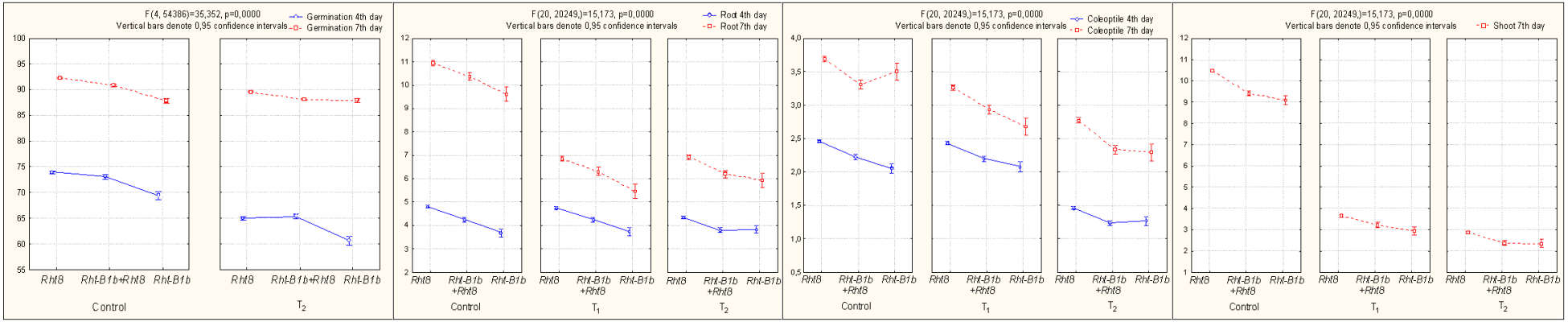

Fig. 2: Germination and seedling traits in controls and under PEG-induced 3-day-long (T_1) and 7-day-long (T_2) drought stress conditions in Bulgarian bread wheat cultivars - carriers of *Rht8* (46), *Rht-B1b* (5), or *Rht-B1b+Rht8* (17) genes.

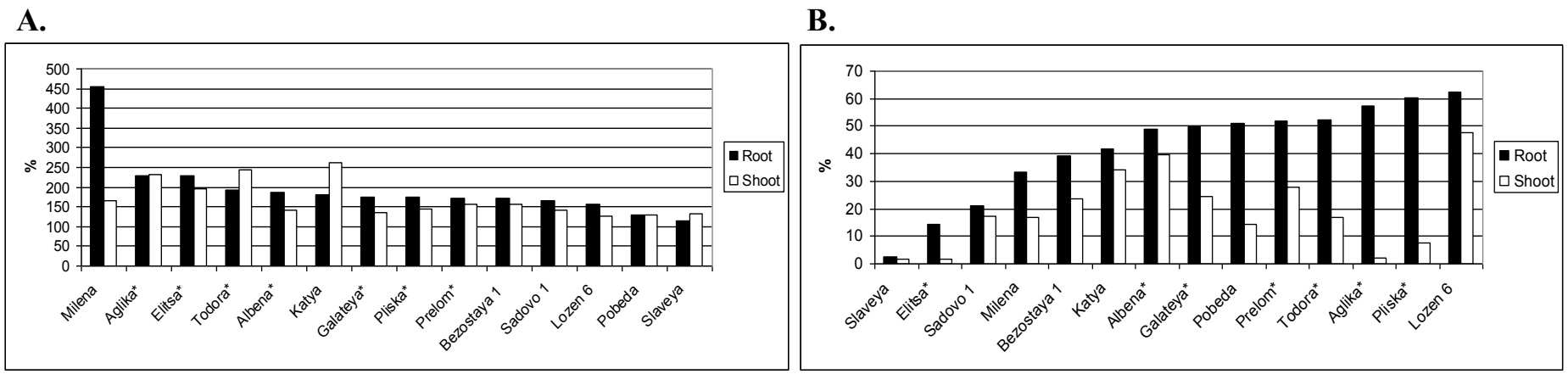

Fig. 3: Variation in proline (A) and malondialdehyde (B) content in 7-day-old seedlings of 14 Bulgarian bread wheat cultivars in response to 3-day-long PEG-induced drought stress (* - GA-I cultivars)

Accumulation of proline and MDA

In response to stress plants accumulate 'compatible solutes', such as proline, which play crucial role as osmoprotectants and radical scavengers (Delauney and Verma 1993). The accumulation of MDA is an indicator of lipid peroxidation and membrane destabilization as a result of the drought-induced oxidative burst (Smirnoff 1995). A number of studies provide evidence that there is a positive correlation between tolerance to stress and proline accumulation, while negative correlation exists between the stress tolerance and the increase in the MDA content (Nayyar 2003/4). For all 14 cultivars, an increase in the proline and MDA content was found in both roots and shoots (Fig. 3). The MDA accumulation in roots was higher than in shoots. Considerable variation was observed among the studied cultivars, suggesting that there exist various mechanisms contributing to tolerance. Generally, GA-insensitive cultivars showed higher MDA increase, particularly in roots.

Acknowledgements

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Detection of loci controlling pre-harvest sprouting and dormancy in the Triticeae

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Dormancy and pre-harvest sprouting (PHS) are prevalent problems in the Triticeae. Pre-harvest sprouting decreases the grain quality and the yield of all cereals. Sprouting is influenced by a combination of factors, e.g. water uptake, grain dormancy, and the mobilisation of stem reserves. In addition, there is a strong interaction with environmental conditions. Wheat, barley, and rye are in study to detect loci controlling these traits. A comparative QTL mapping of different populations can help to find the responsible genome region(s) for pre-harvest sprouting and dormancy in the Triticeae.

Material and methods

For wheat the recombinant inbred lines of the ITMI mapping population were used for the pre-harvest sprouting and dormancy test. For barley the doubled haploid lines of the Oregon Wolfe Barley (OWB) and the Steptoe/Morex (SxM) population were studied. In addition, for the detection of comparable loci in rye the set of Chinese Spring/Imperial wheat/rye addition lines is under investigation.

From all lines five ears directly after harvest were placed in a plate full of wet sand for 14 days. After 14 days a replication with overripe ears followed. For the interpretation of the data a rating of seven score points (1 = resistant, 7 = highly susceptible) was used. Furthermore, a dormancy test was performed. 60 seeds directly harvested and fresh threshed were tested under two different temperature conditions (Lohwasser et al. 2005).

The computer analysis for detecting the QTLs was done with the programme QGENE (Nelson 1997).

Results and discussion

For wheat and barley completely different results were found (tab. 1, fig. 1). For wheat major QTLs could be detected on chromosome 3AL for dormancy and on chromosome 4AL for pre-harvest sprouting and dormancy (Lohwasser et al. 2005).

Table 1: Detected QTLs for wheat and barley

Population	PHS		Dormancy	
	Chromosome	LOD-score	Chromosome	LOD-score
ITMI			3AL	3.46
	4AL	11.72	4AL	6.56
OWB	5H (centromere)	7.08	5H (centromere)	3.92
	5HL	3.01		
	7H (centromere)	6.15	7H (centromere)	6.74
S x M	5H (centromere)	9.90	5H (centromere)	18.89
	5HL	5.02		

For both barley mapping populations major QTLs could be localized in the centromere region and on the long arm on chromosome 5H and in the centromere region of chromosome 7H (tab. 1, fig. 2).

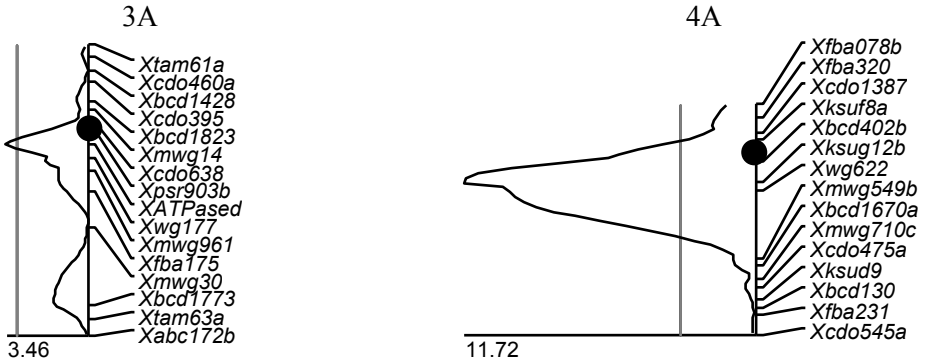

Fig. 1: ITMI population, QTLs for dormancy (left) and PHS (right) (● centromere region)

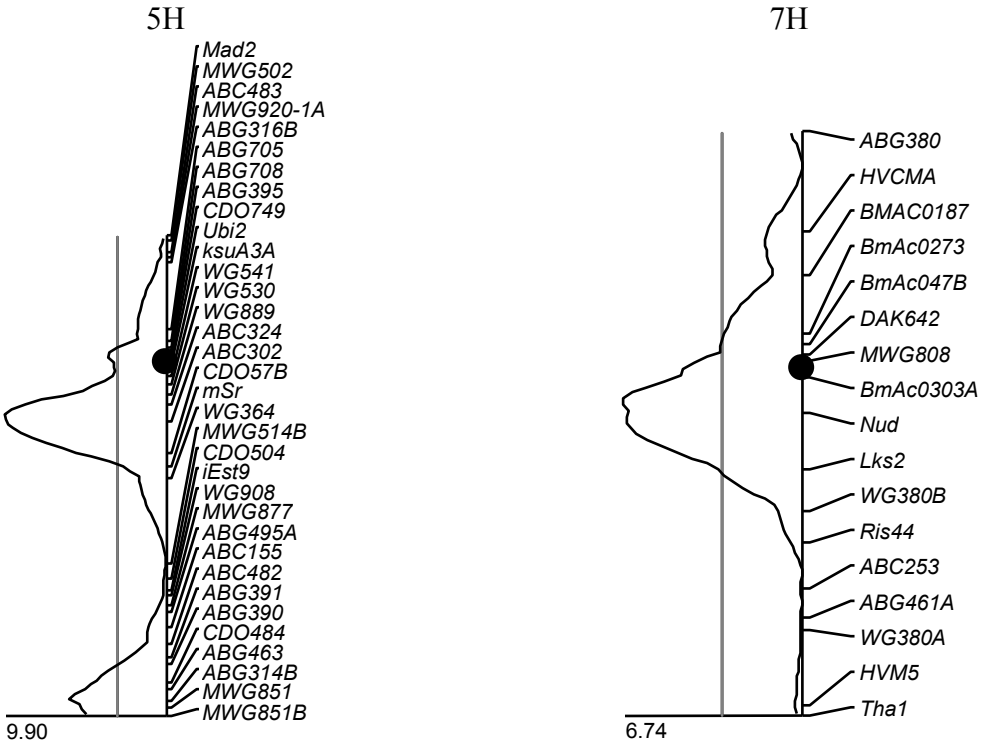

Fig. 2: QTLs for PHS in the S x M population (left) and dormancy in the OWB population (right) (● centromere region)

In order to detect comparable loci in rye the set of Chinese Spring/Imperial wheat/rye addition lines is under investigation.

Acknowledgements

We thank Renate Voss and Annette Marlow for excellent technical assistance.

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The relationship between vernalization requirement and cold tolerance among the set of reciprocal substitution lines for homoeologous group 5 chromosomes between two winter wheat varieties, Bezostaya 1 and Mironovskaya 808

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Winter wheat needs a low temperature treatment for a certain period (vernalization) in order to change development from the vegetative to reproductive stages. This vernalization response, evolved as an adaptation to protect plants from early flowering during unfavourable winter conditions. It is controlled by the *Vrn-1* and *Vrn-2* genes that are located on the long arms of the homoeologous group 5 chromosomes, but the most important are *Vrn-A1* and *Vrn-A2* (Law et al. 1976; Maystrenko 1980; Yan et al., 2004). Expression of *Vrn-A1* is genetically controlled by the gene *Vrn-A2* producing a repressor, and is unblocked by a period of cold temperatures in winter varieties to allow transition to flowering. In spring varieties the control of flowering by the *Vrn- A2* repressor is disabled due to different mutations in the *Vrn-A1 genes* promoter (Yan et al. 2003, 2004).

There is a lot of evidence that the processes involved in vernalization and cold tolerance are interrelated (Fowler et al. 1999, Prášil et al. 2005). The genes controlling frost resistance (*Fr 1, Fr 2, Cor*) were also located on the long arms of homoeologous group 5 chromosomes (5A, 5D), closely linked to the *Vrn-1* genes, by means of molecular markers (Galiba et al. 1995. Sutka 2001, Snape et al. 2001, Vágújfalvi et al. 2004), and relationships between all these important genes have been a subject of special interest.

Winter wheat has to survive unfavourable winter temperatures but the level of frost tolerance is not constant and is dependent on both genotypic and environmental factors (Fowler et al., 1999; Mahfoozi et al., 2001). In autumn, it is primarily low temperature that induces frost tolerance in wheat. A high level of tolerance is maintained over winter, but wheat plants must also be able to re-establish sufficient frost tolerance following occasional warm periods that result in deacclimation (Gusta and Fowler, 1979; Prášil and Zámečník,1991). The ability of wheat plants to maintain frost tolerance decreases after the vegetative / reproductive transition (Mahfoozi et al., 2001a, b), but mechanisms exist which slow down the rate of phenological development and extend the vegetative phase. These involve a requirement for vernalization and responses to photoperiod (day-length sensitivity).

The *wcs120* (wheat cold specific) gene family are located on the group 6 chromosomes. They belong to the Cor/Lea super family that encode a group of highly abundant proteins ranging in size from 12 to 200 kDa, that are co-ordinately regulated by low temperature, and accumulate to high levels in freezing-tolerant wheat plants. It was suggested that *wcs120* proteins could be used as the molecular markers for frost tolerance in the Gramineae.

The goal of our long-term study at the Crop Research Institute (CRI, formerly RICP, Prague) has been to find out more about relationship between cold tolerance and vernalization requirement (VR). For that purpose, reciprocal substitution lines have been used between two winter wheat genotypes for chromosomes carrying respective recessive loci *vrn-A1, vrn-B1* and *vrn-D1* (chromosomes 5A, 5B, 5D) that code for contrasting vernalisation requirements. The varieties Mironovskaya 808 and Bezostaya 1 were chosen since they had been shown earlier to have genetically determined differences in heading time caused by different vernalisation requirement and different sensitivity to photoperiod (Košner, 1992), and there is

also difference in their frost tolerance. From these varieties, 6 substitution lines were created namely Mironovskaya 808 (Bezostaya 1 5A), Mironovskaya 808 (Bezostaya 1 5B), Mironovskaya 808 (Bezostaya 1 5D); and the reciprocal Bezostaya 1 (Mironovskaya 808 5A), Bezostaya 1 (Mironovskaya 808 5B), Bezostaya 1 (Mironovskaya 808 5D). In the tables and figures, Mironovskaya 808 is abbreviated as MIR., Bezostaya 1 as BEZ., and these abbreviations are also used in the descriptions of substitution lines as MIR. (BEZ. 5A) and so on.

Material and methods

Material

Reciprocal substitution lines for homoeologous group 5 chromosomes between two winter wheat varieties Mironovskaya 808 (MIR) and Bezostaya 1 (BEZ) with contrasting vernalization and photoperiod responses, and a different frost tolerance, carrying different recessive alleles of genes *vrn-A1, vrn-B1* and *vrn-D1* (Košner, Pánková 2001*)* were used. Correctness of chromosome substitutions has been verified at the molecular level using SSR analyses.

Vernalization requirement (VR) assesment

Seeds of the substitution lines were germinated at one-week intervals and vernalized at temperatures of +1°C to 3°C so that treatments from 0 to 8 weeks of vernalization were obtained. Plants were sown in the field and heading time was recorded to establish vernalization requirement.

Frost tolerance (FT) test

Seeds were germinated at a temperature of 20°C for four days and then exposed to cold acclimation. After three-week or ten-week acclimation, the plants of each substitution line and parental variety were split into groups of 10 plants and exposed to six different frost intensities for 24 hours. Frost tolerance expressed as LT 50, was calculated according to the model of.Janáček, Prášil 1991.

Molecular study of wcs proteins

Total soluble proteins were extracted in Tris buffer, separated on 2D SDS-PAGE and visualized by silverstaining to determine accummulation levels of *wcs* proteins.

Results and discussion

Higher vernalization requirement and winter survival were observed in MIR than in BEZ. This corresponds to the accumulation levels of WCS proteins. Vernalization requirement was significantly affected by the substituted alleles of *vrn-1*. Substitution lines carrying chromosomes 5A and 5D of BEZ as well as the line carrying chromosome 5B of MIR showed a lower vernalization requirement and winter survival than the corresponding parental variety (Fig.1). The substitution lines did not exhibit a modified induction of frost tolerance levels, but a changed duration of frost tolerance corresponded to the *vrn-1* allele present on the substituted chromosome (Fig.1).

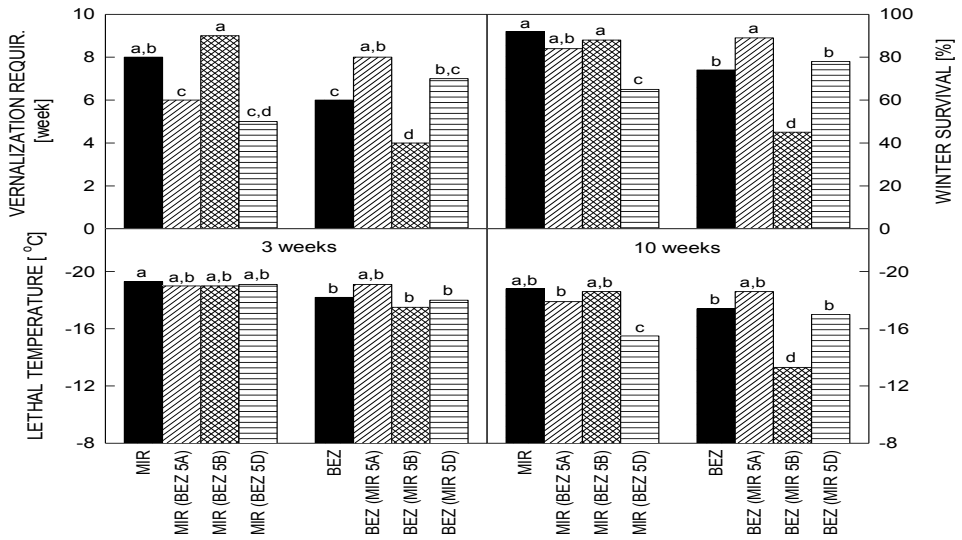

Fig. 1: Vernalization requirement and winter survival (induction and maintenance of frost tolerance) within the set of reciprocal substitution lines between Bezostaya 1 and Mironovskaya 808 for homoeologous group 5 chromosomes (Prášil et al. 2005).

Concentration of WCS120 proteins increased during cold acclimation in both parental varieties (Fig.2). Results showed that MIR had a higher accumulation of three WCS120 proteins (WCS120, WCS66 and WCS40) than BEZ (Fig.2).The results support the hypothesis that genes for vernalization requirement act as a master switch regulating the duration of low temperature induced frost tolerance.

Acknowledgements

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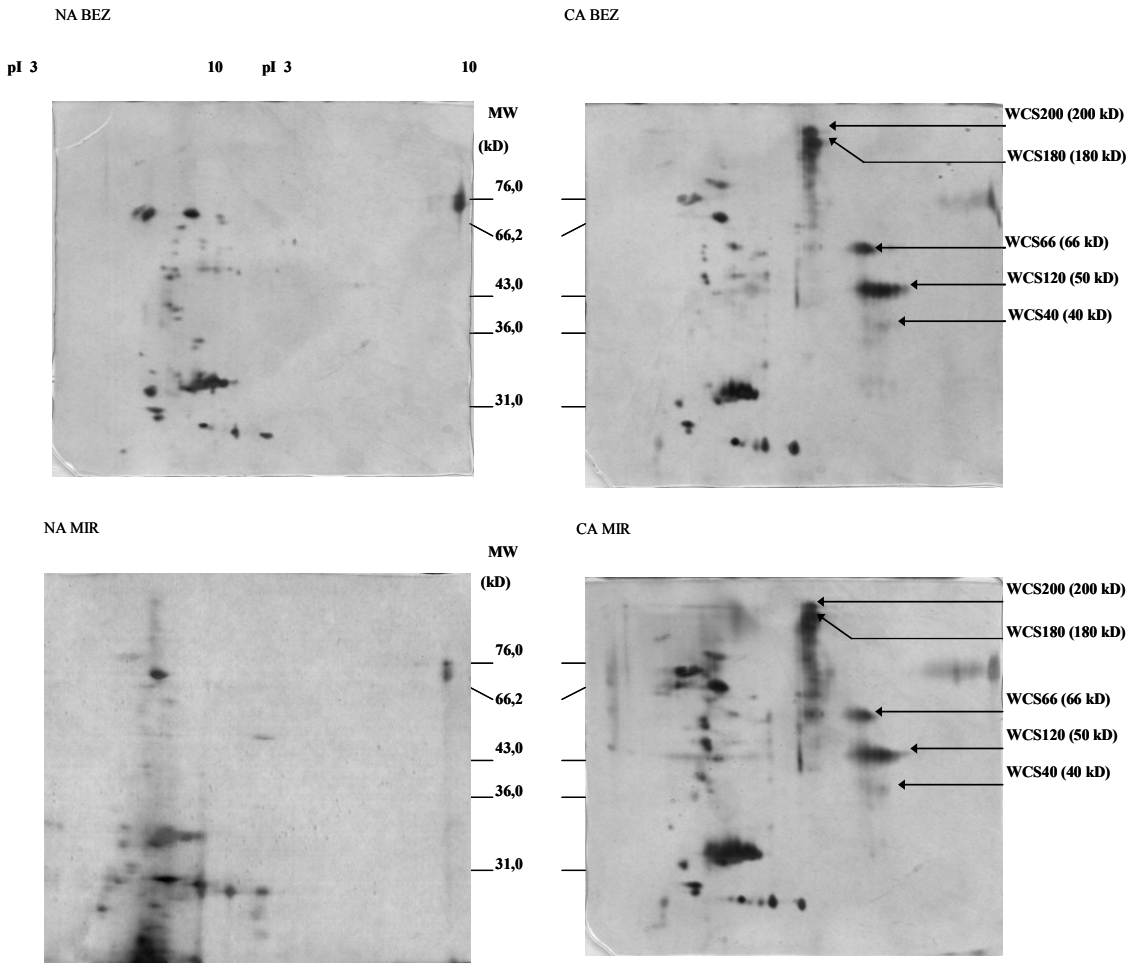

Fig. 2: Comparison of 2-DE gels of proteins (soluble upon boiling) extracted from leaf tissue of non-acclimated (NA) and cold-acclimated (CA) winter wheat Bezostaya 1 (BEZ) and Mironovskaya 808 (MIR). 2-DE gels were silver-stained (Vítámvás et al. 2007)

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Comparative mapping of lipoxygenase loci in wheat and barley

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Lipoxygenase (linoleat: oxygen oxydoreductase EC 1.13.12, LOX) catalyzes dioxidation of non-saturated fatty acids with formation of superoxide radicals. Many isoforms of LOX both bonded to cell membranes and soluble with different subcellular localization assume a poly-functionality of the enzyme and the involvement in various physiological processes of plant cell. The main role of LOX consists in being of a part of defence response mechanisms realizing under different types of stresses.

Using the recombinant inbred lines of ITMI population the QTL was identified on the short arm of 4B chromosome near the marker *Xbcd1262* responsible for polymorphism on specific LOX activity (Pshenichnikova et al. in press) (Fig.1). This result corresponds to the data obtained using recombinant inbred lines of durum wheat (Nachit et al. 2001). In a similar position of 4B chromosome one of the clusters for defense response was found earlier (Li et al. 1999). It may point on a linked inheritance of LOX gene with this cluster in 4B chromosome.

A minor QTL for LOX activity was detected on 7B chromosome near the marker *Xksud2a* firstly for the wheat in ITMI population (Pshenichnikova et al. in press) (Fig.2). In a comparable position the QTL was localized associated with yellow pigment in durum wheat (Elouafi et al. 2001). One of the LOX functions is the degradation of this pigment. In the largest cluster of defence response genes on the long arm of 7B chromosome two genes of traumatin, *Tha1* and *Tha2* (Li et al. 1999) were mapped. This protein is a product of LOX pathway. Possibly, a coincidence of QTLs positions for several physiologically linked characters is not a mere chance and may be the indirect confirmation of LOX gene existence on the long arm of 7B chromosome in wheat as well as its linkage with the genes for defence response.

The mapped positions of LOX genes of barley on 4H and 7H chromosomes (Van Mechelen et al. 1999) coincides with the QTLs both on chromosomes 4B and 7B found in our work (Fig.1, 2). The gene *LOXC* on 7H chromosome of barley is structural; possibly, there is the structural gene of LOX in 7B chromosome of wheat. May be this gene expresses only under the certain conditions or (and) the coding isoforms are so scanty that have not been still identified.

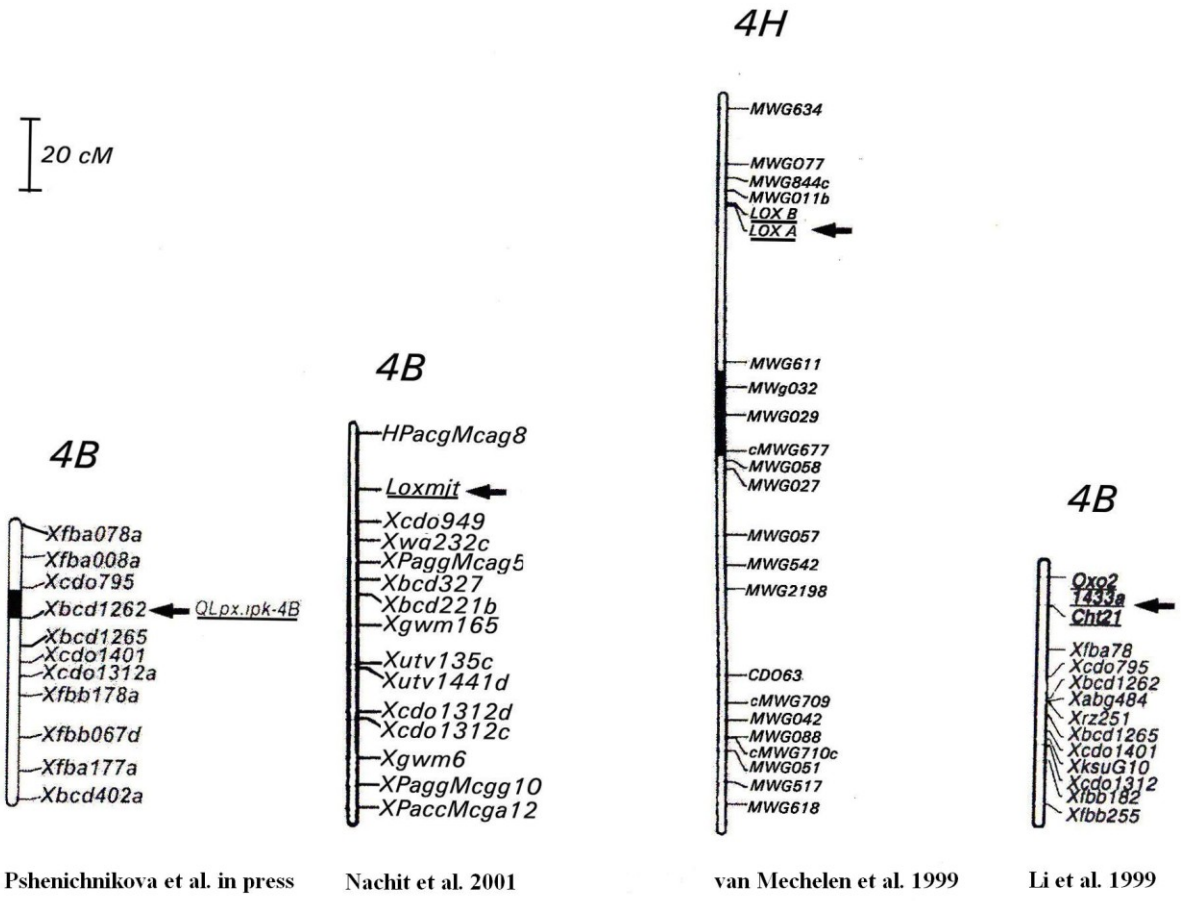

Fig. 1: Comparative maps of 4B chromosome of bread and durum wheat and 4H chromosome of barley

Locus of LOX mapped on chromosome 5A (Li et al. 1999) is situated near the marker *Xrz395* which linked with *VrnA1* gene on another map (Nelson et al. 1995) using the same markers (Fig.3). Galiba with co-authors (1995) mapped on 5A chromosome the vernalization gene *VrnA1* linked with the genes for frost resistance (*FrA1*) and for abscisic acid synthesis (*ABA2.1*) with use of other markers. On 5B chromosome vernalization gene was mapped near the marker *Xgwm604* (Fig.3). The map of Röder with co-authors (1998) serves as a "mediator" between the map of Galiba et al. and maps of Nelson et al. and Li et al. because it contains several common markers. The marker *Xgwm604* on this map is situated near the marker *Xcdo1326* which presented on both maps, on the first map- near the *VrnA1* gene and on the latter - near the gene for LOX. Therefore, comparing the maps using both the common and different markers allows supposing that the lipoxygenase gene on the long arm of 5A chromosome neighbours to vernalization, frost resistance and abscisic acid genes and in 5B chromosome neighbours to vernalization gene.

On the base of the comparative mapping it may be supposed the similar positioning of loci for soluble forms of lipoxygenase on homoeologous group 4 and 7 chromosomes in bread and durum wheat and barley. Possibly, they are localised in linkage groups with different defence response genes forming big functional units for adaptation of plants to different kinds of stresses.

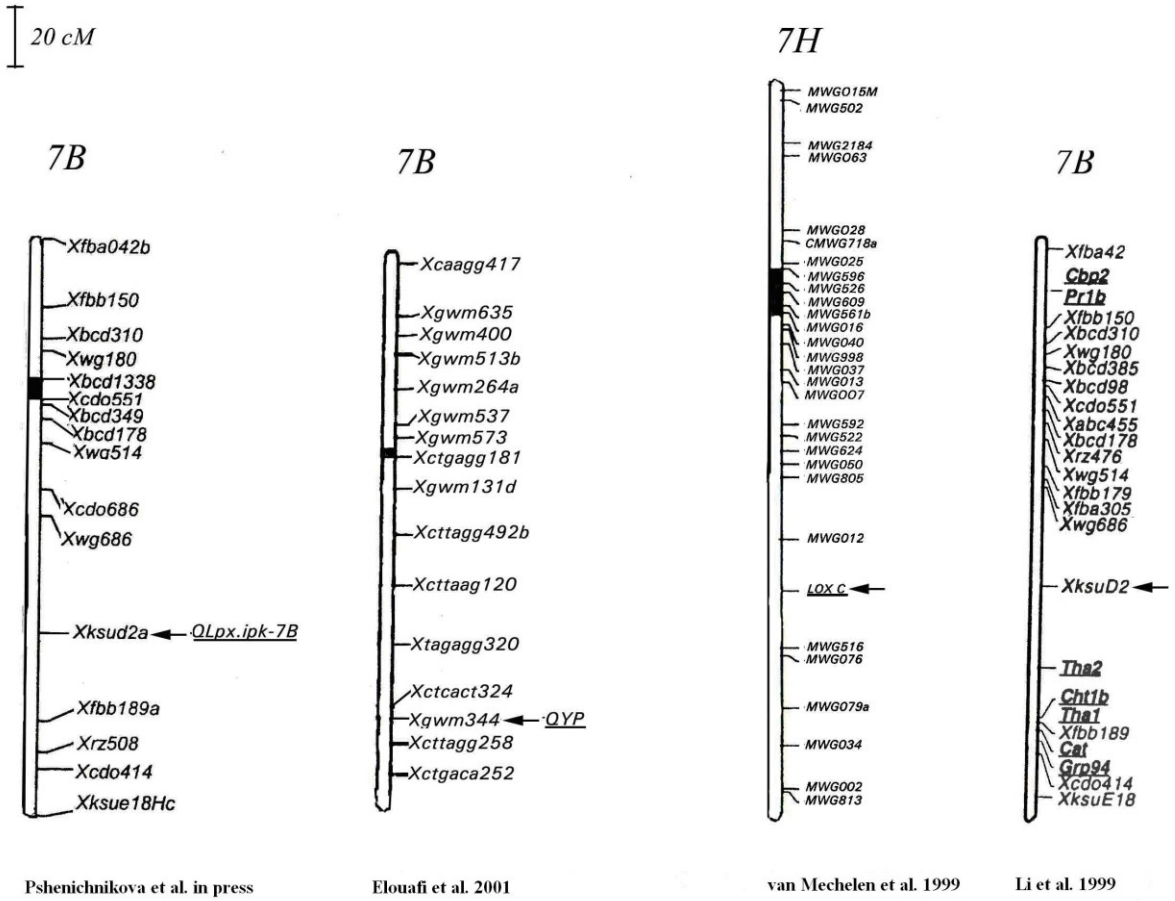

Fig. 2: Comparative maps of 7B chromosome of bread and durum wheat and 7H chromosome of barley

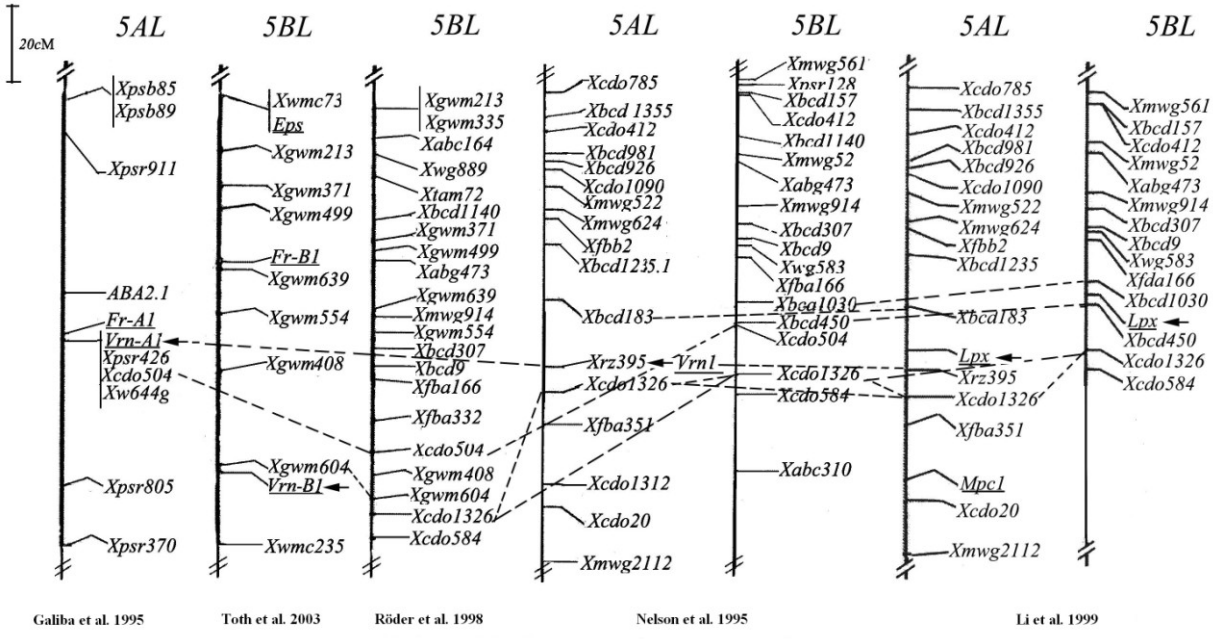

Fig. 3: Comparative maps of the long arms of 5A and 5B chromosomes of bread wheat

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Phenotypic peculiarities of spike in hybrids F_1 and F_2 between *Trtitcum spelta* L. and wheat line with introgression from *Aegilops speltoides* Tausch

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Spelta type of spike found in some hexaploid wheats (AABBDD, 2n=42) is a complex of morphological traits characterizing with glumes keeledness, rachis toughness, low spike density and poor threshing. This genetic complex is fully expressed in the species *Triticum spelta* L. and as considered nowadays is controlled with the locus *q* located in 5AL chromosome (MacGene, 2003). The early genetic investigations of speltoidy were carried out in 20th years of 20th century. Russian geneticist Yu. Philipchenko studied the trait in details in crosses with different hexaploid wheat genotypes (Philipchenko, 1979, 2nd edition). He gave a symbol *S* to the gene for this character from *T. spelta* and proved it to be dominant with the main effect on spike length. Also he showed that this gene manifests very early in the development, on 37-40 day after sowing.

Discovery of orthologous series of genes in cereal genomes allows suspecting the existence of homoeoallelic genes in genomes of diploid relatives of hexaploid species. Dr I. Lapochkina with collaborators (Institute of Agriculture of Central Regions of Non-Chernozem Zone of Russia, Nemchinovka, Moscow Region, Russia) developed the "Arsenal" collection of bread wheat (Lapochkina, 2001) which includes the lines with introgression from the winter accession of *Aegilops speltoides* Tausch developed on the base of spring cultivar 'Rodina'. The line 84/98^{w} from this collection is characterized with the winter type of development and has awned and speltoid spike. Presence of these traits indicates on a possible introgression from *Aegilops speltoides* genome into chromosome 5A of wheat.

The aim of the work was to investigate the possibility of existence of homoeologous gene for speltoid spike in introgression line 84/98^{w} in crosses with the genotypes carrying the known locus *q* in 5AL chromosome, *T. spelta* (accession k-24724, VIR, S-Petersburg) and substitution line Chinese Spring/*T. spelta* 5A.

Table 1: Morphometric parameters of spike of introgressive line 84/98^{w}, *T. spelta* L. and their F_1 and F_2 hybrids

Genotypes	Spike characteristics							
	Spike length, cm		Number of spikelets		Length of rachis segment, mm		Density index	
	0 ± s	Range	**0 ± s**	range	**0 ± s**	range	**0 ± s**	range
T.spelta L. k-24724	11,6±0,8	10,0 - 13,0	15,8±1,0	15-18	6,2±0,6	5-7	13,6±0,8	12,5-15,0
84/98^{w} Introgressive line	10,4±0,8	9,0 – 12,0	17,3±1,6	15-20	5,5±0,5	5-6	16,7±1,5	13,6-18,2
F1 84/98^{w} x *T.spelta* L.	15,8±1,2***	14,0 - 18,0	23,2±1,3***	21-15	6,1±0,6*^{a}	5-7	14,7±0,6***	13,9-15,8
F2 84/98^{w} x *T.spelta* (population)	12,3±2,8	7,0 - 17,5	21,4±4,3	15-30	5,1±1,0	3-8	18,0±4,1	11,6-31,4

*** - P<0,001 (difference from both parents) *^{a}- P<0,05 (from the line 84/98w)

Table 2: Morphometric parameters of spike of introgressive line 84/98^{w}, substitution line Chinese Spring/*T. spelta* 5A and their F_1 and F_2 hybrids

Genotypes	Spike characteristics							
	Spike length, cm		Number of spikelets		Length of rachis segment, mm		Density index	
	0 ± s	range	**0 ± s**	range	**0 ± s**	range	**0 ± s**	range
CS/*T.spelta* L. 5A	10,3±0,4	9,0-11,5	20,7±0,0	18-22	4,3±0,0	4-5	20,2±0,6	19,0-21,1
84/98^{w} Introgressive line	10,3±0,4	9,5-12,0	13,5±0,7	12-15	7,1±0,7	6-8	13,1±0,2	11,8-15,0
F1 84/98^{w} x CS/*T.spelta* 5A	13,1±0,7***	11,5-14,5	20,8±0,7***#	19-23	6,0±0,7***	5-7	15,9±0,4***	14,5-17,7
F2 84/98^{w} x CS/*T.spelta* 5A (population)	9,9±2,0	5,0-14,0	19,5±2,8	13-26	4,4±0,9	2-7	20,1±2,8	14,0-28,6

*** - P<0,001; # - differ from 84/98^{w} only

In the cross with the accession of *Triticum spelta* L. it was found that the spikes of F_1 hybrids significantly exceeded the parental forms for such spike parameters as spike length and number of spikelets (Table 1). Density index (number of spikelets on 10 cm of spike) has the intermediate meaning. In F_2 positive and negative transgressions for all spike traits were observed comparing to the parental forms. The population means for spike length and for number of spikelets exceeded the parental means. Segregation of "superspeltoid" forms with F_1 phenotype was observed again in F_2 generation approximately in ratio 1:1.

In the cross between the line $84/98^w$ and the substitution line CS/*Triticum spelta* 5A (Table 2) a similar effect was observed although not in all cases. Spike length increased significantly comparing to the both parents, the number of spikelets increased only comparing to the introgression line. Length of rachis segment and density index had intermediate meanings. Again positive and negative transgressions were observed comparing to the parents and segregation of "superspeltoid forms" in F_2 generation. At the same time, the F_2 means of this cross for the traits studied did not differ very much from the parents.

A significant increase of measured spike parameters in crosses between the line $84/98^w$ carrying the introgression from *Ae. speltoides* and *T. spelta* accession allows to suppose the additive interaction of two homoeolallelic genes in 5A chromosome responsible for the separate characteristics of speltoid spike. A lesser effect was found in the cross of the introgression line with bread wheat substitution line. It may be explained by the influence of bread wheat genome on the expression of speltoid characteristics.

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Study and utilization of spontaneous spring mutations of wheat, rye and triticale in Siberia

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Since 1999 about 2800 spontaneous mutant plants S_1 of wheat, rye and triticale able to transit from vegetative to reproductive development without cold treatment at the juvenile stages of ontogenesis (vernalization), have been collected from the winter crops populations sown in spring. Among the S_2 of these plants we didn't notice any morphological segregation. Most of the S_2 generations had spring-winter segregations, which proved that the parental S_1 plants were heterozygous and the mutant genes were dominant (Styopochkin, 2000; 2001). Thus, the spring mutant plants had uncertain genetic formula in the early generations $S_2 - S_3$ and were

either homozygous or heterozygous for the habit-controlling genes, which hindered using these plants for genetic analysis of the trait.

We have developed a scheme of genetic analysis to identify the dominant mutant genes responsible for the spring habit of the plants carrying unknown dose of the dominant mutant gene in the early generations. Using the scheme we found that seven studied rye plants had the same mutant gene (Stepochkin, Artemova, 2006). The scheme as well may be used for the genetic analysis of triticale and wheat mutants and includes three steps of crosses.

At the first step, the heterozygotes for two studied genes are obtained:

1. (a) $A_1A_1 \times a_1a_1 \rightarrow A_1a_1$ or $A_1a_1 \times a_1a_1 \rightarrow A_1a_1 + a_1a_1$;

(b) $A_2A_2 \times a_2a_2 \rightarrow A_2a_2$ or $A_2a_2 \times a_2a_2 \rightarrow A_2a_2 + a_2a_2$.

At the second step, the recessive homozygotes are removed (they don't come to the reproductive stage) and the (a) and (b) types of heterozygous plants are crossed with each other:

2. (a) A_1 and A_2 alleles of the same locus:

$A_1a_1 \times A_2a_2 \rightarrow A_1A_2 + A_1a_2 + A_2a_1 + a_1a_2$;

(b) A_1 and A_2 alleles of different loci:

$A_1a_1a_2a_2 \times A_2a_2a_1a_1 \rightarrow A_1a_1A_2a_2 + A_1a_1a_2a_2 + a_1a_1A_2a_2 + a_1a_1a_2a_2$.

The recessive homozygotes are removed and at the third step, the spring plants are either self-pollinated or crossed with the recessive homozygotes.

3. (a) If the dominant alleles A_1 and A_2 belong to the same locus, about one-third of the progeny of the spring plants does not segregate in the subsequent progeny yielded by the crosses of the parents with unknown genetic formula, because theoretically one-third of the progeny obtained at the second step, which become the parental forms in the crosses at the third step, are expected to have been the dominant AA homozygotes. In two-thirds of the families (the original parental plant is heterozygous), the expected segregation for spring and winter plants is 3 : 1, when they are the progeny of self-pollination of wheat or triticale plants (or of a free cross with other spring rye plants of the population) and 1 : 1, when these families are the progeny of crosses with the winter plants carrying recessive genes.

(b) If A_1 and A_2 alleles belong to different loci, segregation is expected in the progeny of all parental plants studied.

In 2003 we started the crosses according to this scheme. At the first step emasculated spikes of a winter wheat variety Novosibirskaya 32 were pollinated by two mutant forms S_3 Lutescens 105/5 and S_3 Lutescens 105/7, and four tester-lines Skorospelka 3b-*Vrn1*, Skorospelka 3b-*Vrn2,* Skorospelka 3b-*Vrn3* and Triple Dirk F (*Vrn4*). Then at the second step the heterozygous F_1 carrying a dominant mutant gene were crossed with the heterozygous F_1 carrying a dominant *Vrn* gene of the tester-lines. The progeny of these crosses were self-pollinated at the third step of the scheme and the seeds from each plant were sown separately in the field in spring to count spring-winter segregations in the progeny (families) F_2 hybrids of the last artificial crosses. We took into consideration only those families of the hybrids, which contained at least 33 or more plants.

Two non-segregated families were found in the combinations where the mutant wheat Lutescens 105/5 and the tester-line Skorospelka 3b-*Vrn1* took part and four non-segregated families occurred in the combinations where the mutant wheat Lutescens 105/7 and the tester-line Skorospelka 3b-*Vrn2* were involved (Table 1).

Table 1: The number of segregating and non-segregating families of wheat in the self-pollinated offspring F_2 obtained by three-step crosses between carriers of the dominant mutant genes and tester-lines

Code of the original combinations at the second step of crosses	*The number of families F_2*			$\chi^2_{2:1}$
	segregating	nonsegregating	total	
(N.32 x L.105/5) × (N.32 x S.3b-*Vrn1*)	10	2	12	1.50
(N.32 x L.105/5) × (N.32 x S.3b-*Vrn2*)	11	0	11	5.50
(N.32 x L.105/5) × (N.32 x S.3b-*Vrn3*)	10	0	10	5.00
(N.32 x L.105/5) × (N.32 x Triple DirkF-*Vrn4*)	14	0	14	7.00
(N.32 x L.105/7) × (N.32 x S.3b-*Vrn1*)	8	0	8	4.00
(N.32 x L.105/7) × (N.32 x S.3b-*Vrn2*)	10	4	14	0.14
(N.32 x L.105/7) × (N.32 x S.3b-*Vrn3*)	8	0	8	4.00
(N.32 x L.105/7) × (N.32 x Triple DirkF-*Vrn4*)	13	0	13	6.50
χ^2_{05} =				***3,84***

The results show, that the mutant gene of the line Lutescens 105/5 seems to be one of the alleles of the *Vrn1* gene of the tester-line Skorospelka 3b-*Vrn1*. The mutant gene of the second line Lutescens 105/7 appears to be one of the alleles of the *Vrn 2* gene of the tester-line Skorospelka 3b-*Vrn2*.

Crosses between these two mutant lines also proved that their dominant genes have different locations (Table 2).

Table 2: Number of spring and winter plants in F_2 of two mutant wheat forms Lutescens 105/5 and Lutescens 105/7

Code of the combinations	Number of plants		$\chi^2_{15:1}$	$\chi^2_{3:1}$
	spring	winter		
Lut. 105/5 × Lut. 105/7	59	5	0.14	10.08
Lut. 105/7 × Lut. 105/5	103	13	2.17	11.77
$\chi^2_{0.05}$			3.84	

These two mutant genes have different expressions. The vegetative period length (VPL) of the two wheat mutant forms is different. One of them, Lutescens 105/7, has the longest VPL, which lasts 100 – 110 days. The VPL of the second mutant line is 20 - 25 days shorter (Figure 1).

Some mutant forms of the other two crops have also different VPL. The VPL of the mutant tetraploid rye is 23 – 27 days longer, than that of the mutant diploid rye and the spring diploid rye variety Onokhoiskaya. The variety matures 3 – 5 days earlier than the diploid mutant rye. One of the mutant hexaploid triticale UK 30 ripens 1 – 3 days earlier than the other mutant triticale O.312 and their VPL is 10 – 15 days longer than that of Onochoiskaya.

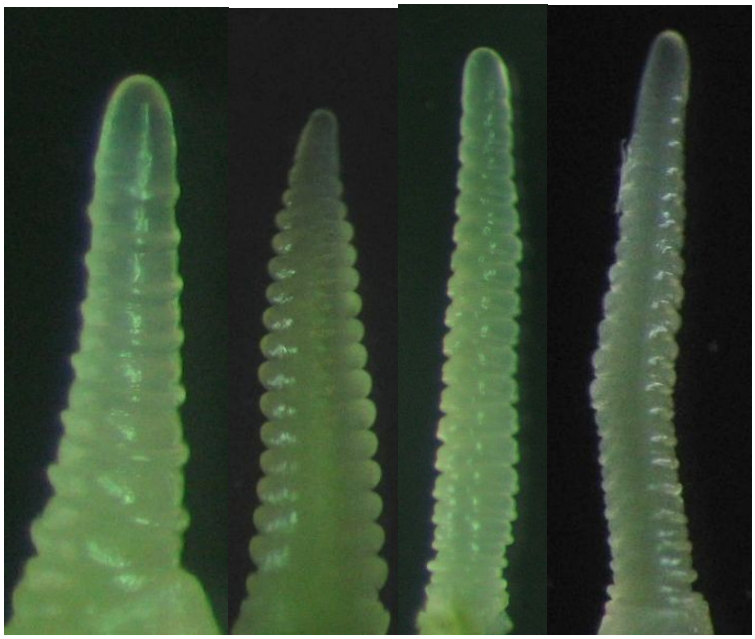
In 2006 we studied the reaction of these mutant forms on the short (12 hours) and long (18 hours) day length. The plants were grown in the chamber of artificial climate “Biotron-4”. The length of the plant apexes and juvenile spikes was measured after 25, 30, 35, 45 and 55 days of the experiment.

The critical 4th stage of transition from vegetative to reproductive development of plants starts according to Cooperman (Cooperman et al 1955) when the juvenile spikelets begin forming (figure 2). The mutant forms studied came to this stage differently in the day-length

experiments. It took a longer period of time for all plants to reach this stage in the 12-hour experiment than in the long-day (18 hours) one.

a b

Fig.1: Wheat mutants: a - Lutescens 105/5; b - Lutescens 105/7



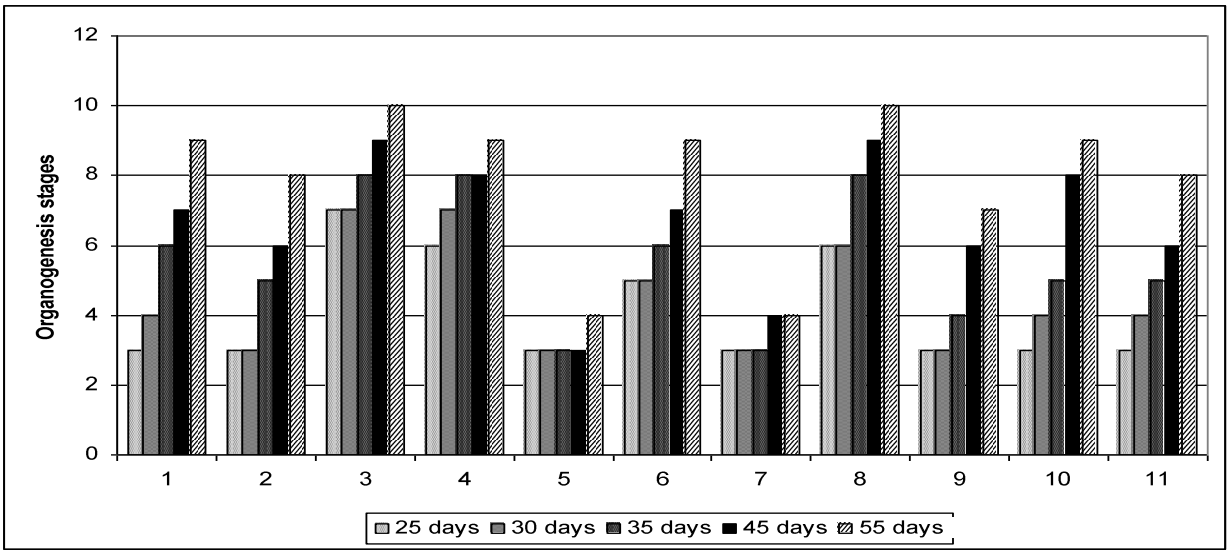
1 2 3 4

Fig.2: Stage IV of organogenesis (the beginning of juvenile spikelets forming) critical to transition from vegetative to reproductive development. Juvenile spikes of mutant forms: 1 – wheat Lutescent 105/5; 2 – hexaploid triticale UK 30; 3 - rye 2n; 4 - rye 4n

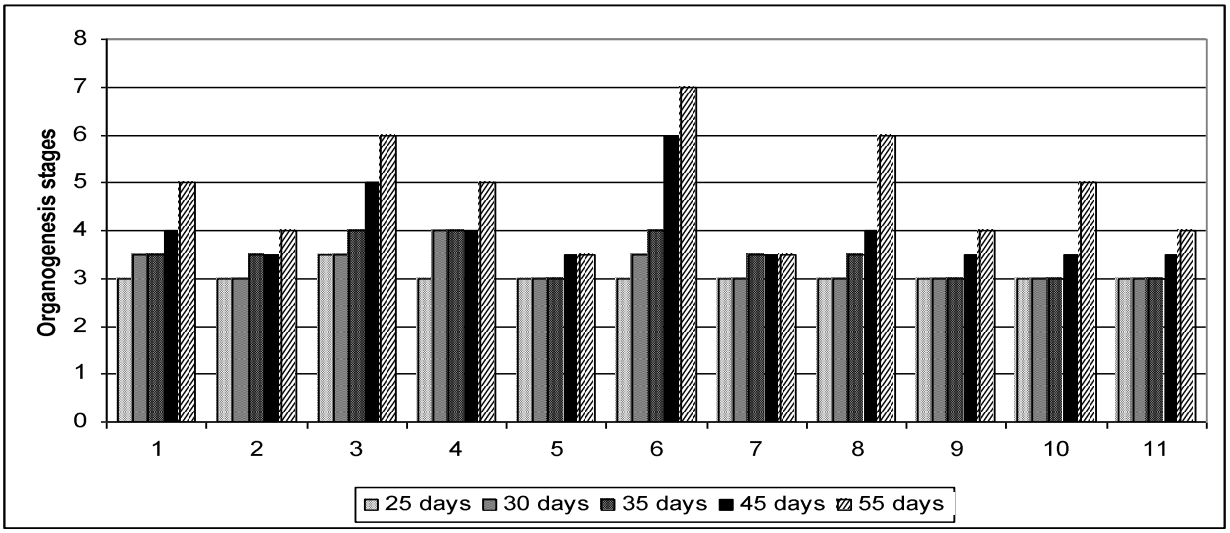
Two diploid ryes, the mutant wheat Lutescens 105/5 and near-isogenic line Triple Dirk D (*Vrn1*) transited to the reproductive development less than in 25 days in the long-day experiment (figure 3A).

The short day delayed the development of all forms studied. The early third organogenesis stage (the lower part of the short apex begins to form the juvenile nodes, internodes and

leaves of the juvenile stem) of the mutant forms: triticale O.312, rye 4n, wheat Lutescens 105/7 and near-isogenic lines of the wheat Triple Dirk lasted more than 25 days. The mutant wheat Lutescens 105/5 was less sensitive to the short day exposure, than the rest forms (figure 3B). One can suppose that the line may have one of the *Ppd* genes, that causes insensitivity to the day length. The mutant diploid rye doesn't differ much from the diploid variety Onokhoiskaya. Perhaps the mutant gene is one of the alleles of the *Vrn* gene of Onokhoiskaya. On the whole, the development rate of these two ryes and the wheat line Triple Dirk D (Vrn1) is similar. We suppose that both ryes have a *Vrn* gene orthologous to the *Vrn1* gene of the wheat.



A



B

Fig.3: Diagrams of the organogenesis stages of plant development of three crops after 25, 30, 35, 45 and 55 days of 18-hour (A) and 12-hour (B) experiments. 1 - Mutant triticale UK30; 2 - Mutant triticale O.312; 3 - Rye Onokhoiskaya; 4 - Mutant rye 2n; 5 - Mutant rye 4n; 6 - Mutant wheat Lutescens 105/5; 7 - Mutant wheat Lutescens 105/7; 8 - Wheat Triple Dirk D (*Vrn1*); 9 - Wheat Triple Dirk B (*Vrn2*); 10 - Wheat Triple Dirk E (*Vrn3*); 11 - Wheat Triple Dirk F (*Vrn4*).

The mutant wheat Lutescens 105/7 and the mutant tetraploid rye remained at the late third stage of organogenesis (elongation of the apex) in the short-day experiment and had reached the fourth stage by the end of the long-day one. We think that the mutant wheat line Lutescens 105/7 has one of the weak *Vrn2* gene alleles. Among the near isogenic wheat lines Triple Dirk B (*Vrn2*) has the longest vegetative development period, however it develops faster than Lutescens 105/7.
In our experiments the near-isogenic wheat lines started to differ in the rate of development at the early stages of ontogenesis. The complete distinction in their development rate had been notable by the 55th day of the long-day experiment. Plants of the line Triple Dirk D (Vrn1) began to develop embryo and endosperm after pollination (10th stage of organogenesis), while plants of the line Triple Dirk E (Vrn3) were flowering (9th stage), the main spikes of the plants of the line Triple Dirk F (Vrn4) were emerging out of the flag leaf (8th stage), and plants of the line Triple Dirk B (Vrn2) remained at 7th stage of organogenesis.

The mutant triticale UK 30 develops faster than O.312. They may have a dominant mutant *Vrn* gene allele of the A, B or R genomes. It is known, that the 5th homeological chromosome group of these genomes carries the genes, controlling the transition from vegetative to reproductive (Law et al, 1976; Kato et al, 1993). As a common wheat took part in their origin, they also might have a translocation fragment or a whole chromosome of the D genome carrying an allele of a *Vrn* gene. The collection of triticale mutants in Siberian Research Institute of plant growing and selection counts several hundreds forms and it is possible, that some of them carry different mutant genes.

To identify the mutant genes in triticale, it is necessary to create triticale tester-lines on each *Vrn* gene on the base of the spring wheat lines carrying one of the genes Vrn1, Vrn2, Vrn3 and Vrn4 and a spring rye carrying a *Vrn* gene. According to new classification the *Vrn* genes are denoted as *Vrn-A1a, Vrn-B1a, Vrn-D1a and Vrn-D5a* for common wheat *(*McIntosh et al, 2004*)* and *Vrn-R1a* for rye. We have already started to create them by crossing: 1) winter 6x triticale × near-isogenic lines Triple Dirk carrying one of the *Vrn* genes and 2) near-isogenic lines Triple Dirk × winter 2n rye with consequent colchicine treatment to double the chromosome number of the wheat-rye hybrids.

The frequency of mutant plants occurrence in the winter crops populations is different. The mutant spring plants emergency frequency in some populations of winter crops was very high. It reached up to 3 : 1000 plants in the population of winter triticale Altaiskoe 2 and 5 : 1000 plants in the population of winter diploid rye Korotkostebelnaya 69.

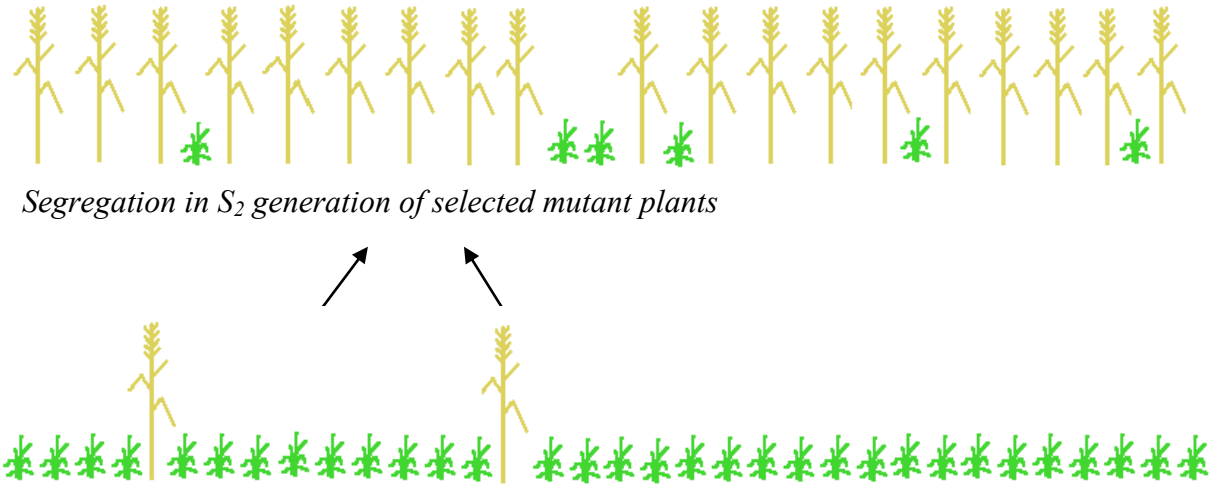
Spring triticale and rye have never been grown either at the experimental plots or at the nearby fields. That guaranteed the reliability of the mutant selection results. The winter wheat variety Lutescens 105 has some morphological distinctions, which make it different from spring wheat varieties and lines, grown at the location. All mutant wheat plants S_1 had typical for the original winter population features, which excluded an impurity. Thus we are certain, that the spring forms of the three crops occurred as a result of spontaneous mutations.

As the offspring of the most S_2 plants segregated (Figure 4) we decided to use the three-step scheme of crosses for the genetic analysis to identify the dominant mutant genes. In cross-pollinating plants, it is extremely difficult to differentiate the dominant genes and to identify them using tester lines. The proposed scheme of analysis is not overly simple, but it makes possible to avoid some time-consuming difficulties such as homozygotization of the forms for the dominant gene or development of new lines. The three-step crosses scheme allows to carry out a genetic analysis of dominant mutants of the early ($S_1 – S_3$) generations.

We think that it is possible to use the process of spontaneous spring mutation of winter crops of non-Siberian origin to introduce them in Siberia. Winter hardiness of all varieties that were bred in the areas with mild winters is weak for Siberia. The collection of them usually perishes under severe Siberian winter conditions. However, the varieties may carry valuable

genes controlling resistance to diseases, grain yield, grain quality, etc. and Siberian breeders cannot directly use their germplasm into their breeding programs. Using the spontaneous spring mutations that take place in the populations of winter crops may help overcome the problem.

First, it is necessary to select the mutant plants according to scheme 4 and obtain dominant homozygous lines of wheat and triticale. Then the collection of the mutant lines must be tested in the field plots for using in the process of the spring crops selection.



Segregation in S_2 generation of selected mutant plants

Selection of S_1 spontaneous mutant plants in the winter populations of wheat, rye and triticale sown in spring

Fig. 4: The scheme of selection of S_1 spontaneous mutant plants and segregation in S_2 generation

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Hybrid lines of wheat, resistant to *Puccinia*

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Hybridization till now is the basic method of wheat improvement. With its help probably not only the decision of some theoretical questions, in particular, questions on an origin of wheat, but also the decision of problems of resistance of wheat to various illnesses. N.I.Vavilov (1967) repeatedly emphasized, that for wheat selection crucial importance has use of a world variety of wheat.

The most full review of various genetic researches by many species of wheat is submitted in V.F.Dorofeeva's collective monography with employees "Wheat of the world ", left for the first time in 1976 in Leningrad (Saint Petersburg), under edition of academician ARAAS of D.D.Brezhneva, and since then sustained already some editions. In it " … long-term experience of domestic and world selection of wheat is generalized, specific genetic resources of species *Triticum L.* are shown, to law of variability and inheritance of useful signs at intraspecific and interspecific crossings, etc. " (Dorofeev at al., 1976).

The purpose of researches was studying of alloplasmic lines of winter soft wheat resistant to races *Puccinia.*

Material and methods

As a material for researches wheat species have served: *Triticum aestivum L.* (sort Saratovskaya-29) and *T.kiharae Dorof. et Migusch.*, hybrids F_1 - F_4, BC_1 – BC_5, BC_4F_4 , received from crossing these species.

Crossings of all species, sorts, lines and hybrids carried out on the standard methods, with some updatings (Khailenko, 2004). 5-10 ears of each combination were castrated and pollinated. Ears of sterile hybrid plants of all generations pollinated without castration. For this purpose the most part of ears put on individual pergament isolators on a measure of an output 2/3 ears from a vagina of a leaf. Pollinated with the help of a twel-method, with cutting or without cutting spicate and floral scales of ears of mother's forms. Pollination was carried out on a measure of maturing stigmas in flowers, with 2-3 times, within 10-15 days. Before pollination, with the help of cytological methods, controlled of fertility and viability of pollen grains in flowers of hybrid plants.

During flowering visually was determined the type of sterility shown at hybrid plants – on a configuration and functioning of anthers.

Crop was made on fields of SPCAaPG. All parental forms, populations of hybrids and lines were raised in a field with the area of a feed of plants 5 x 30 sm. In all field experiences observed a mode of agrotechnical actions standard for the given region. The estimation by illnesses was carried out on international 5 ball scale, experts - phytopathologest on natural and infectious fields of SPCAaPG.

Results and discussion

At use of species *T.kiharae Dorof. et Migusch.* as the mother form at pollination by its various sorts of species *T.aestivum L.* the most viable have been received grains and the highest hit ratio – from 33,33 % up to 73,68 %. The hit ratio already in F_1 reaches 60 %, and with increase in number of backcrosses grows up to 65-70 % in BC_5- BC_6 (at artificial pollination under individual pergament isolators), and at restoration of fertility in generations F_6-F_8 – up to 87 %. But at free pollination setting of hybrid grains in these combinations does not exceed 5-7 % as species *T.kiharae* has not only sign CMS, but also sign GMS.

At sterile plants BC_5 of combination *T.kiharae* x Saratovskaya-29 the quantity of setted grains at free pollination made 2,77 %, in spite of the fact, that ears blossomed openly 15 day, and at artificial pollination by a fatherly sort the quantity of setted grains grew up to 15,05 %. Anthers in all ears had the form “dovetail ”, characteristic for sign of CMS and " star-shaped" the form of pollen.

In combination *T.kiharae* x Saratovskaya-29 setting of hybrid grains in F_0 made 72,58 %, sharply fell up to 25,19 % and 24,07 % in F_1 and BC_1, in F_2 raised – up to 30,56 % a little, but went down in BC_2 – to 15,0 %, and in BC_3 changed from 30,73 % up to 60,12 %, on the average making 45,43 %, and even at free and controllable pollination in BC_2F_3 and BC_3F_4 changed from 43,24 % up to 57,47 %, i.e. did not exceed 60 %. In control ears of plants BC_1-BC_3 which closed by individual pergament isolators and has been not pollinated, setting of hybrid grains it was equal 0, and at plants BC_2F_3 in 1993 only in one such ear (from 152 closed and not pollinated) has taken place restoration of pollen fertility – setting of hybrid grains has made 31,43 %. Probably, also, that also stabilization female gametophytes in this case began. But also, more probably that we was observed not influence of separate genes-restorers of fertility, but *epigenetic control all of mechanism of fertility restoration.*

On dates to V. F. Dorofeev (1976), species *T.kiharae Dorof.et Migush.* and *T.timopheevii Zhuk.* are genetically incompatible with hexaploid species of wheat, however in the first generations in our experiences setting of hybrid grains in combination *T.kiharae Dorof.et Migush.* x Saratovskaya-29 reached 74 %, and in combination *T.timopheevii Zhuk. v. viticulosum* x Saratovskaya-29 changed from 25 % up to 40 % .

Such sharp fluctuations of the hit ratio in F_1 and BC_5-BC_7 testify that plants of backcrosses lines and sterile analogues have genotypic distinctions including on genes of CMS, and also, probably, and on genes of fertility restoration. Hence, all further work with sterile analogues is necessary for carrying out on separate families, and among families with high setting of hybrid grains it is possible to search for restorers of fertility.

In combination *T.kiharae* x Saratovskaya-29 observed segregation on sterile and fertile plants, since F_3. Sterile and fertile plants concerned to varietes *lutescens* and *velutinum* on colouring and pubescence of scales, but the form of ear had intermediate type even in BC_4 - BC_7. In this combination in the first generation the percent of setting in reciprocal crossings did not exceed 30%.

At sterile plants the percent of setting in BC_4 at artificial pollination changed from 14,35% up to 52,25%, at fertile plants it reached 80%.

On appearance of plants of combinations *T.kiharae* x Saratovskaya-29 (F_2) and Saratovskaya-29 x *T.kiharae* (F_2) gave segregation on awned and awnless forms, had non-pubescence and pubescence ear’s scales. Colouring of ears was white, and stems – white and anthocyan.

Below the botanical description of hybrid forms to some morphological signs is resulted.

Combinations T.kiharae x Saratovskaya-29 and Saratovskaya-29 x T.kiharae:

Awnless forms. Pubescence and smooth ear's scales; ears of intermediate type, but close to *T.kiharae*; keel tooth was short, sharp, a shoulder of spicate scales was direct, rather narrow; a culm under an ear was hollow; a grain was large, red, glassy, similar to grain *T.kiharae*, but with wrinkled endosperm; at non-pubescence forms thrashing it is complicated, at pubescence – it is very difficult.

Awned forms. All plants relatives on appearance to *T.kiharae* have pubescence ears of intermediate type; keel tooth was short, stupid; a shoulder spicate scales was wide, direct; a culm under an ear was hollow; grains were red, large, glassy, is similar on grain *T.kiharae*, but with wrinkled endosperm; thrashing it is extremely complicated.

Plants $F_3(BC_3)$ of combination *T.kiharae* x Saratovskaya-29 had white, with weak arista points ears, basically non-pubescence spicate scales; stems were weak anthocyan and white colouring; keel tooth of spicate scales was short, sharp or rostral, a shoulder of spicate scales was rather narrow, direct; a culm under an ear was hollow; grains were dark red, lengthened, glassy. Ears on appearance came nearer to ears of sort Saratovskaya-29.

Let's notice, however, once again, that species *T.kiharae* on the literary data is considered genetically incompatible with species *T.aestivum*, but at us not only the collection of hybrid lines from combinations *T.kiharae* x Saratovskaya-29 is created, but also the part of lines from grown-ups backcrosses has passed successful tests for resistance to races Puccinia in SRAI and SPCAaPG.

During a field seasons on infectious and natural background the estimation of all plants on resistance to a yellow and brown rust and Septoria has been carried out. To a brown rust appeared resistant 67 lines and hybrids winter soft wheat (0 – 2/10), to yellow - 56 (0 – 2/10), to Septoria – practically all plants of winter crop. The negative selection sign which is passing from father to son – wrinkled endosperm, however for genetic researches can be marker's sign.

Thus, already in the first years of reception of alloplasmic lines of wheat we had seeds, having different genotypes that could be of interest for carrying out genetic, cytogenetic, cytoembryological researches on problems of the remote hybridization of plants.

Work on reception and studying of wheat alloplasmic lines on cytoplasms of species *Triticum L.* has been continued before reception BC_8-BC_{17}, then selection lines and a collection sterile both fertile of alloplasmic and backcrosses lines have been incorporated. The part of lines from a collection was annually transferred selection establishments of Republic Kazakhstan.

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The study of quantitative traits for yield and product quality in Saratovskaya 29 introgressive lines developed by the use of amphidiploid *T. timopheevii x Ae. Tauschii*

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Introgression of genes determining resistance to various diseases is one of the effective strategies for developing immune commercial cultivars of common wheat. Many of the wild wheat relatives and synthetic wheats represent a potential source of resistance genes. Immune lines developed on the base of cv. Saratovskaya 29 (S29) common wheat cultivar possess the resistance genes for leaf rust (*Lr*), stem rust (*Sr*) and powdery mildew (*Pm*), introgressed from synthetic hexaploid wheat *T. timopheevii x Ae. tauschii* ($GGA^bA^bD^{sq}D^{sq}$) of Dr. Savov (Bulgaria). Analysis of ten quantitative traits for yield and product quality showed that immune lines do not differ from cultivar-recipient S29 (Laikova et al., 2004 a, b). Chromosome localization of synthetic wheat material in genome of S29 immune lines was determined by the means of microsatellite (SSR) markers (Laikova, Leonova et al., 2001). Hybridological analysis revealed that resistance genes in S29 immune lines are not allelic to the known efficient *Lr* genes.

Literature data evidenced that a transfer of disease resistance genes from wild relatives into cultivated hexaploid wheat are accompanied by linkage drug which negatively influence on the yield traits and protein quality (Krupnov et al., 2004)

The influence of alien genetic material on the appearance of quantitative trait loci of yield traits and product quality was studied in immune line of different backcross selections (BC_5F_4- line X, BC_8F_4- XI, BC_9F_4- VI, VIII) in comparison with S29. Plant material was cultivated on experimental plots of Institute of Cytology and Genetics SB RAS near Novosibirsk in 2001, 2003 years. Genotype-by-environment interactions for some agronomic traits (ear length, spikelets number per ear, grain number and grain weight per spike) were calculated using variance analysis. The influence of environmental factors on quantitative traits of S29 cultivar was studied during two years. Student's test (*t*) was used for statistical analysis. It was shown that the main important yield traits – ear length, spikelets number, ear compactness, are dependent of environmental factors. On the other hand environment doesn't effect on the grain number and grain weight (table 1).

The influence of genotype (r_{wg}) and random factors on the agronomic traits of immune lines estimated by variance analysis is illustrated in table 2. *F* test was performed for estimation of the effect of random factors and genotype on agronomic traits (Vasil'eva, 1999). There was no significant difference in ear length between immune lines XI (BC_8F_4) and VI (BC_9F_4) and S29 cultivar (r_{wr}=0.01 and 0.11, respectively). For line X (BC_5F_4) the difference in this trait comparing with S29 was affected by genetic factors, introgressed from synthetic wheat (r_{wr}=0.92).

In immune lines BC_8F_4 (XI) and BC_9F_4 (VIII) the increase in spikelets number of head ear was observed comparing with initial cultivar S29. These differences have been caused by genetic factors (r_{wr}=0.81 and 0.59, respectively). In line VI (BC_9F_4) the appearance of this trait depends on the random factors (r_{wr}=0.21). Reduction in grain number was found in lines X and VI, but in line VI genotype effects on the trait to a greater extent than environmental factors (r_{wr}=0.89).

Table 1: Influence of environmental factors on agronomic traits of cv. Saratovskaya 29 (Novosibirsk 2001, 2003)

M- Trait	X ± m		*t*
	2001	2003	
Ear length, *cm*	9,7±0,6	6,5±0,1	5.08***
Spikelets/spike	15,8±0,7	12,4±0,2	4.66***
Ear compactness density	16,3±0,7	19,2±0,4	-3.58***
Grains/spike	41±5,8	35,5±1,2	0.96
Grain weight/spike, *g*	1,61±0,27	1,19±0,06	1.50

***P<0.001.

Table 2: Analysis of variance. Influence of alien genetic material from *T. timopheevii* x *Ae. tauschii* on the expression of some quantitative traits of immunity lines derived from different backcrosses, from compared with cv. Saratovskaya 29, Novosibirsk 2001

Trait	S29	Line X (BC_5F_4)			Line XI (BC_8F_4)			Line VI (BC_9F_4)		
	X±m	X±m	r_{wg}	***F***	X±m	r_{wg}	***F***	X±m	r_{wg}	***F***
Spike length, cm	9,7±0,6	8,8±0,4	0,92	*** **69**	9,6±5,5	0,01	1,0	9,6±0,7	0,11	* **4,33**
Spikelets/ spike	15,8±0,7	16,6±0,8	0,05	2,4	17,3±0,7	0,81	*** **106**	16,8±1	0,21	* **11,2**
Grains/ spike	41±5,8	36,5±4,8	0,20	* **7,1**	42,3±6,7	0,07	2,7	35,0±8	0,89	*** **200**
Grain mass/ spike, *g*	1,61±0,3	1,34±0,2	-	0,6	1,46±0,3	0,45	*** **22,1**	1,32±0,3	0,25	** **9,6**

*P<0,05; **P<0,01; ***P<0,001; r_{wg} – influence of genotype on trait expression.

Thus, the data of variance analysis establishes the influence of genetic factors on some quantitative traits in immune lines BC_5, BC_8 and BC_9 selections.

The estimation of grain technological properties of immune lines has been made. Physical parameters of grain of S29 cultivar and immune lines are presented in figure 1.

Alveograph data (figure 1a) evidenced that S29 is characterized by high indexes: force of a flour (W=350 joule), viscoelasticity of the dough (P=119mm), stretchability (extensibility) of the dough (L=98), P/L=1.21. Immune line VII exceeded the initial cultivar S29 on all of these parameters for 20%, 36% and 48%, respectively. The line III also exceeded the S29 on force of a flour and elasticity, but conceded in P/L parameter. The other immune lines met the requirements for strong and valuable wheats.

The data received by farinograpf seem to be the most stable physical parameters of wheat flour (figure 1b). S29 variety is characterized by high water-absorbing ability of the flour (A), time of the dough development (B), dough stability to mixing (C), dough strength (B+C), a degree of the dough stability (E) and index of valorimeter (VD). Among the studied line (X and XI) exceeded the S29 on all parameters obtained by farinograpf. The data of valorimetric estimations of the dough quality evidenced that immune lines meet the requirements for strong and valuable wheats.

a) Alveograph curves **b) Farinograpf curves**

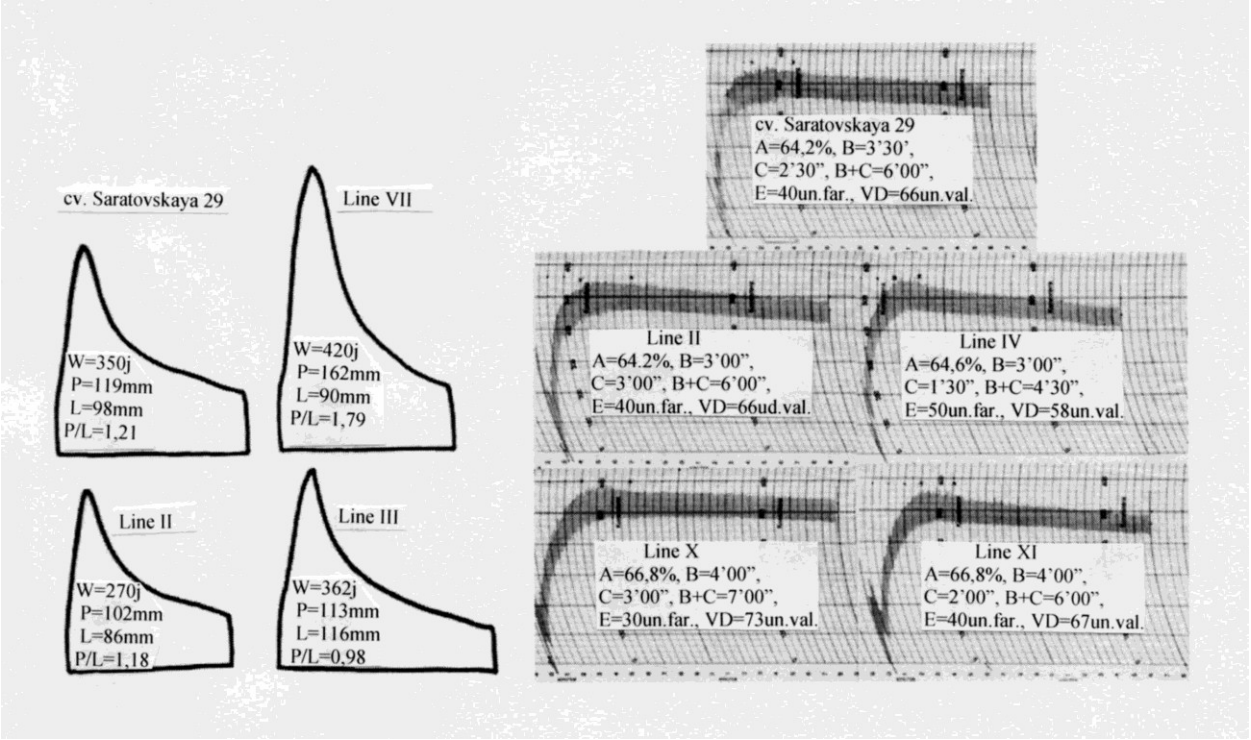

Fig. 1: Alveograph (a) and farinograpf (b) curves of the some immunity lines and cv. Saratovskaya 29, Novosibirsk 2001

Thus, genetic material introgressed from synthetic hexaploid wheat [*T. aestivum* x (*T. timopheevii* x *Ae. tauschii*)] did not effect negatively on technological and physical properties of grain of initial wheat cultivar Saratovskaya 29. These immune lines can be used as donors of useful traits in breeding programmers.

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The diversity of bread wheat samples with alien genetic material from wild relatives for genes of resistance to leaf rust

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In selection of bread wheat the problem of resistance to fungus diseases is most acute. The genetic diversity of bread wheat cultivars on genes of resistance to leaf rust is limited. In Russian cultivars of winter wheat, the researchers identified only 5 genes of resistance (*Lr1, Lr3, Lr10, Lr23, Lr26*) overcome by majority of pathogen races widespread in the territory of Russia. In spring wheat cultivars genes *Lr2, Lr14b, Lr16, Lr19,* and *Lr25* (Kolomiets et al. 2004) were additionally identified. It is possible to increase the genetic diversity of bred cultivars with respect to genes of resistance at the expense of genofond of wild wheat relatives and original material obtained with its participation. Our investigations are aimed to genotyping of samples from the "Arsenal" collection of spring bread wheat with respect to genes of resistance to leaf rust in order to use them in future, in selective programs for immunity. The "Arsenal" collection, developed in the Agriculture Institute of Non-Chernozem Zone laboratory of genetics and cytology (1981-1996) by distant hybridization of common wheat with radiation of its relatives' pollen, contains more than 220 genotypes of bread wheat with alien genetic materials of *Aegilops speltoides, Ae.triuncialis, T.kiharae* and *S.cereale* species (Lapochkina 2001).

Material and methods

Twenty-three hexaploid lines of spring bread wheat of different origin from the "Arsenal" collection were investigated. To identify the genes of resistance, STS markers were used linked with genes *Lr1, Lr9, Lr10, Lr21, Lr24, Lr35, Lr37*. The PCR analysis was carried out according to the protocol for each marker. Simultaneously, the genes of resistance in spring wheat lines were identified by phytopathological testing. For this aim we used 25 test isolates of leaf rust with the known virulence genotype from different regions of Russia and 47 tester lines with single *Lr* genes.

Results and discussion

Phytopathological testing of potential donors of resistance to leaf rust and molecular-genetic methods, as well as the use of tests for allelism complemented each other. The combination of these methods allowed to expand the spectrum of identified genes, and in some cases to exercise double control over a gene of resistance (Table 1). In several cases, however, methods yielded discordant results. The use of molecular markers to effective genes *Lr9* and *Lr24* let us breed four samples with gene *Lr9* (76/00i, 79/00i, 119/00i, 120/00i) and two samples with gene *Lr24* (120/00i, 122/00i-1). However, while conducting a test for allelism with samples 76/00i and 79/00i to gene *Lr9* in hybrid population F_2, segregation into resistant and susceptible plants was observed. That is, the presence of *Lr9* gene itself did not mean its expression in a genotype of lines with alien genetic material.

The sample with alien genetic material *T. kiharae* 120/00i has an unique combination of 6 genes of resistance, uniting juvenile genes of resistance *Lr1, Lr9, Lr10, Lr21, Lr24* with a

gene of resistance of an adult plant *Lr37*. In the sample 119/00i of the same origin the genes *Lr1* and *Lr12* were additionally identified, but the highly effective gene *Lr24* was absent.

Some wheat genotypes are of special value because of a rare combination of genes, such as *Lr10* with *Lr13* (136/00i). This gene combination, as well as a combination of genes of resistance of an adult plant *Lr12+Lr34* with gene *Lr21* (79/00i) provide field resistance under the conditions of the Non-Chernozem Zone. The damage rate does not exceed 10-20%. The combination of juvenile genes of resistance *Lr27+31* with genes of resistance of an adult plant *Lr12*, *Lr34* and gene *Lr35* (102/00i) guarantee immunity and durable resistance.

Table 1: Gene of resistance to leaf rust identified by PCR method and test pathotypes of *P. triticina*

Samples	Genes postulated by							
	STS -markers							test pathotypes of *P. triticina*
	Lr1	*Lr9*	*Lr10*	*Lr21*	*Lr24*	*Lr35*	*Lr37*	
72/00i						+		type of reaction 0; 1; genes are not postulated
76/00i		?+	+	+				presence genes of adult plant
79/00i		?+		+				*Lr12, Lr34*
82/00i			+					*Lr10, Lr26+*
100/00i								*Lr43++*
101/00i			+	+				presence genes of adult plant
102/00i			+	+		+		*Lr27+31+; Lr34, Lr12*
118/00i	+		+	+				*Lr10, Lr14b+*
119/00i	+	+	+	+			+	*Lr1+, Lr12*
120/00i	+	+	+	+	+		+	*Lr10+++, Lr37*
122/00i-1					+			*Lr26+++*
122/00i-2	?-							*?Lr1+,Lr25+,Lr44*
127/00i	+		+	+				*Lr28+; Lr10+*
129/00i	+		+	+			+	*Lr10+++, Lr37+*
130/00i	+		+	+				*Lr10*
136/00i	+		?-	+				*?Lr10, Lr14b++, Lr13++*
141/00i-1								*Lr16+*
142/00i								*Lr26,LrTt*
145/00i			+	+		+		0, 0;
149/00i			?-					*Lr10, Lr26, Lr44++*
к-62905	+		+	+				*Lr1, Lr10*

In other cases when one or several genes of resistance are identified which are not effective *Lr16+* (141/00i -1), *Lr10, Lr26, Lr44++* (149/00i), *Lr1+, Lr25+, Lr44* (122/00i -2), the genotype resistance is most probably determined by additional unidentified genes (maybe by new ones transferred from wheat relatives).

For only two accessions of the collection, 90/00i and k-62903, which displayed a recessive inheritance of resistance, neither phytopathogenic testing (resistant response type 0, 0; 1 upon interactions with the test pathotypes) nor PCR analysis identified the brown rust resistance gene. The reasons for it can be not sufficiently wide spectrum of test pathotypes as far as genes of virulence are concerned, the limited the number of STS markers or the presence in the samples of new genes of resistance, transferred from *Ae.speltoides*, which are not identified by the enumerated methods.

The value samples of bread wheat from the "Arsenal" collection consists in the combination of broad genetic basis as far as genes of resistance to leaf rust are concerned (the pyramid of the genes of resistance) with sufficiently high ear productivity (1.5-2.0 g) and seed size (half of the studied samples has weight of 1,000 grains more than 40 g), as well as height grain quality. The saturation of donors with genes of resistance to leaf rust, including effective, and rare combination of genes determine possibility of their universal use in selection programs for immunity in all regions of the Russian Federation.

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Preliminary assays for the presence of *Ryd 2* resistance gene to BYDV in some inter-specific triploid hybrids (barley DH lines x *H.bulbosum* 4x) and parental genotypes

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Barley yellow dwarf luteoviruses are globally recognized as one of the most widespread and damaging diseases of cereal crops. In barley (*H.vulgare* L.) *Ryd2* gene, located on chromosome 3H has been the only BYDV-resistance gene used in breeding programs. Paltridge et al. (1998) have developed a closely linked co-dominant PCR-based marker for *Ryd2* gene, designated YLM. The YLM shows excellent potential as a tool for selecting *Ryd2* carrying segregants in barley breeding program. On the other hand, the wild species *Hordeum bulbosum* L.(secondary gene pool) is now considered as a potential resource of novel resistance genes, including the BYDV resistance, for barley improvement by introgression breeding (Pickering et al. 2004 a, b).

The programme started at NARDI-Fundulea in 1999 is focused on improving pest and disease resistance of the cultivated barley (*H.vulgare*, genom V) by introgression of valuable genes from wild species *H.bulbosum* (genom B). In this programme diploid (VB, 2n=2x=14), triploid (VBB, 2n=3x=21) and tetraploid (VVBB, 2n=4x=28) hybrids were obtained from the crosses between diploid and tetraploid cytotypes of the two species. In two consecutive years

(2005 -2006) with drastic natural infection, *H.bulbosum* 4x and some triploid hybrids hardly showed BYDV phenotypic symptoms.

In the present paper we report preliminary screening of an *H.bulbosum* 4x accession and some inter-specific hybrids for presence of Ryd2 by PCR with marker designated YLM.

Materials and methods

Inter-specific hybridization for triploid hybrids (VBB) development was carried out using eight winter two-rowed barley DHLs: DH 32-16, -17, -19, -22, -27, 28 and DH 32-29, selected for high crossability and a natural tetraploid cytotype of *H.bulbosum* (Mihailescu and Giura 2001).

Partial fertile triploids, cytogenetically selected by MI analysis were backcrossed to barley DHLs and putative obtained substitution and/or recombinant lines (SLs or RLs) were phenotypically characterized and cytologically checked.

PCR assay for presence/absence of *Ryd 2* gene was performed with primers: 5' CAG GAG CTG GTG AAA TAG TGC CT 3' and 5'TTA AAG GGC TCC GTG AAG C 3' (Harsh Raman and Barbara J. Read, 1999) in 25µl (1x buffer, dNTPs 0,2 mM, primers 25 µM, 1U AmpliTaq GOLD, 1,5 mM, $MgCl_2$ and 50-60 ng DNA) using a termocycler Applied Biosystem 9600, with amplification programme: 95°C-10 min, (94°C-1min.; 60°C- 1min.; 72°C- 2 min.) 36 cycles and 72°C-10 min. Electrophoretic analyses of the PCR products were performed using 3% agarose gel and 0,5x TBE electrophoresis buffer with ethidium bromide stained.

Results

Significant improvement of crossability and *in vitro* triploid hybrid regeneration were obtained by using high crossable selected homozygous DHLs (obtained by *Bulbosum* system), compared with varieties and lines in crosses with tetraploid *H. bulbosum.*

Out of five cytogenetically selected triploids, four: TH-14, TH-17, TH-28 and TH-218, with partial pollen fertility (11.7% - 40.9 %) were backcrossed to DHLs for obtaining putative substitution or recombinant lines (SLs or RLs) (Table 1).

Table 1: Metaphase I analysis of triploid hybrids

Hybrids	I	II			III	IV	V	Chiasma/PMC	Autosynd. PMC (%)
		Total	rod	ring					
TH-14	4.4	5.5	1.0	4.5	1.8	0.07	-	14.16	7.1
TH-17	5.6	5.5	0.9	4.6	1.4	0.02	-	13.20	28.8
TH-28	3.9	4.4	0.8	3.6	2.2	0.2	0.02	15.48	9.6
TH-218	5.5	5.8	0.8	5.0	1.3	-	-	13.67	22.0
TH-89	5.5	4.5	1.4	3.1	0.7	-	-	9.30	33.3

PCR based assay showed (Table 2) the presence or absence of the PCR product linked to *Ryd 2* gene. Our results, have distinguished absence of PCR product for *Ryd 2* gene and a different electrophoresis pattern in *H. bulbosum* 4x. The resistance *Ryd 2* gene to BYDV is present in triploid hybrids, absent in tetraploid hybrids, suggesting that the *Ryd 2* gene could be present in the maternal parent, respectively barley DHLs.

Table 2: Presence/absence of PCR product associated with resistance gene Ryd2

Lane	GENOTYPE	*Ryd2 +/ Ryd2-*
1	H. bulbosum 4x/2	-
2	VBB (Hv.169 / 5 / 33)	+
3	VBB (DH 77 - 2x / 24 / 102)	-
4	VBB (DH 77 - 2x / 25 / 116)	-
5	VBB (Hv.169 / 6 / 36)	+
6	VBB (LS 130 / 140 / 2)	+
7	VVBB (DH 77 - 4x / 21)	-
8	VVBB (DH 77 - 4x / 18)	-
9	VVBB (DH 77 - 4x / 13)	-
10	VVBB (DH 77 - 4x / 10)	-
11	VVBB (DH 77 - 4x / 9)	-
12	VBB (LS 36 / 87)	+
13	VBB (DH 32 / 26)	+
14	VBB (LS 129 / 105)	+
15	LS 200A3 / 7*	+
16	LS 181P158 / 2*	+
17	VVBB (DH 77 - 4x / 23)	-
18	H. bulbosum 4x/1	-

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Prolamin analysis of common wheat introgression lines with leaf rust resistance from *Triticum timopheevii*

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Remote hybridization is one of the most efficient methods to impart disease resistance to common wheat (*Triticum aestivum* L.) varieties. *Triticum timopheevi* Zhuk. (Tt) is the most immune wheat species, which is used in breeding programs (Dorofeev 1987). In particular, E.B. Budashkina and N.P. Kalinina developed a collection of introgression *T. aestivum* lines bearing the leaf rust resistance character from Tt (Budashkina and Kalinina 1998). This collection is stored at the Institute of Cytology and Genetics and partly in Gatersleben Gene Bank (Germany). It is under comprehensive study Leonova et al. 2004). Other commercially

important characters of new wheat lines obtained by remote hybridization are also of great theoretical and practical interest. They include bread-making quality (BMQ), a multiple trait. First of all, it is determined by the amount and quality of gluten (Wrigley 1996). The best known gluten components are: high-molecular-weight and low-molecular-weight (HMW and LMW) glutenin subunits and gliadins.

Our purpose was to establish the major storage proteins of gluten in introgression lines risen from common wheat varieties and Tt (stored in Budashkina and Kalinina's collection (Budashkina and Kalinina 1998)), to compare them with the parents, and to describe Tt-related products.

Materials and methods

Experiments were performed with generation 18 of 11 cytologically stable introgression lines (2n=42) raised from common wheat cultivars and highly resistant to brown rust of wheat (causative agent *Puccinia recondita* Erikss.). The parental variety Tt, cultivars Saratovskaya 29 (S29), Novosibirskaya 67 (N67), Skala (Sk), Irtyshanka 10 (Ir10), Tselinnaya 20 (C20), and lines 821, 832, 837, 676, 728, 732, 175, 67, 140, 191, 206 were examined. Plants were grown in the field near Novosibirsk (2004) and in a greenhouse. Storage proteins were assayed in duplicate in sifted flour samples from lines and parents, in individual grains from 10 plants of introgression lines, and in grains of polymorphic varieties to determine the parental accession. The same flour samples (55 g) were involved in bread-making quality tests (paper in press). High-molecular-weight and low-molecular-weight glutenin subunits were analyzed by SDS-PAGE and gliadins by SDS-PAGE and A-PAGE according to (Obukhova et al. 2001). Electrophoretic images were analyzed with the GelPro Analyzer 4.0 program. The R_f values and peak densities in the densitograms of parental accessions and introgression lines were compared. The allelic states of loci Glu-1, Glu-2, Glu-3, Gli-1, and Gli-2 were determined with the use of corresponding catalogues and reference varieties and lines and compared with those reported in Payne and Lawrence (1983), Metakovsky (1991) and Gupta and Shepherd (1990).

Results and discussion

T.timopheevii v. viticulosum, (2n=28; $A^{Tt}A^{Tt}GG$): None of the HMW glutenins of Tt matches the mobilities of glutenins from cultivars studied. In the range of B-type glutenins of cultivars Sk and Ir10, two protein bands match Tt proteins but are very poorly stained. The pattern of Tt gliadins contains 27 bands: 8 ω, 5 γ, 9 β, and 5 α. *Triticum timopheevii* protein bands detected in introgression lines are marked in the densitogram (Fig. 1). We analyzed storage protein patterns in flour of varieties from collections of line authors and compared the results with data from the literature. According to our results, S29 contains ca. 10% genotype Glu-B1b, alien to this cultivar according to (Morgunov et al. 1990). Cultivars N67 and Ir10 were heterogeneous in HMW glutenin, according to (Morgunov et al. 1990).

Lines 191 and 206: Lines 191 and 206 do not differ from each other or the parental variety (C20) in HMW or LMW glutenin subunit patterns (Table 1). Line 206 shows the absence of four bands (6B) of six. We conclude that the newly formed Gli-2 locus resulted from integration of part of gliadin genes controlled by chromosomes 6B (TA) and 6G (Tt). The products of the Tt genome are indicated in Fig. The products of chromosomes 1A, 1B, 1D, 6A, and 6D were the same as in C20 (Table). The density of α-gliadin band (6A) increased except for the combined (6D+6A) band.

Lines 821, 832, and 837: Lines 821 and 832 retain the allelic states of all glutenin (Glu-1, Glu-2, and Glu-3) and gliadin (Gli-1 and Gli-2) loci existing in parental S29. The composition

of prolamins (glutenins and gliadins) of line 837 indicates that the line is heterogeneous and originated from two varieties, S29 and N67.

Lines 676, 728, and 732: The origin of line 676 from N67 is confirmed by its HMW and LMW glutenin composition, which is the same as in the cultivar. The flour sample of N67 corresponds to the Gli-D2a allele, which is in agreement with (Sozinov et al. 1986). However, flour of line 676 lacks the fastest β- band (6D). The absence of this band from N67 conforms with data of E.V. Metakovsky (private communication). It may be related to polymorphism of N67 in the Gli-D2 locus. We found N67 grains corresponding to parental genotypes of 676. Comparison of the gliadin patterns of line 676 and cv. N67 shows one difference: in the density of the β- band (6D+6B), having R_f= 0.606. We found neither disappearance of products of chromosomes 6B of the parental variety nor appearance of new products. Line 732 was obtained from cv. N67, genotype Glu-A1 (subunit 2*). Its gliadin and LMW glutenin patterns are the same as in line 676. We found that the line 728 originated from Pyrothrix 28 rather than from N67, as has been considered before.

Lines 67 and 140: These lines were derived from cv. Ir10, biotype Glu-A1c (null). It should be noted that the source accession of the flour sample was maintained as a pure line with the HMW glutenin pattern exceeding (Glu-1 score = 7) that of either of the lines (Glu-1 scores = 5) [11]. Line 140 shows no changes in glutenins, ω-gliadin, or γ-gliadin patterns. Neither introgression products from Tt in the range of two β-bands (6B+6D) or α-gliadins (6A), nor appearance of unknown bands were found, but the density of indicated bands was elevated in comparison with the cultivar. Line 67 shows two additional Tt proteins within the range of B-type LMW glutenins: the products of the Glu-G3 locus (Fig. and Table). Study of gliadin gene products in line 67 by A-PAGE revealed appearance of products of the Tt genome and disappearance of the products of Ir10 chromosome 1B. We interpret these deviations from the parental cultivar as introgression of the Gli-G1 locus from the Tt genome (Fig. 1 and Table). Judging from the appearance of two products of the Tt Gli-G2 locus (indicated in Fig.), line 67 obtained only a portion of this locus, located on Tt chromosomes 6G.

Line 175: The comparison of the gliadin patterns in line 175 and parents (Sk and Tt) was done. It was found that all ω-gliadins disappeared except two bands (1D), and the whole set of ω-gliadins expressed by Tt chromosome 1G appeared. Analysis of the gliadin fraction of line 175 by SDS-PAGE confirms introgression of a gene from chromosomes 1ATt according to the ω-fraction. For this band (ω and γ fractions), characteristic to Tt genome, we found an allele in *T. aestivum* cv. Pyrothrix 28 (Gli-A1p) (Metakovsky 1991). It was difficult to study β-gliadins in flour of line 175. They were not detected in the flour sample because of polymorphism for these proteins. Products of chromosomes 6G of Tt were found in A-PAGE gliadin patterns of ten individual grains from different plants. Six line 175 grains had two Tt products: the third γ-band and the first β-band, attributed by us to chromosomes 6G in lines 191 and 206. The remaining four grains had three Tt products: the first, second and third β-gliadins, attributed by us to chromosomes 6G. The products of Tt chromosomes revealed in line 175 are indicated in Fig. Line 175 retained the components of HMW glutenins and some LMW glutenins (only Glu-2, D-type) of the parental cultivar. In the range of B-type LMW glutenins, two bands of the cultivar disappeared. Instead, three Tt bands appeared. The LMW glutenin bands of line 175 were attributed to chromosomes by comparison with line 67. These products are likely to be related to the Glu-A^{Tt}3 and Glu-G3 loci of Tt, because line 175 shows changes in Gli-A1 and Gli-B1, linked to Glu-A3 and Glu-B3. The composition of triplet proteins (locus Tri-1) in line 175 also changed (data not shown). The widespread changes touching a segment of ~ 50 cM of chromosome 1AS in line 175 suggest substitution of the short arm 1AS/1A^{Tt}S.

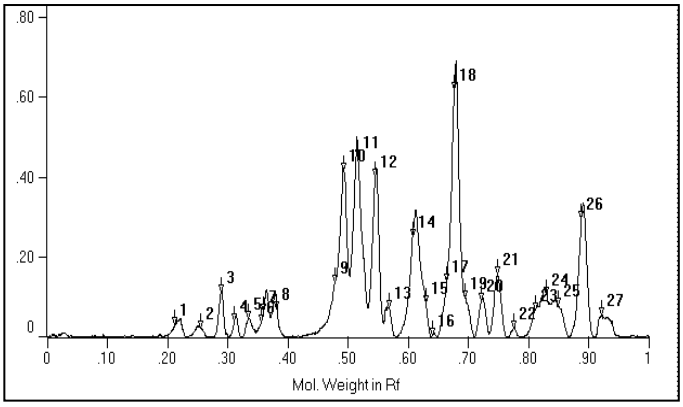

Fig. 1: The densitogam of gliadin proteins of Tt from electrophoregram

Locus Gli-A^t1 controls protein N12 (Rf=0.544), which was found in L175. Gli-G1 controls proteins NN 1-8,10, 11, 20 (Rf= 0.211, 0.253, 0.287, 0.309, 0.332, 0.354, 0.357, 0.380, 0.491, 0.515, 0.721, correspondingly). These products were found in the lines 67 and 175. Gli-G2 controls bands 13-16, 18 (Rf=0.566, 0.606, 0.628, 0.641, 0.677). Bands 13, 14, 18 were found in the lines 206 and 191. The line 67 possesses the bands 14 and 15. The bands 13 and 14 were found in individual grains of L175; other individual grains have bands 14, 15 and 16.

Table 1: Allele variants of storage proteins

Cultivar, line	Locus, chromosome, arm														
	Glu-1			Glu-2		Glu-3			Gli-1			Gli-2		
	1AL	1BL	1DL	1BS	1DS	1AS	1BS	1DS	1AS	1BS	1DS	6AS	6BS	6DS
S29	b+a#	c+b#							i+f			q+s	s+q	
L821	b	c							i			q	s	
L832	b	c							f			q	s	
N67**	a+b													a+a*
L676	a													a*
L732	b													a*
Sk**						not determined			k	b		k	k	
L175						Tt	Tt	Sk	Tt	Tt		**k**	Tt*	
Ir10**	b+c					not determined				b		k	k	
L67	c					Ir10	Tt	Ir10		Tt			Tt*	
L140	c											**k**	**k**	
C20**						not determined						q	d	
L206												**q**	Tt*	
L191												**q**	Tt*	

Empty cells show no changes as compared with parent cultivars.
-Alleles are absent in S29 [9].
** - Presence of additional fast ω−gliadins in cultivars as compared with Metakovsky (1991) and Payne et al. (1987). The same ω−gliadins were conserved in the lines originated from these cultivars.
Tt*- lines have new Gli-2 integration loci that involve individual genes of two loci: from the cultivars and from Tt, controlled by arms 6BS and 6GS.
a*- the fastest band is absent.
Bold letters mark alleles with enhanced expression.

Conclusions

All lines inherited the HMW glutenin pattern from their parental cultivars. This factor is favorable for the BMQ of introgression lines. An impairment of BMQ in comparison with the parental cultivars is expected only in lines 67 and 175 because on the increasing amount of S-poor ω-gliadins. Lines 676, 732, 821, 832 and 140 show no introgression products. Line 175 is the result of substitution of chromosome arms 1AS/1A^{Tt}S (supposedly) and two homeoelogical recombinations in chromosomes 1 and 6 involving two linked loci (Gli-B1/Gli-G1 and Glu-B3/Glu-G3) and individual genes rather than whole loci (Gli-B2 and Gli-G2). Line 67 is the result of homoeological recombination at the level of two linked loci Gli-B1/Gli-G1 and Glu-B3/Glu-G3 and at the level of individual genes of the Gli-G2 locus. Lines 191 and 206 show a homoeological recombination between individual genes within the Gli-2 locus. Neither of the lines studied shows products absent from the parental accessions, which is indicative of simple (homeological recombination) mechanisms of formation of new cytologically stable hexaploid wheat accessions characterized by these traits.

Thus, study of storage proteins in individual grains from different plants revealed polymorphism in lines. One kind of polymorphism is inherited from polymorphic parental cultivars (S29, Ir10, and N67). Another kind of polymorphism in lines 67 and 175 appears to be related to the polymorphism for ω-gliadins in Tt. The third kind of polymorphism, observed in lines 67 and 175, is related to different introgressions of gene material from Tt. Therefore, not all lines studied can be considered pure according to the characters examined. The originations of all lines were validated except for the lines 837 and 728. The line 837 has two parent cultivars Saratovskaya 29 and Novosibirskaya 67. The parent of the line 728 was Pyrothrix 28 instead of Novosibirskaya 67.

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Fusarium species associated with FHB on wheat isolated in the Czech Republic and their molecular diversity

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The *Fusarium* species in wheat and other small-grain cereals are predominantly found in all over Europe (Bottalico & Perrone 2002). The main causative species of Fusarium head blight are *F. graminearum*, *F. culmorum*, *F. poae* and *F. avenaceum*. The disease is linked to seedling blight and brown foot rot and forms part of a cycle of Fusarium related diseases on cereals (Parry et al., 1995). Fusarium head blight and ear rot reduce grain yield and the harvested grain is often contaminated with mycotoxins such as trichotecenes and zeralenone (Lee et al., 2002).

An important component of the effort to manage these pathogens is knowledge of their population genetic structure. The variability is intensively studied especially in *F. graminearum* (Láday et al., 2004, Carter et al., 2002, Tóth et al., 2005) and *F. culmorum* (Tóth et al., 2004, Mishra et al., 2003).

Materials and methods

In total, 72 samples were processed. For isolating of micromycetes were used cultivation methods. The level of *Fusarium* infection in wheat ears was detected by bonit system 0-9 (0-health ears, 9-infected ears by *Fusarium* species). The wheat kernels were surface washed in NaClO (for a period of 3 minutes shaken) and follow 3× washed in sterile water (for a period of 1 minute). The following media for isolation (Atlas and Parks 1997) were used: Pentachloronitrobenzene agar (PCNB) and Potato Dextrose Agar (PDA). The Petri dishes were incubated at 20-25°C. The media for identification were: Synthetic nutrient-poor agar Nährstoffarmer Agar (SNA), PDA and Oatmeal Agar (OA).

DNA from 190 monosporic isolates was extracted using CTAB method (Leišová et al., 2005).

AFLP analyses were carried out according to the AFLP™ Plant Mapping Protocol (Applied Biosystems, Foster City, CA USA). 15 combinations of MseI and EcoRI primers (marked with FAM, NED and JOE fluorescent dye) were used. The PCR products were denatured and separated by capillary electrophoresis on the genetic analyzer ABI PRISM 310 (Perkin-Elmer, Foster City, CA USA). Chromatograms were processed by the software GENESCAN and GENOTYPER. The date was statistically analyzed using Statistica for Windows (StatSoft, Inc., Prague CZ).

Results and discussion

The regions with the most frequent infection and also DON content were South-west Bohemia, North-east Bohemia, North and East Moravia. The regions with the lowest frequency were south and central Moravia and the region around Prague.

15 primer pairs were used to score the variability of 190 *Fusarium* isolates. 39 – 76 length polymorphic DNA fragments of 60 – 500 bp in size were detected. A total of 727 AFLP polymorphic bands were scored with all *Fusarium* isolates, which corresponds to an average of 50 polymorphic bands per primer combination. The AFLP analysis clearly distinguished 5

separated clusters of *Fusarium* species and a cluster of undetermined fungi. These are further mycologically determined. The method was found to be an useful and reliably tool to score the variability of *Fusarium* species associated with FHB. The most abundant *Fusarium* species was *Fusarium graminearum* (57%) the others were determined in these frequencies: *F. culmorum* (14%), *F. poae* (11, 5%), *F. avenaceum* (12%), F. equiseti (0, 5%) and undetermined species (5%).

The interspecific variability of determined species and the relations with the region and the production of DON or NIV are further analyzed.

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Analysis of bread wheat near-isogenic lines with respect to *Gli-* loci using microsatellites and *Gli*-allele-specific markers

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Near- isogenic lines are valuable material for quantitative and qualitative genetic analysis and for marking agronomically-important genes using molecular markers. One of the effective methods for identification and differentiation of near-isogenic lines is microsatellite analyse.

Microsatellites (or SSRs) are highly polymorphic loci that have been mapped with high density on all chromosomes of the *Triticum aestivum* L. genome and characterized as codominant markers (Röder et al. 1995, 1998; Bryan et al. 1997, Somers et al. 2004). Microsatellites represent a highly reproducible and suitable marker system for mapping agronomically important genes / QTLs (Korzun et al. 1997, 1998, Börner et al. 2002),

studying the genetic diversity among bread wheat varieties and related species (Plashke et al. 1995; Fahima et al. 1998; Chebotar and Syvolap, 2001; Röder et al. 2002), verifying the identity of cytogenetic stocks (Korzun et al. 1997, Salina et al., 2003) and in the study of near-isogenic lines (Khlestkina et al. 2000).

The aim of this work was to analysis of the isogenity of the six lines bread wheat (*Triticum aestivum* L.) developed on the basis of the winter wheat variety Bezostaya 1 differing by the alleles of *Gli*-loci (Kopus, 1994). These lines are of interest for studying the fine structure of gliadin loci.

Materials and methods

Six presumably near-isogenic lines of bread wheat with different alleles at the *Gli-1* loci: *Gli-D1-5* (1D5), *Gli-B1-3* (1B3), *Gli-B1g* (1B4), *Gli-A1-1* (1A1), *Gli-D1-4* (1D4), *Gli-B1-12* (1B12), which had been created by Dr. M. Kopus (1994) (Donskoy center, Rostovskiy region, Zernograd, Russia) on the basis of the wheat variety Bezostaya 1, were explored. The lines were created as a result of six backcrosses and selection using gliadin storage protein markers. The varieties of Krimka mestnaya (alleles *GliD-1-4*, *Gli-A1-1*), Levent (*GliB-1-12*), Zg2689/74 (*Gli-B1g*), Triumph (*GliD-1-5*), Aurora (*GliB-1-3*) at crossing with Bezostaya 1 were used as the donors of allelic variants of storage proteins loci. In the research the varieties Bezostaya 1, Odesskaya krasnokolosaya and line B-16 with the wheat-rye 1RS.1BL translocation were also involved.

DNA was isolated from 5-day-old seedlings by the standard CTAB procedure. PCR-reactions with microsatellites were performed as described by Röder et al. (1998). Electrophoresis of products of amplification with microsatellite markers was carried out in 10% denaturing polyacrylamide gels with 6M Urea in 1xTBE.

Amplification with allele-specific primers GligA1.1, GligA1.2, GligB1.1, GligD1.1, GligD1.2, which had been developed on the bassis of SNPs in γ-gliadin genes, was conducted according to Zhang et al. (2003). The products of amplification fractionated in 8% non-denaturing PAAG. For visualization of amplification products, $AgNO_3$ staining was used in accordance with "Silver sequence TM DNA Sequencing System Technical Manual" (Promega).

Results and discussion

The microsatellite analysis of loci located on different chromosomes *Xgwm550* (1B), *Xgwm153* (1B), *Xgwm140* (1B), *Xgwm408* (5B), *Xgwm257* (2B), *Xgwm291* (5A), *Xgwm126* (5A), *Xgwm186* (5A), *Xgwm156* (5A) *Xgwm437* (7D), *Xgwm155* (3A), *Xgwm190* (5D), *Xgwm261* (2D) showed no interlinear polymorphism. The analysis of lines with the microsatellite marker for 1R chromosome (*Secale cereale* L.) *Rems1303* tested products of amplification, which are specific for rye (Khlestkina et al., 2004), in the Gli-B1-3 line, thus confirming the presence of rye translocation in 1B chromosome of this line.

Analysis of the lines with allele-specific primers permitted us to reveal unique combinations of alleles at *Gli-A1*, *Gli-B1*, *Gli-D1* loci for each line. The exceptions were the lines Gli-B1-3 (1B3) and B-16 containing the 1RS.1BL translocation, which gave negative results for markers GliB1.1 and GliB1.2. The results are shown in Table 1. We compared the results of allele-specific PCR with the data of electrophoresis of storage proteins. The analysis of seven gliadin loci (*Gli-A1*, *Gli-B1*, *Gli-D1*, *Gli-A3*, *Gli-A2*, *Gli-B2*, *Gli-D2*) and three loci, that code the high molecular subunits of glutenins (*Glu-A1*, *Glu-B1*, *Glu-D1*), has shown that these lines differ in certain loci coding storage proteins (Kozub and Sozinov, 2000). The comparative analysis shows that using allele-specific primers GliD1.1, Gli-D1.2 it is

impossible to distinguish between alleles *Gli-D1-1* (Bezostaya 1) and *Gli-D1-4*, which is specific for lines Gli-D1-4 (1D4) and B-16 according to electrophoresis of gliadins, though allele *Gli-D1-5* specific for line 1D5 was clearly differentiated. The similar situations were revealed for alleles *Gli-B1g* and *Gli-B1-15* (Odesskaya krasnokolosaya), alleles *Gli-A1-4* (Bezostaya 1) and *Gli-A1-9*, *Gli-A1-10* (B16, Odesskaya krasnokolosaya) that could not be distinguished by allele-specific PCR and clearly separated by electrophoresis of storage proteins.

Table 1: Alleles at loci *GliA1*, *GliB1* and *GliD1* in genotypes of near-isogenic lines of bread wheat, which had been created on the basis of the variety Bezostaya 1

Varieties / Lines	Loci		
	GliA1	*GliB1*	*GliD1*
B-16	*GliA1.1*	-	*GliD1.2*
Odesskaya krasnokolosaya	*GliA1.1*	*GliB1 .2*	*GliD1.2*
Bezostaya 1	*GliA1.1*	*GliB1 .1*	*GliD1.2*
Gli-D1-5(1D5)	*GliA1.1*	*GliB1 .1*	*GliD1.1*
Gli-B1-3(1B3)	*GliA1.1*	-	*GliD1.2*
Gli-B1g(1B4)	*GliA1.1*	*GliB1 .2*	*GliD1.2*
Gli-A1-1(1A1)	*GliA1.2*	*GliB1 .1*	*GliD1.2*
Gli-D1-4(1D4)	*GliA1.1*	*GliB1 .1*	*GliD1.2*
Gli-B1-12(1B12)	*GliA1.1*	New allele	*GliD1.2*

We have demonstrated that allele-specific primers, developed by Zhang et al. (2003), have some limitations for marker-assisted breeding but could be used in parallel with proteins markers.

We can summarize that the level of substitution of genetic material of donor after six backcrosses is very high and these lines are practically the analogs of the recipient Bezostaya1.

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Diversity of Siberian bread wheat cultivars on grain quality in dependence of the breeding period

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Grain quality is a very important agronomic trait determining the end-use of bread wheat cultivars. It is composed of many separate components with manifestation depending both from the genetic factors and plant growth conditions. Combining of high grain quality with high productivity in one wheat genotype for years was a desirable although not easy aim of breeders. In this work the collection of Siberian bread wheat cultivars obtained on 80 years time period was investigate in order to reveal the effect of breeders' efforts on improvement of grain quality. Earlier the diversity of this collection was studied using a set of 22 molecular markers located on 19 bread wheat chromosomes (Khlestkina et al. 2004). Correlations of these markers with some grain quality parameters were investigated.

Materials and methods

21 old cultivars obtained in 1920-1960 years and 17 modern cultivars obtained in 1960-2000 years in Siberia were grown in 2005 and 2006 years near Novosibirsk, East Siberia. The first season was favourable for temperature and precipitation regimes during grain filling and gluten formation (July and August) promoting the full realization of cultivars' potential for technological parameters. A second season was characterized by precipitations shortage in July and low temperature and excessive precipitations in August which is not favourable for the formation of gluten with good quality. The following grain parameters were studied: weight of 1000 grains (g), gluten content in grain (%), flour particles size (mk) and vitreousness (%). (Anonimous, 1988).

Results and discussion

It was found (Table 1) that the old cultivars have significantly low meanings of vitreousness and particles size in both environment. In favourable environment the old cultivars had a significantly lower weight of 1000 grains and higher gluten content in grain. In 2006 the tendency was the same also the differences were not significant.

All the traits studied are quantitative; therefore, for further analysis the cultivars were divided into three groups with low, intermediate and high meanings of every trait. It was found that for weight of 1000 grains the old cultivars constituted the majority in the group with low weight in 2005 year. The modern cultivars prevailed in the groups with intermediate and high meaning. But in the unfavourable conditions they showed the decrease of weight of 1000 grains and constituted the majority in the low-weight group. For particles size (Fig.1**)** and vitreousness the old cultivars formed the majority in the group with low meaning in both years. In the group with high meanings the modern cultivars prevailed. However, under the conditions unfavourable for formation of high quality gluten they showed the decrease of these parameters and moved to the group with intermediate meanings. For gluten content in grain (Fig.2) the opposite situation was observed: the majority of the group with high meaning of the trait constituted the old cultivars in both weather conditions. The modern cultivars always prevailed in the group with low gluten content.

Table 1: Technological parameters of grain in old and modern Siberian cultivars in 2005 (favourable) and 2006 (unfavourable) seasons

Groups of cultivars, year	Weight of 1000 grains, g	Vitreousness, %	Gluten content, %	Particle size, mk
Modern 2005	26,0±2,9	83,4±6,6	33,4±2,0	22,4±2,6
Old 2005	23,1±2,3*	73,6±13,3*	37,3±3,7**	17,5±4,3**
Modern 2006	27,9±4,0	88,3±3,3	37,1±2,5	19,6±2,6
Old 2006	25,1±3,8	75,2± 12,4*	40,0±4,7	15,2± 4,2*
h^2	64,3	88,0	57,7	94,1

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

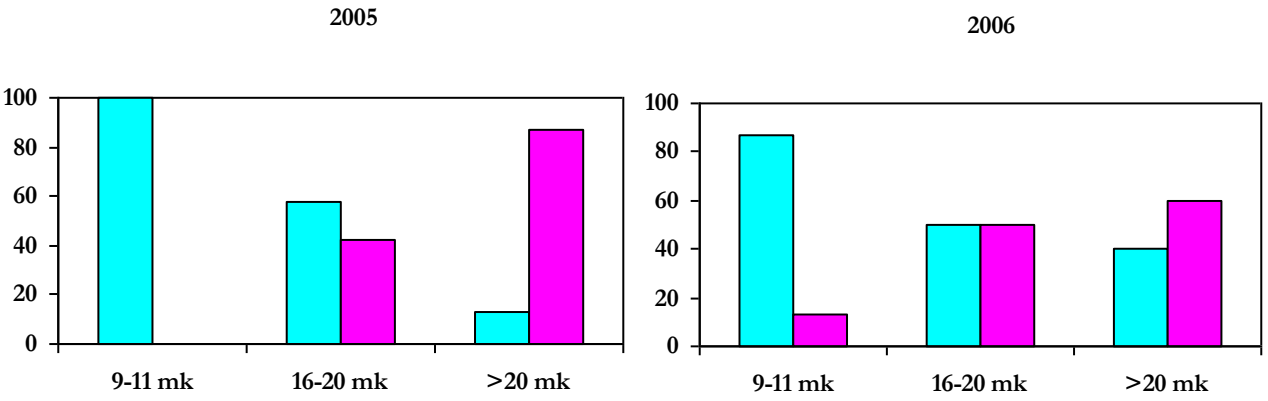

Fig. 1: Distribution (%) of old (■) and modern (■) Siberian cultivars inside groups with low, intermediate and high meanings of flour particle size

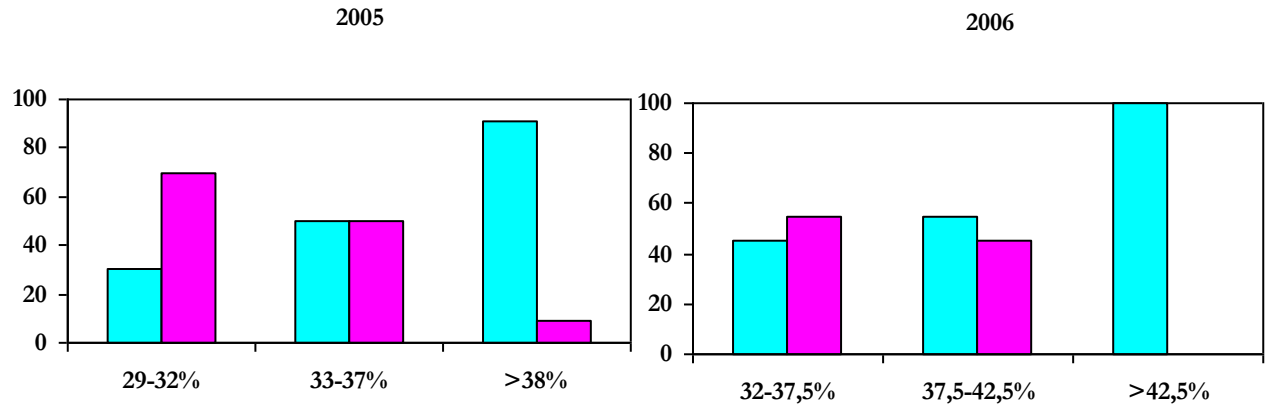

Fig. 2: Distribution (%) of old (■) and modern (■) Siberian cultivars inside groups with low, intermediate and high meanings of gluten content in grain

Compare of the parameters studied with the data of microsatellite analysis of this collection (Khlestkina et al. 2004) showed that 85% of cultivars with high particles size carry the 77 n.p. allele in the locus *Xgwm0003* in 3DL chromosome. This character correlates with vitreousness and 54% of the cultivars with high meanings of the trait carry the same microsatellite allele. It should be noted that earlier in the long arm of chromosome 3A the QTL was mapped in comparable position associated with these two traits in ITMI mapping population of bread wheat (Pshenichnikova et al. 2006). About 66% cultivars of the collection with high gluten content carry the allele of the same length (200 n.p.) in locus *Xgwm0631* of 7AS chromosome. Earlier, in the short arm of the same chromosome the QTL was detected associated with protein and gluten content in grain in mapping population ITMI (Börner et al. 2002a; Börner et al. 2002b).

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Genetic mapping of powdery mildew resistance genes in barley

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Barley is one of the most wide-spread crops in the world and its production is adversely affected by many diseases. In Europe, powdery mildew caused by *Blumeria graminis* f.sp. *hordei* is a common and economically important disease of barley. Resistance to powdery mildew plays a significant role in breeding the crop.

Jørgensen (1994) summarized race-specific genes for barley powdery mildew resistance located at 10 loci on current linkage maps and 31 others present in the literature. Altogether, 85 genes are presented in this review. Since then, this list acquired four race-specific resistance genes. Wild barley (*Hordeum vulgare* ssp. *spontaneum*) represents a promising source of new resistance genes. Mapping of powdery mildew resistance genes by means of genetic markers utilizes the identification of markers linked to the resistance genes and the location of known genes on the barley genetic map. Recently, genetic maps consisting of simple sequence repeat (SSR) markers and amplified fragment length polymorphism (AFLP) markers have been constructed for barley and powdery mildew resistance genes were successfully located on barley chromosomes by these types of markers.

Thirteen wild barley accessions resistant to powdery mildew were studied to find the number of genes conferring the resistance, to find the mode of inheritance and to estimate their

relationships to known powdery mildew resistance loci by means of genetic mapping and DNA markers.

Materials and methods

Thirteen wild barley accessions from the USDA National Small Grains Collection, with resistances to powdery mildew (Dreiseitl and Bockelman 2003, Dreiseitl and Dinoor 2004), and the two-row winter barley variety 'Tiffany' carrying powdery mildew resistance genes *Mla7*, *MlaMu2* were used. F_2 populations after crosses between the variety 'Tiffany' and individual wild barley accessions were developed. A virulent pathotype 0323 of the pathogen was used for the resistance tests. The numbers of plants in the two phenotypic categories (resistant and susceptible) found in F_2 populations were compared with theoretical Mendelian segregation ratios by a chi-square test, and the number of resistance genes in each accession was estimated.

Genomic DNA extractions from leaves of parental and F_2 plants were performed using the Gene Elute Plant Genomic DNA Miniprep Kit (Sigma-Aldrich Co.). Application of 120 SSRs (Ramsay et al. 2000) was focused on the chromosomal locations of individual resistance genes (Řepková et al. 2006). Sequence tagged-site (STS) markers from target chromosomal regions were used for the development of cleaved amplified polymorphic sequence (CAPS) markers linked to the resistance genes of interest. Further CAPSs were developed from *RGH* gene sequences from chromosome 1H (*R*-gene homologs; accession number AF427791, http://www.ncbi.-nlm.nih.gov/). The known or designed (PRIMER3 programme) primers and the following programme was used for the amplification: one cycle for 3 min at 94 ºC; 35 cycles for 1 min at 94 ºC, 1 min at 60 ºC, 1 min at 72 ºC, with a final extension of 5 min at 72ºC. For identification of polymorphic sites between the parent DNAs, the restriction enzymes *Afl* III, *Alu* I, *Ava* II, *Bam*H I, *Ban* II, *Bsa*J I, *Bst*N I, *Dde* I, *Dpn* I, *Dra* I, *Hpa* II, *Hin*f I, *Mbo* I, *Mse* I, *Nla* III, *Pst* I, *Rsa* I, *ScrF* I, *Taq* I and *Xba* I were used for the DNA digestion. Linkage detection was carried out with 100 to 150 plants of each F_2 population. Map Manager QTXb17 package software was used to construct linkage groups and establish orders and map distances for each group of markers.

Results and discussion

The mode of inheritance of all analysed genes was dominant or semi-dominant except two genes in two accessions where they were recessive. We estimated that in five accessions, the resistance was based on one gene; all of them were assigned in the known *Mla* locus on chromosome 1HS. A two-locus model of resistance was shown by genetic analysis of seven F_2 populations and in one accession, the resistance was based on three independent genes. Our genetic analyses showed that 10 out of 13 accessions contained an allele of the *Mla* locus. It confirmed the unique significance of this locus among other barley loci conditioning resistance to powdery mildew.

The most exact chromosomal positions of resistace genes were assigned for two F_2 populations. In 'Tiffany' x PI466461, one gene coincided with the *Mla* locus with an expected position 8 cM proximal to the *RGH1aI1a* marker designed for the known *RGH1a* gene sequences. The other resistance gene derived from the wild accession was found on the short arm of chromosome 7H. It was mapped close to the sub-telomeric region of this chromosome and is flanked by the markers *Bmag0021* and *EBmag0794* at the distances of 4 and 9 cM, respectively. Until now, neither a dominant/semi-dominant major gene nor a quantitative trait locus conferring powdery mildew resistance has been located on the short arm of chromosome 7H of barley. *Mlt* gene with recessive resistance allele was located in the same

chromosomal region. The two determined resistance genes originated from *H. vulgare* ssp. *spontaneum* support additive effect on resistance due to their functional cooperation.

In 'Tiffany' x PI466197, molecular analysis revealed a highly significant linkage with the markers *Bmac0213* and *MGB402* on the short arm of chromosome 1H, which is the position consistent with the *Mla* locus. The other gene was located between the markers *Bmac0134* and *MWG878* on the short arm of chromosome 2H, which might be a newly identified gene for powdery mildew resistance originated from *H. vulgare* ssp. *spontaneum*.

Altogether 18 CAPSs have been developed from known STSs located mainly on the chromosomes 2HS and 7HS and from *RGH* sequences from chromosome 1HS. CAPS markers exploit single base pair differences in the target regions of genomes of both parents used for the cross. As a matter of fact, these DNA differences result in single nucleotide polymorphisms and they are generally common in genomes of all organisms, including plants. Their appearance is far more frequent in comparison with changes in repetitive sequences in the case of microsatellites and they are therefore very useful for more precise mapping of particular resistance genes. The prospect of our work is to find markers tightly linked to resistance genes so that breeders could use them for marker-assisted selection.

Acknowledgements

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Business meeting - ongoing and future co-operation within EWAC

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The business meeting was chaired by the members of the International Organising Committee Tatyana Pshenichnikova, Andreas Börner, Victor Korzun and John Snape. The following topics were discussed:

- Poster award
- Maintenance of present and development of new stocks (Need for an updated inventory?)
- Ongoing and new co-operative projects (Cost Project – Marta Molnar-Lang)
- Publication of Proceedings
- The 15th EWAC Conference (where and when)

For the first time the organisers proposed awarding a prize for the best poster presented at the Conference. A group of referees consisting of John Snape, Victor Korzun and Andreas Börner evaluated all posters and came to the unanimous decision that the presentation of Elena Khlestkina and co-authors was the winner. Beside the scientific value and arrangement, the poster was chosen because it nicely demonstrated results of co-operative efforts, a basic intention of EWAC. Elena took the award with pleasure and expressed her deep thanks to the International Organising Committee for being selected.

Continuing the business meeting, the maintenance of present and development of new stocks was discussed. During 40 years of EWAC activities a lot of materials have been developed, several of which have been lost in the mean time. The last inventory of (cyto)genetic stocks was created in the early 1990s and published by Andreas Börner and Tony Worland in the EWAC Newsletter of 1995, twelve years ago. The participants agreed that this inventory needs to be updated. A special section of or link via the webpage of EWAC (www.ewac.eu) may be created. A questionnaire will be prepared by Andreas Börner together with Zbynec Milec and distributed by Caroline Munnings. Before doing this, there is a need to update but also to improve the link to the members of EWAC. Zbynec and Caroline will take care of this.

Due to the kind efforts of Zbynec Milec and with the help of other EWAC members the webpage of the co-operative was significantly improved since the Prague meeting. Starting with the home page there are special sections for: Mission, History, Members, Meetings, News, Newsletters, Contacts and Links. Both the 2003 and 2006 Newsletters are accessible via the webpage – the present one will be as well.

Marta Molnar-Lang reported about her activities in getting additional funding for the EWAC Network. In 2006, Marta prepared a proposal within the frame of 'COST Action' entitled: 'Development, maintenance and use of cereal genetic materials for molecular genetic studies'. The aim of the proposed COST Action was to co-ordinate the development, maintenance and utilisation of genetic defined materials for genetic research on cereals, principally wheat, barley and rye in Europe. More than 800 proposals were submitted. Unfortunately, ours was not successful. The proposal was re-submitted in early spring 2007 and is under evaluation now. Other possibilities for additional funding were mentioned by John Snape. There is an Organisation called 'EPSO' (The European Plant Science Organisation) providing money for

scientific meetings. A further possibility may be the Marie Curie-Foundation supporting training programmes.

John Snape suggested dedicating the Proceedings of the 40th Anniversary EWAC conference to Colin Law's 75th birthday to be held at November 18, 2007. The participants agreed. In order to meet this date, the manuscripts have to be submitted to Andreas Börner by June 30, latest.

Finally the place and the time for the next EWAC meeting was discussed. During the conference Boris Kobiljski from Novi Sad, Serbia but also Krzysztof Kowalczyk from Lublin, Poland indicated their interests in hosting the next EWAC Conference. During the business meeting, Krzysztof repeated his intention but pointed out that he would be even more interested to organise the Conference after the next, which may come together with the retirement of Danuta Miazga, being an active EWAC member for many years. The participants were attracted by this idea and Boris was asked to give his proposal for the next meeting. After a five minutes PowerPoint presentation illustrating the proposed venue it was agreed that the 15th EWAC conference should be held in Novi Sad, where, in fact, the idea of creating the co-operative was born in 1966. As an appropriate time the year 2010 (spring) was decided.