European Wheat Aneuploid Co-operative

Newsletter

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Proceedings of the 13th International EWAC Conference

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Edited by
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and
The John Innes Centre, Norwich Research Park, Colney, Norwich, UK

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Preface

A. Börner (Secretary, EWAC)

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The 13th International EWAC Conference was held in Prague, the Capital of the Czech Republic from June 27 to July 1, 2005. More than 30 participants from 12 countries attended. For the first time a web-site was created allowing information gathering and registration on-line (http://www.vurv.cz/ewac05). The web-site will be maintained, publishing all abstracts and full papers of the oral and poster presentations as well as forthcoming information's about following EWAC activities.

The Conference started with a minute's silence in memory of three EWAC members who passed away since the last conference was held in Norwich in 2002 – Dieter Mettin, Steve Petrovic and Enrique Suarez. We have lost excellent scientists, supportive colleagues and good friends attending many of the former meetings. Their activities within the European Wheat Aneuploid Co-operative are documented in numerous contributions to the EWAC Newsletters published in recent years. We always will cherish their friendship and remember their impact on wheat genetics.

During the Conference an intensive discussion about the present situation and the future of EWAC was held. In order to broaden the field of research activities and to get more people interested in EWAC, the participants decided to change the name of the co-operative into: **'EWAC – The European Cereals Genetics Co-operative'**. Another point of discussion was the establishment of a linkage between EWAC and CIMMYT not least in the context of the global 'Generation Challenge Program', an initiative with the aim to use advances in molecular biology to harness the rich global heritage of plant genetic resources. In consequence Tom Payne announced the offer of CIMMYT to host the 14th EWAC Conference in Turkey, in Spring 2007. We look forward to that conference celebrating the 40th Anniversary of EWAC.

The history and the current status of wheat breeding in the Czech Republic

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Wheat is a predominant crop in the Czech Republic. At present, the wheat growing area is around 800 thousand ha. The importance of wheat and thus its cropping area has grown continually, by up to 40%, since 1938. The main reason for the increase was the growing cost-effectiveness of the crop due to increasing yields. There were mainly two factors that influenced the increase of the yield potential of wheat: breeding, and the revolution in the use of industrial fertilizers. The average yield of wheat was 1.1 to 1.25 t / ha, and increased up to 5.07 t / ha in the eighties and reached 5.84 t / ha in the favourable conditions of 2004.

The utilization of industrial fertilizers has been similar whilst the consumption of 10 – 15 Kg of added nutrients per ha at the beginning of the 20th century increased to 68 kg of added nutrients / ha in 1960, and then up to 262.6 kg of added nutrients / ha by the end of the eighties. Such an increase of consumption of industrial fertilizers brought about the need for breeding new forms of wheat, resistant to lodging and capable to utilizing the high doses of fertilizers.

The breeding of winter wheat started at the beginning of the 20th century in the area of Bohemia and Moravia. The first breeding trials had already started at the end of the 19th century with the aim of increasing the utility of some crops, mainly of spring barley and sugar beet. The first development of wheat breeding took place between the years 1914 – 1920. In the preceding period, mostly Czech landraces of red wheat or alternative wheat were grown in our country. To a limited extent, some foreign varieties were grown, such as German square heads in Bohemia or Hungarian, mainly the ´Banátka´ variety. Of the local varieties, there was „Hanácká bělka“, improved by bulk selection at Třebechovice near Holešov. Bulk and individual selection were the predominant techniques of the newly developing breeding programmes. The first breeders were landowners that had originally been interested in multiplying landraces. There were, particularly, four breeding stations in Bohemia:

1) Chlumec nad Cidlinou
2) Seed production station at Semčice
3) Selecta company with central station at Stupice
4) Breeding station of R. Stanky at Kaštice near Podbořany

In addition, there were four breeding stations in Moravia as well:

1) Breeding station Židlochovice, originally Branišovice
2) Sugar refinery Velké Pavlovice
3) Lichtenstein seed production stations Valtice and Lednice
4) Regional farm Stará Ves – Regional institute for improving plants in Přerov

The primary objectives of breeding were improving the existing wheat varieties adapted for the quality of soil or climate, with an improved rust resistance, a better grain quality and frost resistance. In the thirties, there were many quality local varieties that, though, were inclined to lodging. The first variety produced by artificial crossing was started in 1923.

Breeding of wheat, mainly by selection or limited crossing of landraces with imported foreign varieties was done at private breeding stations between 1918 and 1939. Some smaller

agricultural companies did multiply imported varieties, mostly originating from Germany; state research institutes were not much involved in breeding.

From 1921 to 1939, in total 193 new original varieties of winter wheat were approved. Of them, 86 were obtained by selection, 8 originated from private station material, 23 were selected from imported materials, and 46 were obtained due to crossing, 30 originated from multiplying imported varieties.

During The Second World War, 13 varieties of winter wheat were approved.

Since 1945, the activities of breeding companies were restored in the whole area of Czechoslovakia. In Bohemia and Moravia, there were 11 stations dealing with breeding of cereals. In 1945, and subsequently in 1952, big waves of restrictions took place on all the varieties that had been approved during the war occupation, and on most of the pre-war approved varieties, too. The first efficient wheat varieties were approved in 1954 – Kaštická osinatka, Lada, Pavlovická 198, Diana I above all.

The winter wheat variety, „Kaštická osinatka" represented a shift in yield, and it was followed by „Diana I". In 1963, an extreme occurrence of yellow rust lead to an infection of almost all of the Czech varieties. In infected fields, the yield even decreased by 50%. To achieve an improvement of winter wheat production, foreign varieties were introduced and approved: "Hadmerslebener Qualitas", „Fanal" (GDR), Jubilar (Western Germany) and mainly „Mironovskaya 808", „Bezostaya 1", "Belocerkevskaya", „Jubilejnaya", „Kavkaz", „Aurora", „Iljičovka" (USSR), and later „Sava", „Zlatna Dolina" (Yugoslavia) and „Grana" (Poland).

In this period, it was difficult for the local breeders to compete with the imported varieties. In 1967, no local variety was grown in Slovakia while about 15-20% of the total wheat area contained local varieties, mainly Draga, Iva, Diana II in Bohemia and Moravia.

From 1964 to 1976 breeding for resistance intensified at the RICP, Ruzyně. Dr.P.Bartoš verified the race spectre of rusts and co-operated with breeding stations on production of genetic resources for subsequent utilization. In breeding programmes, Soviet, French and German varieties and new breeding combinations with the required disease resistance were used. The first Czech variety with enhanced yellow rust resistance was Slavia, and it was followed by Vala, Hela, Regina, and others; in Slovakia, there were mainly Amika and Solaris. The introduced varieties, except the flexible „Mironovskaya 808", became less important continually in Czechoslovakia. They were replaced exclusively by the varieties coming from the Czechoslovak breeding programmes since 1985.

From 1948 to 1996, in total, 39 varieties of winter wheat were approved in Bohemia and Moravia.

Viewed from an organizational structure, 1948 was the date when all private breeding stations were nationalized and associated within the state enterprise, Oseva. In 1951 three research institutes were founded that contributed significantly to the development of Czech breeding: Research Institute of Crop Production, Ruzyně, and the institutes in Kroměříž (Moravia) and Piešťany (Slovakia).

In the fifties and the sixties of the last century, breeding took place particularly in Kaštice, Čejč and in VUŘ Semčice. Following the specialization of breeding activities in the sixties, the new breeding of winter wheat was gradually concentrated at Stupice and Úhřetice breeding stations, and at Hrubčice and Branišovice breeding stations in Moravia.

These stations were active until the beginning of the nineties when privatization of breeding was under way, and they gave the rise to the private companies: In Bohemia Selgen Praha a.s. included Stupice and Úhřetice. In Moravia both the breeding stations, Hrubčice a Branišovice originally set up the Morstar a.s but the Hrubčice station was sold directly to its the management to form the Plant Select Hrubčice later. Whereas Selgen has not changed its

proprietary structure since 1993, foreign investors bought both Moravian stations later: The Plant Select Hrubčice is owned by the Dutch company, Cebeco Seeds. The breeding station Branišovice has been a part of a French company, RAGT, following the period 1997-2004 when it was owned by Monsanto, an American company. The phase of mutual close co-operation between the stations was thus replaced by competition.

The geographical location of these four stations and the specialization on some particular problems was sensible, as long as the main objective was to assure the appropriate composition of varieties co-ordinated by the State. At present, when the primary aim is the share of the market, such a ´division´ of labour is impossible, there only is co-operation on solving some particular problems.

Following 1990, the local breeders had to adapt to quite different conditions. While in the preceding years the local varieties had a dominant role, the present is characteristic of the gradual liberalization of the market for varieties, and after having become accustomed to mutual competition we have had to also get accustomed to the competition of foreign varieties. The recent developments show that it is not easy and the local breeders must learn to cope with this phenomenon. The dominant recent factor has mainly been a penetration particularly of newly bred German varieties at the State variety trials.

At the end of the sixties and the beginning of the seventies a certain crisis had to be overcome when the typical extensive local varieties could not keep up with the revolution in application of industrial fertilizers and when they were mostly replaced by the introduced Soviet and German varieties. Following that, a period of a big boom and success of local breeding occurred. From 1975 to 1992, 19 varieties of Czech origin, 14 varieties of Slovak origin, 4 Yugoslavian varieties, 2 Soviet varieties and one German variety, Kormorán, were approved in Czechoslovakia.

There was a contrasting pattern in the period from 2001 to 2005 when in total 24 varieties – 5 of the Czech origin, 15 of German origin, 3 Dutch, 1 French variety and none of Slovak origin were approved.

There is a question of whether the success of the eighties and of the nineties was truly brought about by the outstanding quality of local varieties, or there was an artificial effect of not allowing the varieties (for political reasons) to enter our market.

Breeding is a long-term process and the mistakes that were made in the past manifest themselves more than elsewhere in the present. One of the present problems of the local breeders is the choice of partners for cross breeding in the preceding era.

The collective (group) of four breeding stations – Branišovice, Hrubčice, Stupice and Úhřetice carried out, in total, 1209 crosses in 1989. In 77% of combinations, a local variety or advanced local new breeding line was used as a parent. The varieties coming from Slovakia were used in 15% of crosses. Materials originating from western Europe found limited use, most of them, up to 5%, being German and Austrian varieties, while the rest - about 3%, were varieties coming from Romania, Poland and the USSR.

The lack of information and impossibility of normal communication, particularly with breeding and research institutes in western Europe until 1989, and only the gradual opening to the World since 1990, logically ended up in a utilisation mainly of native lines in crossings because the boom of Soviet varieties of the sixties had been overcome. The Czech breeders indeed reflected the success of newly bred, highly productive and frequently resistant varieties, coming particularly from German, French or Dutch breeding, and they changed the parents in their crosses. In 2004, from information of our colleagues, local varieties and lines were used in 25 % of crosses, whilst German, English, Dutch and French resources were used in 35%, 10%, 12% and 9% of crosses, respectively. Varieties from Russia, Turkey,

CIMMYT, China, Ukraine, Hungary and Yugoslavia were used to a lesser extent. The Slovakian varieties were used in only 0.5% of crosses.

Local varieties are used primarily as sources of frost resistance in crossings with the above-mentioned varieties.

We are convinced that the mutual collaboration of the breeders in some fields, such as joint testing of diseases in regional tests, specifying of techniques for testing and the possibility to utilize unconditionally the best European varieties, will lead to breeding of new Czech varieties that will continue the successful tradition of the past epochs.

Acknowledgements

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The use of precise genetic stocks for gene mapping: results obtained within EWAC

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The development of precise genetic stocks in bread wheat pioneered by Prof. E. Sears in cv. 'Chinese Spring' promoted accumulation of extensive knowledge about chromosomal location of genes for many traits. Foundation of European Wheat Aneuploid Co-operative (EWAC) in 1967 was aimed to join the efforts of wheat cytogeneticists in the development of new plant material as well as to exchange information and cytogenetically verified stocks (Worland, 2001). A special vehicle was established, EWAC Newsletter for operative publication of recent results of the studies fulfilled by different scientific teams. At the same time, in every issue the communication about genetic analysis of different traits obtained using the newly developed cytogenetic collections were published. The main methods of investigations were monosomic and reciprocal monosomic analysis and use of intervarietal chromosome substitutions. Enormous data about chromosomal location of genes for morphological, biochemical and physiological characters in bread wheat was published in EWAC Newsletters for a period of about forty years of EWAC history.

In the framework of the common EWAC projects, Russian scientists have developed two aneuploid sets in cvs. Saratovskaya 29 (S29) and Diamant 2 (Dm2) and a set of intervarietal and alien substitution lines (Maystrenko et al. 1970-1971; Maystrenko et al. 1986). These lines were purposefully used for determination of chromosomal location of different genes of bread wheat (Arbuzova et al. 1996). Nowadays, elaboration of molecular techniques allows the establishment of precise map positions of the earlier discovered genes. Since the above mentioned publications, part of the genes described have been mapped (Table 1). This work is being continued. It is based on using both earlier developed cytogenetic methods and modern molecular methods. In this paper the examples, results and perspectives of this approach are presented.

Table 1: The use of precise genetic stocks in bread wheat for discovering and mapping new genes (partially from Arbuzova et al. 1996)

Symbol	Character	Chromosome	Mapping information
Vrn1 *Vrn2a* *Vrn2b* *Vrn3*	Response to vernalisation	5A 5B 5B 5D	------ Mapped (Leonova et al. 2003) ------
Hl1	Hairy leaf	4B	Mapped (Dobrovolskaya et al. submitted)
Pa	Pubescent auricles	4B	------
Fe1 *Fe2*	Response to iron deficiency	7DL 7DS	------
Pc2 *Pan1*	Purple stem Purple anthers	7DS	Mapping
Pp1 *Pp2*	Purple pericarp	7B 6A	Mapped (unpublished)
Rg3	Red glume colour	1AS	Mapped (unpublished)
B1a *B1b* *B1c*	Inhibitor of awns	5AL	-----
Eg1	Elongated glume	7AL	Mapped (Wang et al. 2002)

1. Genes for leaf hairiness

Leaf hairiness is an important morphological trait involved in adaptation of plants to drought (Johnson 1975). This trait is differently expressed in wheat and among its wide relatives indicating polygenic control. One gene, *Hl1*, was identified on chromosome 4B of bread wheat cv. S29 using monosomic analysis (Maystrenko, 1976). It is widely distributed among the cultivars from Siberia, Volga and Kazakhstan steppe. Another type of hairiness was found in a bread wheat line with an introgression from *Aegilops speltoides* (sample k-389, VIR, S-Petersburg). Firstly, genetic and monosomic analyses were made with the cultivars 'Dm2' having glabrous leaves, S29 carrying *Hl1* gene and the monosomic lines of cv. 'Dm2". As a result of the two experiments it was shown that introgressed hairiness is under the control of one gene on chromosome 7B, non-allelic to *Hl1* (Table 2). When the "critical chromosomes" were determined, two F_2 populations for both types of hairiness were established for mapping the genes on corresponding chromosomes. It was found that the gene *Hl1* is situated on the long arm of chromosome 4B near the marker *Xgwm538*, whereas *Hl2* was positioned on chromosome 7BL near the marker *Xgwm537*.

2. Gene for smoky coloration of spike glumes

Another example of the use of such approaches is the discovery and mapping of a new allele of *Rg2* gene responsible for dark, smoky coloration of spike glumes. This trait is characteristic of bread wheat varieties *columbina* and *ceasium* spread among the cultivar assortment of East Siberia. The genetic analysis has shown that this character is controlled by one gene allelic to *Rg2* but introgressed from *Aegilops tauschii*, however the two genes produce different colorations (Pshenichnikova et al. 2005). Monosomic analysis (F_2) using cv. 'S29' lines of homoeologous group 1 chromosomes having white glumes showed that this gene is located on chromosome 1D (Table 3). Only in this monosomic population nullisomic plants have white glumes. Also in these plants gliadin composition was analysed and the absence of the components controlling with *Gli-D1* locus was detected. This confirmed the supposed location of the gene for smoky coloration on the short arm of chromosome 1D where the linkage of *Gli-D1* and *Rg2* loci was earlier discovered (Jones et al 1990). The only difference was that the new coloration was associated with another allele of the gliadin locus. Using molecular markers for chromosome 1DS the map position of the gene for smoky glumes was determined near the marker *Xgwm1223*.

Table 2: Genetic and monosomic analysis (F_2) of leaf hairiness introgressed from *Aegilops speltoides* (only monosomic populations of homoeologous group 7 are presented)

Cross	Phenotype		χ^2	P
	Haired leaves	Glabrous leaves		
IL* x 'Dm2'	129	49	0.48 (3:1)	0.50-0.25
IL x 'S29'	262	21	0.53 (15:1)	0.50-0.25
IL x 'N67'	148	9	0.11 (15:1)	0.75-0.50
Mono 7A 'Dm2' x IL	137	39	0.61 (3:1)	0.50-0.25
Mono 7B 'Dm2' x IL	177	3**	52.3 (3:1)	<0.01
Mono 7D 'Dm2' x IL	139	44	0.12 (3:1)	0.75-0.50

* - introgressed line, **-identified cytologically as nullisomics

Table 3: Monosomic analysis (F_2) of the trait smoky glumes coloration in cv. 'Golubka'

Cross	Phenotype of glumes		χ2 (3:1)	P
	Coloured	White		
F2 Mono 1A S29 × 'Golubka'	114	46	1,2	0,5-0,25
F2 Mono 1B S29 × 'Golubka'	120	49	1,56	0,10-0,25
F2 Mono 1D S29 × 'Golubka'	152	9*	31,9	< 0,01

* - nullisomic plant phenotype

3. Investigations in progress

The same approach is now being applied for the genetic study of anthocyanin pigmentation of different organs in wheat plants. Anthocyanins are involved in protection of plants against low and high temperatures, pathogens and are widely spread among the wild relatives of wheat. Plants containing anthocyanins are known for a high activity of oxidation-reduction enzymes and more intensive photosynthesis. Using genetic and aneuploid analyses Maystrenko and Laikova

(1995) showed that two genes for anthocyanin pigmentation of stem and anthers, *Pc2* and *Pan1* are linked and located on chromosome 7DS. A mapping population have been developed involving the carrier of these genes (cv. 'Novosibirskaya 67') and the cultivar 'Golubka' without pigmentation of stem and anthers. Another mapping population is a cross between a spring wheat line with introgression from *Aegilops speltoides* having coloured stem and anthers and the bread wheat cv. 'Dm2' with uncoloured organs. In this case chromosome 7B is supposed to carry the gene for anthocyanin pigmentation because in previous studies this line was shown to have the 7B/7S recombination (Dobrovolskaya et al., in press).

Another character under study is a speltoid spike shape also introgressed from *Ae. speltoides*. It is known that this trait is characteristic for the hexaploid wheat species *Trticum spelta* and is controlled with the gene *Q*. In our collection we maintain a winter introgession line (WIL) obtained in the genetic background of bread wheat cultivar 'Rodina' having a speltoid spike. The spikes of the F_1 hybrids WIL x *T. spelta* showed a significant increase of length and number of spikelets per spike (Table 4). Also the index of compactness (number of spikelets/spike length) was increased comparing to WIL. In F_2 progeny of this cross, a transgression of spike shape was observed so as the plants with normal spike were segregating. These observations suppose the existence of another gene for speltoidy. Further investigations will include additional genetic analysis of the trait in the cross of WIL with the substitution line Chinese Spring/*T. spelta* 5A. This line was used for mapping the *Q* gene on chromosome 5A (Kato et al. 1998). In addition, monosomic analysis of the trait is being fulfilled now with monosomic lines of chromosome 5A of the spring cvs. S29 and Dm2. After proving the existence of the new gene mapping procedures will be applied to find its intrachromosomal position.

Table 4: Morphometric characteristics of spikes of winter line with introgression from *Ae. speltoides* (WIL), the parental cultivar, *T. spelta* and the F_1 hybrid

Genotypes	Spike characteristics		
	Length, cm	Number of spikelets	Index of compactness
'Rodina', parental	7,8±1,0	18,4±1,0	24,0±2,2
WIL	10,4±0,8	17,3±1,6	16,7±1,5
T. spelta	11,6±0,8	15,8±1,0	13,6±0,8
F1 WIL x *T. spelta*	15,8±1,2***	23,2±1,3***	14,7±0,6***

*** - P<0,001 comparing to the WIL and cv. 'Rodina'

One more example of the utility of both cytogenetic and molecular techniques is a verification of earlier discovered QTLs. Technological parameters of dough were studied in the lines of the ITMI mapping population and its parents. Significant QTLs associated with mixing parameters and physical properties of dough were identified on chromosomes 5B and 7D. Now these chromosomes are being introduced from the lines ITMI-43 carrying the QTLs negatively influencing both traits and ITMI-34 carrying the QTLs with the opposite effect into cvs. 'S29' and 'Dm2' using appropriate monosomic lines. The recipient cultivars have contrasting technological properties. After backcrossing the significance of the identified QTLs will be checked.

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Genetic stocks in the 21st century – waste or important tool?

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Introduction

More than fifty years ago, in 1954 Ernie R. Sears published his outstanding paper 'The aneuploids of common wheat' (Sears 1954). Inspired from his findings researchers from all over the world started to develop monosomic series from more than seventy wheat varieties (Worland 1988). In addition, series of chromosome substitution lines, alloplasmic lines, single chromosome recombinant lines, introgression lines, etc. have been created. Using the stocks, many genes or gene complexes could be associated to certain chromosomes, chromosome arms or introgressed segments. Sears (1954) already described the chromosomal location of genes determining the traits pubescent glumes (XIV *syn.* 1A); brown glumes (I *syn.* 1B); red seeds (XVI *syn.* 3D); suppression of the speltoid effect, pubescent nodes, spring habit and inhibition of awn development (IX *syn.* 5A); pistillody (X *syn.* 6B) or red colour of coleoptile and pistillody (XI *syn.* 7A).

The knowledge gathered from the stock investigations often was the prerequisite for the precise gene mapping using molecular markers, decades later. In co-operation with several partners mainly within the framework of EWAC we have mapped a number of genes/QTLs (quantitative trait loci) determining morphological and agronomically important traits including some of those already described by Ernie Sears. In this paper we give examples for the successful molecular mapping of selected genes and QTLs based on the knowledge of former aneuploid research. The importance of genetic stocks is highlighted.

Chromosomal location and gene mapping

Examples given here will focus on genes determining the colour of coleoptiles and grains. Other traits successfully mapped in wheat at IPK but not described in detail here are reduced plant height, vernalisation response, tissue culture response, spike density (*sphaerococcum*), glume colour, glume pubescence, glume elongation, leaf hairiness or disease resistance. Details and references are given in Table 1.

Table 1: Wheat genes/QTLs mapped at IPK mainly based on former aneuploid research

Trait	Gene/QTL	Chromo-some	Reference
Reduced plant height	*Rht12*	5A	Korzun et al. (1997)
	Rht-B1	4B	Börner et al. (1997)
	Rht-D1	4D	Börner et al. (1997)
Vernalisation response	*Vrn-A1*	5A	Korzun et al. (1997)
	Vrn-B1	5B	Leonova et al. (2003)
	QEet.ipk-5D (Vrn-D1)	5D	Börner et al. (2002)
	QFlt.ipk-5D (Vrn-D1)	5D	Börner et al. (2002)
Tissue culture response	*Tcr-B1*	2B	Ben Amer et al. (1997)
Spike density (*sphaerococcum*)	*S1*	3D	Salina et al. (2000)
	S2	3B	Salina et al. (2000)
	S3	3D	Salina et al. (2000)
Glume colour	*Rg-A1 (Bg)*	1A	Khlestkina et al. (2002b, 2006a)
	Rg-B1 (Rg1)	1B	Khlestkina et al. (2002b, 2006a)
	Rg-D1 (Rg2)	1D	Khlestkina et al. (2006a)
	Rg-A1 (Rg3)	1A	Khlestkina et al. (2006a)
	QRg.ipk-1D (Rg2)	1D	Börner et al. (2002)
Glume pubescence	*Hg*	1A	Khlestkina et al. (2002b, 2006a)
Glume elongation	*P-A^{pol}1 (Eg1, P1)*	7A	Wang et al. (2002)
	P-A^{pet}1	7A	Wang et al. (2002)
Leaf/auricle hairiness	*Hl1*	7B	Dobrovolskaya et al. (2006b)
	Hl2^{Aesp}	4B	Dobrovolskaya et al. (2006b)
	QHl.ipk-4B	4B	Dobrovolskaya et al. (2006b)
	QHl.ipk-4D	4D	Dobrovolskaya et al. (2006b)
	QPa.ipk-4B	4B	Dobrovolskaya et al. (2006b)
	QPa.ipk-4D	4D	Dobrovolskaya et al. (2006b)
Rust resistance	*lrTt1*	2A	Leonova et al. (2004)
	Yrns-B1	3B	Börner et al. (2000), Khlestkina et al. (2006b)

Coleoptile colour

As already mentioned, one gene determining the trait coleoptile colour was described by E.R. Sears located on chromosome XI (7A) of the variety 'Hope' (Sears 1954). The location was confirmed by Gale and Flavell (1971) identifying in addition a second major gene for red coleoptile colour on chromosome 7B of 'Hope' by analyzing 'Chinese Spring/Hope' substitution lines. The genes were designated *Rc1* (*Rc-A1*) and *Rc2* (*Rc-B1*), respectively. A third gene designated *Rc3* (*Rc-D1*) was identified on chromosome 7D by Jha (1964). Again

chromosome 7D was detected to carry a gene for red coleoptile by Sutka (1977) performing a monosomic F_2 analysis using the Ukrainian winter wheat variety 'Mironovskaya 808'. Both *Rc3* and *Rc1* were mapped in relation to RFLP markers (Chao et al. 1989) and by using a QTL approach (Nelson et al. 1995), respectively.

Khlestkina et al. (2002a) investigated mapping populations generated from crosses between non coloured wheat accessions and the two intrachromosomal substitution lines 'Chinese Spring/Hope 7A' (*Rc-A1*) and 'Chinese Spring/Hope 7B' (*Rc-B1*) as well as 'Mironovskaya 808' (*Rc-D1*). Using wheat microsatellite markers the authors were able to map the homoeologous series of *Rc* genes on the short arms of the homoeologous group 7 chromosomes (Fig. 1). The mapping data of *Rc-A1* and *Rc-D1* were confirmed by performing a QTL approach analysing recombinant inbred lines of the ITMI (International Triticeae Mapping Initiative) mapping population (Börner et al. 2002).

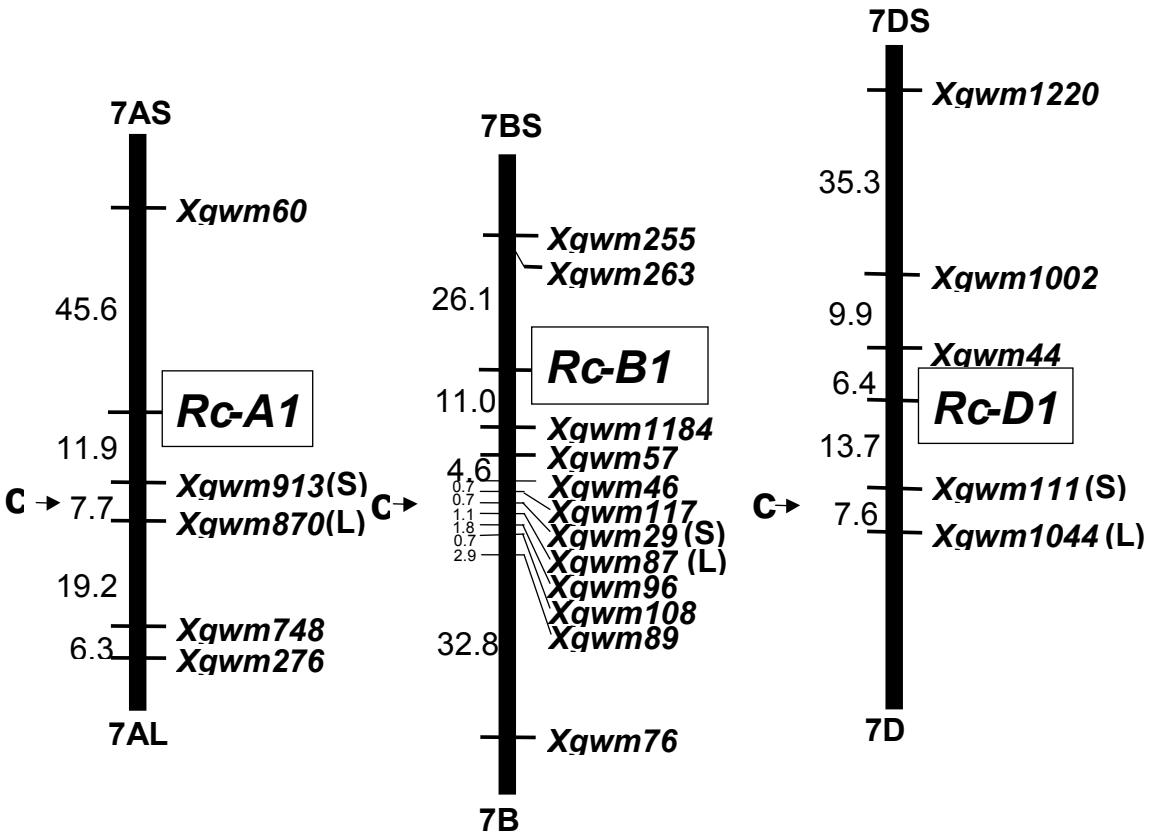

Fig. 1: Partial maps of chromosomes 7A, 7B and 7D showing the map positions of *Rc-A1, Rc-B1* and *Rc-D1* (Khlestkina et al. 2002a)

Purple grain colour

The frequency of purple grained hexaploid wheats is very low. Most probably, the trait has been transferred from purple-grained Ethiopian tetraploid wheats. Two cultivars having purple grains 'Purple Feed' and 'Purple' were originated from Australia and Canada, respectively. Performing monosomic analyses, genes in 'Purple Feed' have been localized on chromosomes 7B (*Pp1*) and 6A (*Pp2*), whereas 'Purple' may carry genes on chromosomes 7B (*Pp1*) and 2A (*Pp3*) (Arbuzova et al. 1998, Arbuzova and Maystrenko, 2000).

F_2 mapping populations were developed based on crosses between 'Purple', 'Purple Feed' and the Russian wheat cultivars 'Novosibirskaya 67' ('N67') and 'Saratovskaya 29' ('S29') having white and red grains, respectively (i.e. 'N67' x 'Purple', 'N67' x 'Purple Feed', 'S29'

x ‘Purple’, ‘S29’ x ‘Purple Feed’) and used for molecular mapping (Dobrovolskaya et al. 2006a).

Although, according to the results of monosomic analysis, the genes controlling purple grain colour in ‘Purple’, were located on chromosomes 2A and 7B (Arbuzova et al. 1998), we obtained a monogenic (3:1) segregation analysing the ‘N67’ x ‘Purple’ cross. SSR marker analysis revealed the location of one gene (*Pp3*) in the centromeric region of chromosome 2A (Fig. 2a).

Testing chromosome 6A and 7B markers in the ‘N67’ x ‘Purple Feed’ cross no linkage to the purple grain colour character was detected, which again was inherited monogenic. However, because the gene controlling purple grain colour in ‘Purple’ was mapped on chromosome 2A, markers of this chromosome were also screened for linkage to the target trait. Here we detected linkage between the colour gene and the microsatellite markers (Fig. 2b). The order and orientation of the mapped markers and the gene were the same as those in the map of the ‘N67’ x ‘Purple’ population (Fig 2a). The two *Pp* genes of Purple (*Pp3*) and ‘Purple Feed’ (*Pp2*) seem to be allelic.

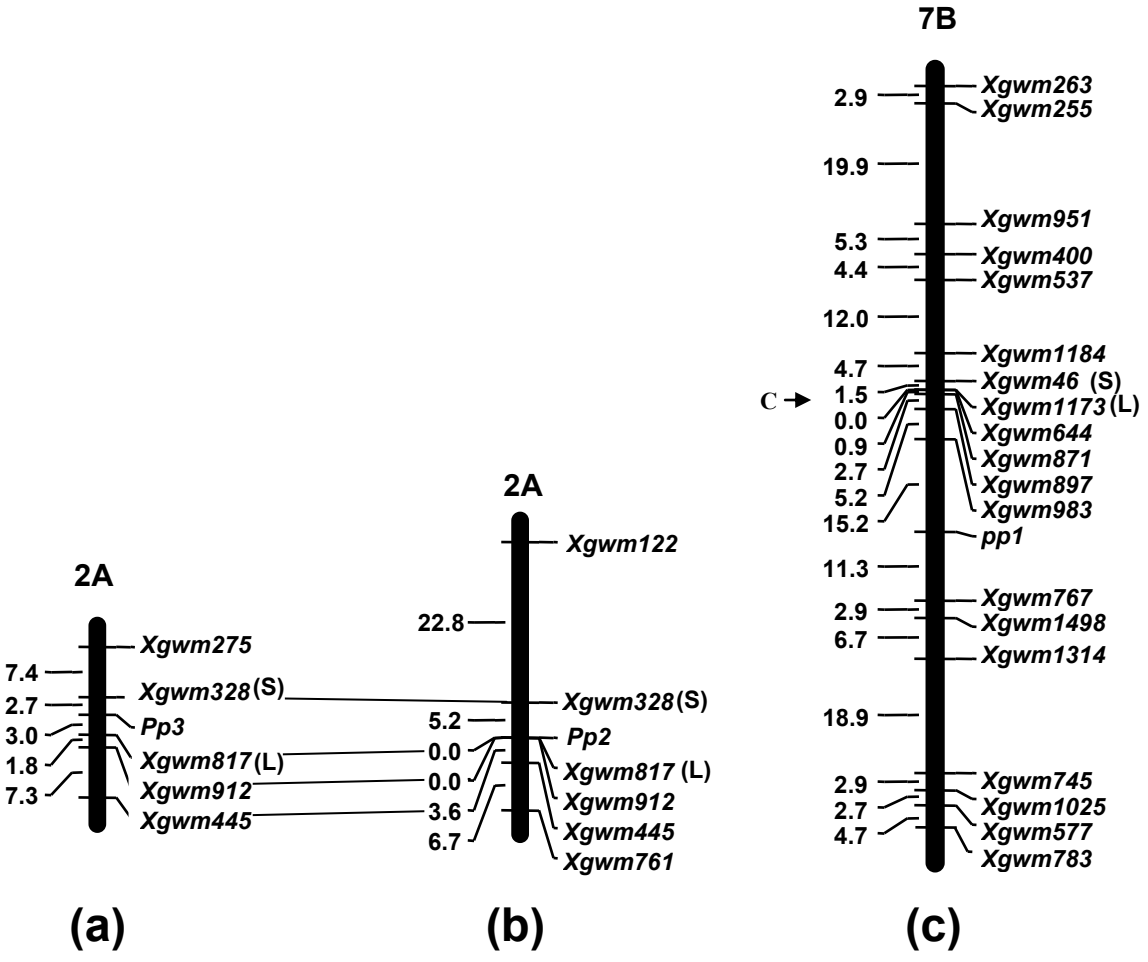

Fig. 2: Molecular linkage maps showing the positions of (a) *Pp3* on chromosome 2A, (b) *Pp2* on chromosome 2A and *pp1* on chromosome 7B (Dobrovolskaya et al. 2006 a)

In contrast to the crosses with ‘N67’, where purple grain colour of ‘Purple’ and ‘Purple Feed’ was inherited as a monofactorial dominant trait, in crosses with ‘S29’ the segregation ratio was close to 9:7, which is characteristic for a complementary dominant gene action. This suggested that the non-purple-grained cultivar ‘S29’ carries a recessive *pp* allele (designated *pp1*), which interacts complementarily with *Pp2* and *Pp3*. Former data of monosomic analysis

demonstrated that chromosome 7B is crucial for the control of purple grain colour in the purple-grained near-isogenic lines of 'S29' (Arbuzova et al. 1998, Arbuzova and Maystrenko 2000). Thus, microsatellite markers mapped on chromosome 7B were chosen for genotyping. As a result *pp1* was located on the long arm of the chromosome 7B (Fig. 2c).

Development and Utilisation of new stocks

More recently an initiative within EWAC was created to focus on three divergent sets of intervarietal substitution lines – 'Cappelle-Desprez/Bezostaya', 'Chinese Spring/Synthetic 6x' and 'Saratovskaya 29/Janetzkis Probat' (Börner et al. 1998). Selected lines were used to develop more precise genetic stocks like single chromosome recombinant DH lines to permit the mapping of genes of interest. Till now new single chromosome recombinant DH lines for 24 chromosomes have been produced or are in preparation (for details see Börner et al. 2006, present Newsletter).

One population comprising seventy-five DHR lines for chromosome 6A originated from the 'Chinese Spring/Synthetic 6A' substitution line was employed for mapping QTLs determining resistance (antixenosis) to greenbug biotype C and to the Russian wheat aphid (Castro et al. 2005). The results are presented in figure 3.

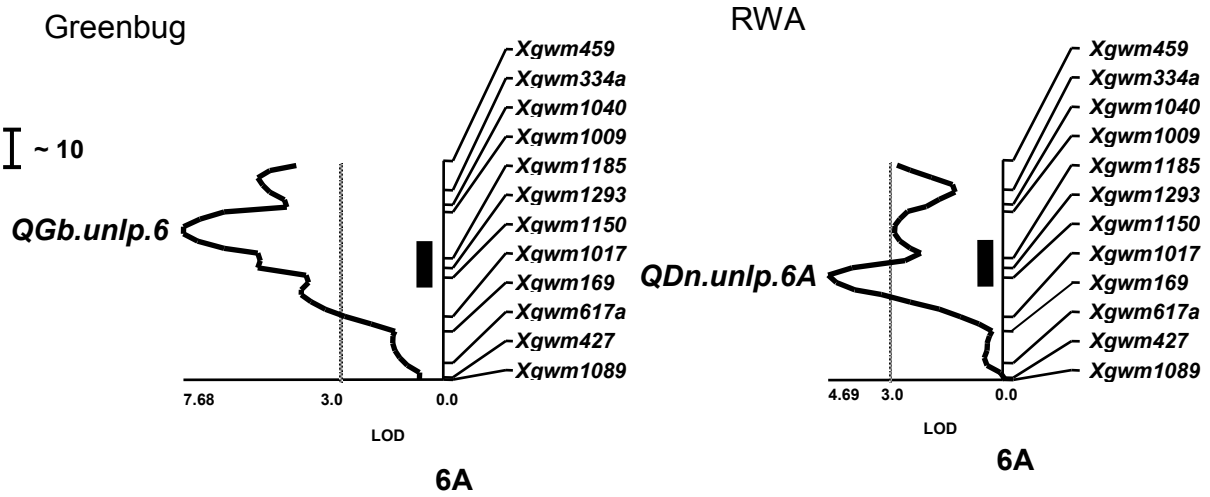

Fig. 3: Linkage maps of chromosome 6A showing the location of QTLs determining resistance to greenbug (left) and Russian wheat aphid (right). Centromere regions are marked by vertical bars (Castro et al. 2005)

Conclusion

The examples presented here clearly underline the importance of genetic stocks for gene localisation and mapping. The information obtained from studies using series of aneuploids, interspecific or intervarietal substitution or introgression lines etc. combined with the knowledge about the more recently detected synteny within the Triticeae, allowing the exploitation of mapping data from related species, significantly increase the efficiency of gene/QTL detection. Using the 'classical' genetic stocks, however, one should keep in mind that some of them may be incorrect as demonstrated for three sets of intervarietal substitution lines by Salina et al. (2003). Verification by using molecular markers becomes necessary. The development of new stocks is in progress, as demonstrated by Börner et al. (2006) in the presentation 'Ongoing and future co-operation within EWAC – business meeting' (present Newsletter).

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What is the restriction today: genotype or phenotype?

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The progress in cereals genomics during the last two decades has been remarkable. In each cereal, this has led to the development of large sets of molecular markers, construction of high density molecular maps (both genetic and physical), generation of large number of expressed sequence tags (ESTs) and sequencing of regions carrying specific genes. Identification and cloning of QTLs including those involved in epistatic and environmental interactions, or those involved in controlling the level of expression of genes (eQTLs) turn out to be a regular procedure in each ordinary molecular biology laboratory. Nevertheless, at the same time the need for precise genetic material (mutant, cytogenetical stock collections, etc.) obviously became to be one of the major limited factors for future developments. Diverse methods of plant genomics, archived results, and their future improvement by using precise genetic material are discussed in this paper.

1. Conventional plant breeding

Conventional cereal breeding is time consuming and very dependant on environmental conditions. Breeding a new variety takes between eight and twelve 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. Genomics, and in particular, molecular marker technology offers such a possibility by adopting a wide range of novel approaches to improving the selection strategies in cereal breeding.

2. Genomics

Already, it now is clear that genomics will achieve a new level of knowledge in current biology, genetics and breeding and will help to more deeply understand not only structural organisation of living organisms, but also their functional organisation. Complex spectrum of methods have been applied by genomics today could be divided into three major groups:
- structural genomics (including genome sequences and expressed sequences tags (ESTs);
- comparative genomics;
- functional genomics (gene expression analysis, reverse genetics, proteomics, metabolic profiling, ect.).

Structural genomics has grown very rapidly as result of the discovery of new technical platforms for high throughput sequencing. In 1999, for example, public databanks (NCBI) contained only eight entries of EST for wheat (*Triticum aestivum* L.) and close to 590 000 sequences have been deposited here till June 2005 (http://www.ncbi.nlm.nih.gov/dbEST/dbEST-summary.html). Now it is a reality to cover by sequencing the full genome of a plant as that the case for Arabidopsis or rice and a true time for thinking about full genome sequencing of more complex plants such as maize or wheat. These days, the main area of research applications in molecular biology has already moved from structural genomics to functional genomics. Nevertheless, between both groups there exists a very tight link and this relation will be extended in the future.

Comparative genomics helps the scientific community to exchange the knowledge from one species to the other, in same cases to save time and resources.

Functional genomics opens up a new view on systems in plants, with most fascinating questions about function of single genes and gene complexes. This became possible by applying a broad spectrum of methods: expression analysis of genes by using array technology, reverse genetics, proteomics, metabolic profiling and etc.

The genomics era has already opened a new perspective for further applications of genetic material. That includes precise high resolution trait and gene mapping; chromosome sorting and development of chromosome specific libraries; gene cloning; genetic modification and gene expression.

3. Use of genomics in plant breeding

In recent years, different marker systems such as Simple Sequence Repeats (SSRs) or microsatellites, Sequence Tagged Sites (STS), Amplified Fragment Length Polymorphisms (AFLPs), Single Nucleotide Polymorphisms (SNPs) and others have been developed and applied to a range crop species including cereals. A large number of cereal studies have used markers as a tool to identify major genes, QTLs, or to introduce new characters into 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). 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. Marker-assisted selection allows plant selection at the juvenile stage from an early generation. For simply inherited traits, conventional PCR, which requires a small amount of DNA, is becoming very useful for screening large populations of segregating progenies. Unfavourable alleles can be eliminated or greatly reduced during the early stages of plant development through MAS, focusing the selection in the field on reduced numbers of mature plants.

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.

Recently, 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. 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 (Fig. 1).

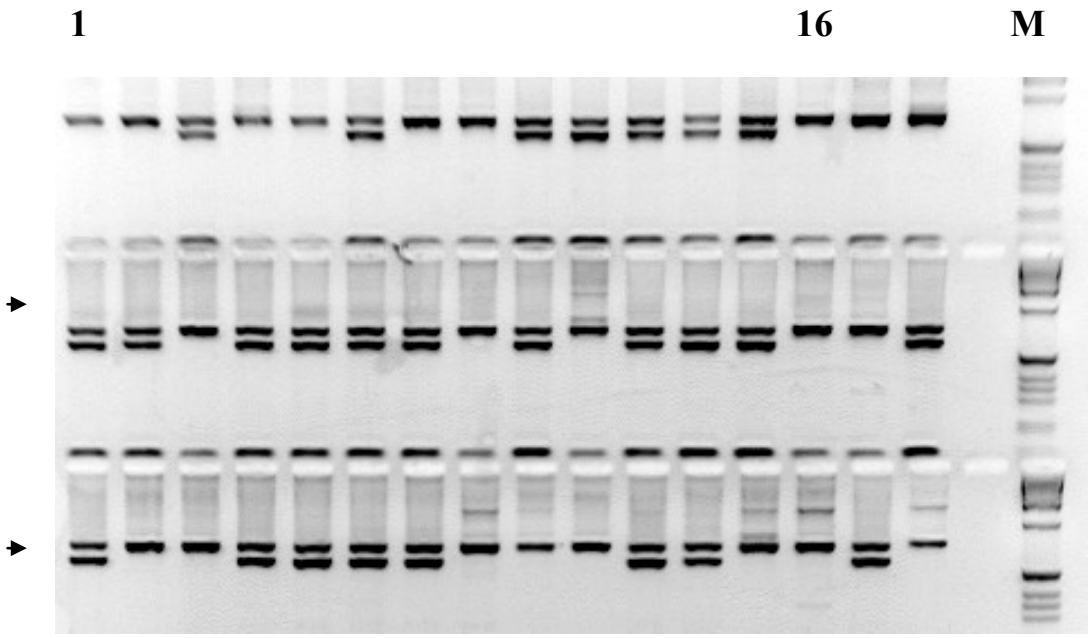

Fig. 1: Application of PCR-based marker SR4R04 for selecting fertile plants. M – 1 kb ladder; lanes 1-48 rye plants, fertile plants detected by two fragments

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

Example 2: Application of molecular markers in breeding for resistance to Fusarium head blight in wheat

Fusarium head blight is a serious disease of wheat (*Triticum aestivum* L.) in humid and semi humid areas of the word. 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. Deoxynivalenols (DON) are harmful to humans, because they are highly heat stable and cannot be eliminated totally once they enter 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 markers closely linked to the major QTL involved in FHB resistance have recently been found (Buerstmayer et al. 2002; Schmolke et al. 2005) and raises the possibility of using MAS for introducing resistance alleles into elite wheat varieties as have been confirmed also by us. However, due 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 is, at the moment, as reported by Miedaner and co-workers (Miedaner et al. 2005), a more sufficient and safe strategy in breeding of a new varieties, combining a high level of yield performance and high level of resistance to Fusarium head blight.

Conclusions

The results, tools and methods that have been achieved by genomics are being evaluated and adapted for plant breeding. There is no doubt that biotechnology is now having a major impact on agriculture and this impact will rapidly grow in the near future. Nevertheless, often the

technology and machinery are not limited factors by genomics, but the plant resources are crucial factors for new discoveries in the structures and functions of plant genomes.

The use of precise cytogenetic stocks in the framework of genomics research will lead to a better understanding of genetics and physiology of crop plants and will drive a continuous progress in crop breeding and, more generally, across different areas of agriculture.

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Development and molecular cytogenetic identification of new winter wheat 'Martonvásári 9 kr1'/winter barley 'Igri' disomic addition lines

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The hybridization of wheat and barley makes it possible to transfer useful characters such as earliness, tolerance to drought and soil salinity, and various nutrition quality parameters from barley into wheat. The first wheat-barley hybrid was produced by Kruse (1973) and not much later a wheat/barley addition line set was developed by Islam et al. (1978). Since the production of the Chinese Spring/Betzes spring wheat/spring barley addition lines there has been no report on the development of a new set of wheat/barley addition lines except the 5H and 6H addition lines produced from a hybrid involving the wheat cultivar 'Shinchunaga' and the barley cultivar 'Nyugoruden' by Koba and co-workers in 1997. However, wheat/rye addition lines have been developed in many cultivar combinations (Shepherd and Islam 1988). Wheat-alien addition lines form the starting point for producing translocations from selected chromosomes and are also suitable genetic materials for genome mapping. As there is a great genetic variability between various barley cultivars for important agronomic traits (two or six-row, winter or spring habit, biotic and abiotic resistance, etc.) it would be advisable to develop addition lines using different barley genotypes in order to map and transfer favourable agronomical characters from barley. Many cultivated barley varieties were used for the pollination of wheat, and the development of addition lines was started from hybrids produced with the German two-rowed winter barley cultivar ‘Igri’ and the Ukrainan six-rowed winter barley cultivar ‘Manas’.

1. Production of new winter wheat 'Martonvásári 9 kr1'/winter barley 'Igri' disomic addition lines

The 'Martonvásári 9 kr1' × 'Igri' hybrid was vigorous and had good tillering ability, which made it possible to collect anthers from young inflorescences for meiotic analysis, to pollinate some spikes with wheat, and to use some developing inflorescences for *in vitro* multiplication (Molnár-Láng et al. 2000). Since the hybrid showed complete male and female sterility, they were multiplied in tissue culture from young inflorescences. 101 plants were regenerated from one initial hybrid plant, of which 92 were raised to maturity. Meiotic chromosome pairing in the anthers of the regenerants was analysed by the Feulgen method and by genomic *in situ* hybridization (GISH). The regenerated hybrids were pollinated with wheat and backcross seeds were developed. Six BC_1 plants were grown from the nine embryos developed after pollinating 4606 flowers on the regenerated Martonvásári 9 kr1 (Mv9 kr1) × Igri hybrids with the wheat line Mv9 kr1. The BC_1 plants were backcrossed again with the wheat line Mv9 kr1 and 24 BC_2 seeds were obtained, 14 of which grew into fertile plants. Plants with 44 chromosomes were selected from the selfed progenies of these. Chromosome pairing was analysed in metaphase I of meiosis to identify plants with 22 bivalents. As several different lines with 44 chromosomes and 22 bivalents in meiosis were selected, the next step was to identify the barley chromosomes added to the wheat background. As a first step the parental genotypes had to be identified under our laboratory conditions using fluorescent *in situ* hybridization (FISH) with the help of different DNA probes.

2. Identification of barley (*Hordeum vulgare* cv. 'Igri') chromosomes using FISH with the help of DNA probes GAA, HvT01 and pTa71

Wheat chromosomes can be identified using the GAA sequence combined with the pAs1 clone (Pedersen and Landgride 1997). All the chromosomes of the line Mv9 kr1 were identified with the help of these sequences in the molecular cytogenetic lab in Martonvásár. The identification of barley chromosomes is a more difficult task. Barley chromosomes can be identified using FISH with the help of the GAA sequences (Pedersen et al. 1996). The GAA FISH hybridization sites resemble the C-banding pattern of barley, especially on chromosomes 4H and 2H. 4H is the most heterochromatic chromosome after C-banding (Jensen and Linde-Laursen 1992; Linc and Molnár-Láng 2003), and the largest number of FISH signals were observed on this chromosome in cultivar 'Igri' in the present work. A symmetrical FISH hybridization pattern was observed on both arms of chromosome 2H, similarly to its C-banding pattern. Chromosomes 5H and 6H both have satellites, but the arm ratio of these two chromosomes is different, which helped their identification. 5H and 6H have strong GAA FISH signals around the centromere, but there were no GAA FISH signals in the NOR region, though strong bands were observed in these regions after C-banding (Linc and Molnár-Láng 2003). The differentiation of chromosomes 3H and 7H was the most difficult, as their FISH hybridization pattern looks very similar. Not all the small bands described by Pedersen et al. (1996) appeared in all experiments; in most cases the strong FISH signals close to the centromere in the proximal regions of both arms could be identified. As the 3H and 7H could not be identified with full certainty in all experiments it was necessary to apply more FISH probes for the identification of the different wheat/barley addition lines.

The FISH identification of barley chromosomes were further confirmed with the help of the subtelomeric repeat HvT01 (Schubert et al. 1998) and the probe pTa71 (Leitch and Heslop-Harrison 1992). Most of the barley chromosome arms had telomeric FISH signals after hybridization with the HvT01 probe, except for the 3H long arm, which had a strong subtelomeric signal and another weak interstitial signal on its long arm. Thus, chromosome

3H could be reliably identified with the help of this probe. The long arms of 2H and 5H have very weak telomeric signals, but these sites could not be seen at all in most of the experiments. Thus the satellited 5H could be differentiated from 6H, the other chromosome with a satellite, which gave a clear signal with this probe on the long arm. The probe pTa71, containing the ribosomal genes, 18S-5.8S-26S, was located on five pairs of barley chromosomes by Leitch and Heslop-Harrison (1992). Two major sites can be observed in the NOR region of chromosomes 5H and 6H. There are minor sites on 1H, 2H and 7H, the site on 1H having more copies than those on chromosomes 7H and 2H. The interstitial signal on the short arm of chromosome 1H could be easily recognized under the present experimental conditions, but the signals on chromosomes 2H and 7H were hardly perceptible. Thus, the probe pTa71 helped the exact identification of 1H.

3. Identification of the barley chromosomes in the addition lines using FISH and SSR markers

The first addition line was identified with the help of the GAA sequence and the pAs1 probe. All the wheat chromosomes could be identified with the help of these sequences and a pair of 4H chromosomes was present. The 4H chromosome could be identified by its strong GAA FISH hybridization signals. The presence of the additional barley chromosome pair was confirmed with genomic *in situ* hybridization (GISH) using total barley genomic DNA directly labelled with Fluorored as a probe. The GISH experiment was carried out on the same slides after washing off the FISH signals. Chromosome 4H was also identified with the help of probe HvT01, which gave telomeric signals on both chromosome arms, while the wheat chromosomes gave no signals. The presence of chromosome 4H in this addition line was also confirmed with SSR markers. Markers HvM 40 and HvM 67, previously mapped on chromosome 4H, gave the expected bands on this line. The 4H Mv9 kr1/Igri addition line has a compact spike with small awn stubs at the top. The spikes have good fertility. The 4H addition line has already been multiplied in our nursery, so a great number of seeds are available for further studies.

The next addition line contains a pair of 2H chromosomes, identified with a combination of the probes GAA and the pAs1 using FISH. The GAA FISH hybridization signals on chromosome 2H are very similar to the C-banding pattern of this chromosome, having symmetrical interstitial hybridization sites on both arms. The presence of 2H was also confirmed with sequential GISH analysis after FISH. When applying HvT01 as a probe, a FISH hybridization signal was observed only on the short arm of the barley chromosomes, which is typical of 2H. The other barley chromosome which has a signal only on the short arm is 5H, but that is a satellited chromosome which could be easily recognized. The SSR marker HvM36 also gave the expected PCR product size to confirm the presence of 2H in this line. This line has a long, loose spike, having fewer seeds/spike than the 4H addition line. The plants are taller than the 4H addition line. This line has also been multiplied in the nursery so a large number of seeds are available for further studies.

The third addition line contains the chromosome 3H. It was first identified using FISH with a combination of the DNA probes GAA and pAs1. The GAA hybridization sites on this chromosome did not allow the unequivocal identification of 3H, as it was mostly hybridization sites close to the centromere that could be recognized, which are very similar to the hybridization signals on 7H. Thus, the probe HvT01 was also used for FISH identification. This probe made it clear that this chromosome was 3H, as it is the only barley chromosome which has a strong subtelomeric signal and a weak interstitial signal besides the telomeric signal on its long arm. The molecular cytogenetic identification was also confirmed with the help of molecular markers. The SSR markers HvM60 and HvM62 gave the expected bands, confirming the presence of the 3H chromosome in this line. The 3H addition line has a very

compact ear with a great number of spikelets per ear. Small awnstubs can be seen at the top of the spikes. The plants are short but have good fertility. This addition line has also been multiplied in the nursery.

The fourth line was the most difficult to identify as it has a disomic addition of the isochromosome 1HS. In this addition line strong GAA FISH hybridization signals were observed around the centromere on the barley chromosome after the identification of all the chromosomes with the GAA sequence and the probe pAs1. It was thought to be 7H, as two telomeric bands were observed with the HvT01 probes at the ends of the chromosome pair. 5H and 6H could be excluded, as there was no satellite, and 2H and 4H on the basis of the GAA pattern, while 3H could be excluded as it has a very characteristic hybridization pattern with HvT01. However, molecular markers tested to confirm the presence of 7H did not give the expected PCR products. FISH hybridization with the probe pTa71 identified this chromosome pair as 1HS isochromosomes. Two minor, perfectly symmetrical, interstitial pTa71 signals were observed on both arms of this chromosome. The strength of this signal coincided with the strength of the signal on the 1H short arm. It thus became clear that this line carries a pair of 1HS isochromosomes. It has a long, loose spike with small awnstubs. This plant was grown in a controlled environment, producing enough seeds for further multiplication.

4. Production and molecular cytogenetic identification of backcross progenies from the winter wheat ‘Asakaze komugi’ × winter barley ‘Manas’ hybrids

The aim of this study was to produce backcross progenies on a new winter wheat (‘Asakaze komugi’) × Ukrainan six-rowed winter barley (‘Manas’) hybrid produced in Martonvásár. As no backcross seeds were obtained on the initial hybrids, young inflorescences of the hybrids were used for *in vitro* multiplication in three consecutive cycles until a backcross progeny was developed. The chromosome constitution of the regenerated hybrids was analysed using GISH after each *in vitro* multiplication cycle in metaphase I of meiosis (Molnár-Láng et al. 2005). The seven barley chromosomes were present even after the third *in vitro* multiplication cycle but abnormalities were observed. Sixteen BC_2 plants shown by GISH analysis to contain one to three complete barley chromosomes, two deletion barley chromosomes and a dicentric wheat-barley translocation were grown to maturity from the single backcross progeny. The barley chromatin was identified using 20 chromosome-specific barley SSR markers. All seven barley chromosomes were represented in the BC_2 plants. A deletion breakpoint at FL ±0.3 on the 5HL chromosome arm facilitated the physical localization of microsatellite markers.

Conclusions

The 2H, 3H and 4H disomic addition lines of winter wheat 'Martonvásári 9 kr1'/winter barley 'Igri' were produced and identified using GISH, FISH and SSR markers. A disomic addition of the 1HS isochromosome was also identified. The 2H, 3H and 4H addition lines have been multiplied in the nursery and characterized morphologically. There are enough seeds available for further studies. Backcross progenies were produced and identified with molecular genetic and cytogenetic methods from the Japanese winter wheat ‘Asakaze komugi’ × Ukrainan six-rowed winter barley ‘Manas’ hybrids. The development of disomic additions is in progress from the fertile BC_2 plants.

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The *Agropyron cristatum* genetic system promoting homoeologous pairing in hybrids between wheat and *A. cristatum.*

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Agropyron is a perennial genus of the *Triticeae* consisting of 10 species, which occur at three ploidy levels, diploid (2*n*=14), autotetraploid (2*n*=28) and autohexaploid (2*n*=42) and which are built on one basic genome, P (Dewey 1984). In addition to being economically important forages, *Agropyron* species have been found to possess potentially valuable traits for wheat improvement, including tolerance to drought (Dewey 1984; Asay and Johnson 1990) and low temperature (Limin and Fowler 1987) and resistance to diseases such as barley yellow dwarf virus (BYDV) (Sharma et al. 1984).

Chen et al. (1989) successfully hybridized *Triticum aestivum* cv. Chinese Spring (2*n*=6*x*=42, AABBDD) and tetraploid *Agropyron cristatum* (2*n*=4*x*=28, PPPP). The analysis of the meiotic behaviour in the F_1 hybrids showed the occurrence in the P genome of a genetic system interfering with the wheat *Ph1* gene recognized as the main factor responsible for the cytological diploid-like behaviour of polyploid wheats by suppressing pairing of homoeologues (Chen et al. 1989). In the backcross progenies, six disomic addition lines (1P to 6P) and five ditelosomic addition lines (2PS, 2PL, 4PS, 5PL and 6PS) were extracted and identified through the use of a set of RFLP probes detecting each homoeologous chromosome

arm in wheat (Chen et al. 1992, 1994). This work did not provide any evidence of structural rearrangements which differentiate the P genome from the A, B and D genomes of wheat.

Different strategies were designed for producing wheat-alien chromosome translocations (Jiang et al. 1994). Among them, induced homoeologous pairing is the method of choice if the gene synteny is conserved. It can be achieved either by deleting the *Ph1* gene or by suppressing its inhibitory activity on chromosome pairing by adding to wheat the chromosome of certain strains of a few species including *A. cristatum*. The present study aimed at assessing the level of homoeologous pairing between wheat and *A. cristatum* chromosomes by using the *Ph* suppressor genetic system in *A. cristatum* and the *ph1b* mutation which is in fact a deletion of a segment with the *Ph1* gene (Sears 1977).

1. Integrity of P chromosomes added in the addition lines

A prerequisite to the evaluation of pairing between P chromosomes and ABD chromosomes was to check that the P added chromosomes had not retained any wheat chromatin in the course of extraction of the addition lines. Each of them was visualized using GISH with total genomic DNA of *A. cristatum*, as a probe. No wheat translocations were detected on the P chromosomes. The hybridization of the probe pTa71, which detects the sites of rDNA, allowed the development of a physical marker for the 5P chromosome.

2. Chromosomal location of the *A. cristatum* genetic system *Ph* suppressor and its potential to induce P-ABD homoeologous pairing

It was planned to assess the level of allosyndetic pairing in the F_1 hybrid '*T. aestivum* / *A. cristatum*' in which meiosis was studied by Chen et al (1989). For an unknown reason, degeneration of PMCs prior to meiosis was observed and consequently the frequency of pairing between the P chromosomes and those of wheat could not be studied.

Meiotic behaviour of the hybrids ($2n$=36 or $2n$=35+t) between the five disomic addition lines (1P, 3P, 4P, 5P and 6P) and the five ditelocentric addition lines (2PS, 2PL, 4PS, 5PL and 6PS) with *Aegilops variabilis* ($2n$=$4x$=28, UUSS) should enable the identification of chromosome(s) carrying the *A. cristatum Ph* suppressor system. Chromosome pairing, if any, could only occur between homoeologous chromosomes. Chromosome configurations in each hybrid were observed and the pairing level for each of them was compared to that of the control hybrid *T. aestivum* cv. CS / *Ae. variabilis*. For each genotype, the chiasmata frequency per PMC (50 cells) was calculated on three plants.

Significant differences on the homoeologous pairing level between the 11 genotypes tested were found. Comparison of means showed that all the genotypes, except those with 2PS and 2PL chromosomes, displayed a significantly higher level of homoeologous pairing than the control. Consequently, all the P chromosomes tested, except 2PS and 2PL chromosomes, seem to promote homoeologous pairing. The *A. cristatum Ph* suppressor system appears polygenic.

However, even if each of the chromosomes 1P, 3P, 4P, 5P and 6P have a promoting effect on homoeologous pairing, i.e. a suppressing effect on the *Ph* genetic system, this effect has the same magnitude as that of the *Ph2* gene on wheat chromosome 3DS (Driscoll 1972) but is much weaker than the effect of the *ph1b* deletion. Indeed, Sears (1977) observed 18.2 chiasmata per cell in the hybrid *T. aestivum ph1b* / *Ae. variabilis*. Here, the highest mean

number of chiasmata per cell is 4.9 (hybrid between the 6P disomic addition line and *Ae. variabilis*), further evidence that the *A. cristatum* system involves only minor genes. So far, *Ph* inhibition has only been genetically studied in *T. speltoides*. In that species, two systems are involved in the promotion of homoeologous pairing, with one system being composed of two major genes segregating independantly of each other and the other being composed of several minor genes modifying the effects of the major genes (Chen and Dvorak 1984). Chen et al. (1994) transferred the two major genes (Ph^I genes) to *T. aestivum*. Although the homoeologous pairing level, in F_1 interspecific hybrids with the Ph^I line, was not as high as with the *ph1b* mutant, it was concluded that the Ph^I lines could be effective for transferring alien genetic variation (Chen et al. 1994).

In the hybrids between the ditelosomic addition lines and *Ae. variabilis*, the P telocentric chromosomes are easily identified in meiotic preparations. Despite the promoting effect on homoeologous pairing of the telocentric chromosomes 4PS, 5PL and 6PS, these have never been observed paired, among 450 cells screened. Genes involved in the *A. cristatum* system are not efficient enough, when used separately, to induce homoeologous pairing between *A. cristatum* and wheat genomes. Nevertheless, as the 7P addition line was not extracted, we cannot exclude the occurrence, on the 7P chromosome, of one or few genes having a major suppressor effect on *Ph1*.

3. Attempts of homoeologous pairing induction using the *ph1b* mutation

The *ph1b* mutation was introduced in monotelosomic addition lines for the telocentric chromosomes 2PL, 5PL and 6PS. Homozygosity for *ph1b* was checked using the PCR test described by Qu et al. (1998). Despite the absence of the *Ph1* gene, the added telocentric chromosomes 2PL and 6PS were not observed paired, among 331 cells screened. Only the telocentric chromosome 5PL was found paired with a pairing frequency of 0.018 among 108 cells screened.

Finally, allosyndetic associations between the *A. cristatum* and wheat genomes, if any, are rare even in the absence of the *Ph1* gene. Our results confirm the observations of Chen et al. (1989) on the meiotic behaviour in different F_1 hybrids between wheat and *A. cristatum*. These authors argued that the high level of chromosome pairing in the F_1 hybrids is likely to be due to homologous pairing within *Agropyron* chromosomes and homoeologous pairing between wheat chromosomes. Allosyndetic associations wheat - *A. cristatum*, if any, are rare in the F_1 hybrids. Under the hypothesis that the co-linearity of the maps of wheat and *A. cristatum* is real, then the absence of allosyndetic pairing would be due to differences in the nature and importance of repeated sequences in the two species.

Consequently, induction of homoeologous pairing between the *A. cristatum* and wheat chromosomes does not appear a suitable method for fully exploiting *A. cristatum* in wheat breeding. Development of translocation lines using ionizing radiation (Sears 1993) could be a better technique to exploit the *A. cristatum* genome in wheat improvement.

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Gene identification by transcript profiling and real time PCR during cold acclimation using wheat 5A chromosome substitution and recombinant lines

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Genetically determined frost tolerance is a function of a cold acclimation process. Several coordinated metabolic changes like altered gene expression, modifications in cellular metabolism, changes in growth rate, result in the induction of frost resistance during acclimation. The expression of most genes is dependent on networks of regulators such as transcription factors. In the model species *Arabidopsis thaliana*, a crucial role in the regulation of genes involved in cold resistance has been demonstrated for a family of transcription factors named C-repeat binding factor (CBF). CBFs have been shown to increase freezing resistance when over-expressed in transgenic plants. *Cbf*-like sequences have been isolated from different species including *T. monococcum*, barley and wheat using the methods of comparative gene mapping. More-over, expression profiling on wheat 5A single chromosome recombinant lines by real time PCR indicated that some of the *Cbf* genes may have an important regulatory role during the cold acclimation process in wheat. A cDNA macroarray study utilizing Chinese Spring and two 5A chromosome substitution lines revealed from 10,297 monitored unigenes that the cold-induced changes in the expression of 162 unigenes were significantly affected by chromosome 5A. The genes most likely involved in the cold acclimation process will be highlighted.

1. Gene identification by real time PCR during cold acclimation

The CBFs are the most extensively studied transcription factors because of their critical role in the regulation of low temperature stress response in *Arabidopsis* (reviewed by Thomashow et al. 2001). Less is known about the orthologs of *Arabidopsis Cbf* genes in cereals. Cold induced transient accumulation of *Cbf* -like transcripts was reported in wheat and rye. The barley *Cbf3* gene was first mapped on barley chromosome 5H and then in the colinear region of chromosome $5A^m$ in einkorn wheat linked to the frost tolerance locus *Fr-*A^m*2* (Vágújfalvi et al. 2003). This same chromosome region was then found to affect frost tolerance on chromosome 5B of common wheat (Tóth et al. 2003) and chromosome 5H of barley. It was shown that the barley locus includes the *HvCbf3*, *HvCbf4* and *HvCBf8* genes (Francia et al. 2004).

1.1. Chromosome 5A plays a key role in the control of *Cbf* gene expression in wheat

To get an overall view of cold induced *Cbf* expression, frost tolerant and sensitive wheat cultivars, and a set of Chinese Spring (Cheyenne) (CS{CNN}) single chromosome substitution lines were compared with Northern analysis using a radioactively labeled probe containing the conserved *AP2* domain and the *Cbf* signatures of the barley *Cbf3* gene. Among the chromosome substitution lines studied, it was found that chromosome 5A showed the same expression level as CNN. This result proved the central role of this chromosome not only in the determination of frost tolerance, but also in the regulation of *Cbf* genes.

1.2. The higher Cbf expression is associated with the *Fr-A2* region on chromosome 5A

Eight recombinant substitution lines (RSLs), originated from the cross between the frost tolerant line CS(CNN5A) and the frost sensitive Chinese Spring (*Triticum spelta*5A), (CS{TSP5A}), were selected to identify the region of chromosome 5A that is responsible for the different gene expression. Five RSLs (38-6, 35-2, 72-6, 74-7, 31-2) expressed the same amount of *Cbf* mRNAs as CS(TSP5A), while three lines (10-2, 19-4 and 46-1), similar to CS(CNN5A), showed a high level of *Cbf* expression (Fig 1).

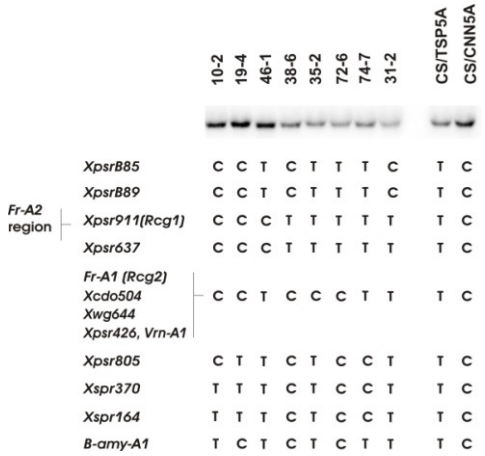

Fig. 1: Northern analysis of CS(TSP5A), CS(CNN5A) and some selected single chromosome recombinant lines after cold stress. T and C indicate the RFLP probes representing TSP or CNN alleles, respectively

The genotypes of the recombinant lines with RFLP markers located in the critical region of chromosome arm 5AL are presented in Fig 1. All the genotypes showing a low level of expression carries *T. spelta* (T) alleles at the *Xpsr911* and *Xpsr637* linked loci. On the other hand, all the lines with high *Cbf* transcript levels share the Cheyenne (C) allele at these loci. The *Xpsr911* locus was previously mapped linked to the *Fr-2* locus in wheat (Vágújfalvi et al. 2000, 2003. This result indicates that the higher level of *Cbf* transcripts is linked to the *Fr-A2* locus on chromosome 5A.

1.3. Three *Cbf* genes are differentially expressed in frost resistant and susceptible genotypes

Gene specific primers for 8 *Cbf* genes, previously mapped in *T. monococcum* on chromosome $5A^m$ (Miller et al. 2005) were designed. RT-PCR analysis showed that all tested *Cbf*s were expressed in CS or CNN, but at different level. The expression of each *Cbf* genes in CS(CNN5A), CS(TSP5A) and in the RSLs 46-1 and 38-6, previously characterized by the different levels of *Cbf* expression in Northern blots, was compared by real-time qRT-PCR. *Cbf1A*, *Cbf1C* and *Cbf7* genes showed significantly higher transcription levels in CS(CNN5A) and in RSL 46-1 than in the RSL 38-6. Based on these results, it was possible to map the differential regulation of these three *Cbf* genes to the *Fr-A2* region.

The RSL 46-1 with the parental lines CS(CNN5A) and CS(TSP5A) were subjected to freezing test. It was found that the difference in *Cbf* expression is linked with the difference in frost tolerance, suggesting the presence of an additional QTL on the long arm of wheat chromosome 5A corresponding to *Fr-2* found both in *T. monococcum* (Vágújfalvi et al., 2003) and barley (Francia et al., 2004).

2. Gene identification by transcript profiling during cold acclimation

For the investigation of the cold-induced changes in transcriptome profiles of plants macro- and microarray methods can be used very efficiently. The effect of cold-treatment on gene expression was monitored most extensively using microarrays in *Arabidopsis*, in rice and in sugarcane to follow cold-induced expression changes (Shinozaki et al. 2003; Zhang et al. 2004; Nogueira et al. 2003). Several genes involved in the stress tolerance of wheat were mapped on chromosome 5A. Thus genes responsible for osmoregulation, frost tolerance and vernalisation requirement, cold-induced abscisic acid and carbohydrate accumulation, genes affecting the levels of heat shock proteins and dehydrins are also localised on this chromosome. Comparing the expression of the cold-responsive *cor14b* gene in the frost-tolerant CS(CNN5A), in the moderately frost-sensitive CS and in the frost-sensitive CS(TSP5A) a relationship between the frost tolerance and gene expression was described (Vágújfalvi et al. 2000). Thus, it was proposed that by comparison of the cold-induced changes in the transcript profile in these 3 genotypes such candidate genes can be found whose induction is influenced by chromosome 5A.

2.1. Materials and methods of the macroarray study

The seeds were germinated at room temperature for 3 d between wet filter papers. The seedlings were cultivated for 10 days in hydroponics using half-strength modified Hoagland nutrient solution (Nagy and Galiba, 1995) in a spring type growth chamber (Conviron PGV-36 chamber, Controlled Env. Ltd., Winnipeg, Canada) at 18/15 °C day/night/ temperature and 75/75% relative humidity, with 16 h illumination at 270 μmol m^{-2} s^{-1}. The sampling was always done at the middle of the light period in order to exclude the effects of circadian changes on the gene expression. Total RNA was extracted using the NucleoSpin RNA L kit from Macherey Nagel (Düren, Germany) and the mRNA was isolated by the Dynabeads

mRNA Purification Kit from Dynal (Oslo, Norway). ^{33}P-labelled second-strand cDNA probes were prepared and hybridization with the barley cDNA membrane was carried out as described previously (Zierold et al., 2005) except for the less stringent wash after the hybridization (1 x SSC, 0.1 % SDS; 0.5 x SSC, 0.1 % SDS). 10450 cDNA sequences (corresponding to 10 297 unigenes) in duplicate were spotted in 6 x 6 subarrays onto two 8 x 12 cm nylon membranes. Three biological repetitions were done with plants grown under identical conditions in independent experiments. Genes differentially expressed in the 3 genotypes were selected using one-factor ANOVA and hierarchical clustering (Program Tigr MeV 3.1, Saeed et al. 2003).

2.2. Results

Comparing the 3 genotypes in the 4 sampling points, the intensity of 173 spots out of 10450 were found to be significantly different at the $P<0.01$ % level. The 173 spots corresponded to 162 unigenes from which 68 had higher expression level in CS(CNN5A) and the remaining 94 in the two frost-sensitive genotypes. Based on the time-course of the expression changes the selected 162 unigenes could be grouped into 10 clusters by K-means clustering. In 4 clusters (54 unigenes) the gene expression was up-regulated, and in 3 clusters (45 unigenes) down-regulated in all three genotypes studied. In 2 clusters (28 unigenes) only in CS(CNN5A), and in 1 cluster (35 unigenes) only in CS and CS(TSP5A) was the expression up-regulated, while down-regulated in the other genotypes. In the cluster with the highest level of cold-induction genes coding for glutathione S-transferase, cold-acclimation protein, Ca-binding protein, late embryogenesis abundant (LEA) proteins, enzymes of carbohydrate metabolism were identified.

Since genes of several LEA proteins are downstream to the transcription factor *CBF2* in the cold-inducible stress response pathway in *Arabidopsis* (Maruyama et al. 2004), and the *Cbf3* gene was mapped on the long arm of the chromosome $5A^m$ of einkorn wheat (Vágújfalvi et al. 2003), it is possible, that the regulation of the LEA genes is influenced by chromosome 5A, especially by the *Fr-A2* region containing the *Cbf* genes. Genes, responsible for cold- induced carbohydrate accumulation were localised in the *Fr-A1 – Vrn-A1* region on the long arm of chromosome 5A (Galiba et al. 1997), so the induction of the carbohydrate metabolism genes described above is most likely regulated by this region. Finally, since it has been proved that the *Fr-A1 – Vrn-A1* and the *Fr-A2* region on chromosome 5A are playing crucial role in the regulation of many genes induced by low temperature, it is reasonable to hypothesise that these two regions are responsible for the regulation of the majority of the chromosome 5A-controlled candidate genes selected by macroarray in this experiment.

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Genetic study of wheat stress resistance, growth habit and flowering time in the Czech Republic; the role of the aneuploid technique

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Wheat disease resistance – breeding and research

Breeding

Whereas empirical breeding for disease resistance based on selection is probably as old as plant breeding itself, beginnings of wheat breeding for disease resistance based on scientific genetic principles date since the first decades of the last century. In 1918 Professor Peklo published the first Czech contribution summarizing experience with breeding for yellow rust resistance. He used Swedish resistant cultivar Kotte Grenadier for crosses with Czech susceptible cultivars and developed the winter wheat Bastard UP that remained resistant for at least 15 years. After a period of empirical resistance breeding specific resistance genes started to be used.

After World War II wheat cultivars from the USSR were introduced to Czechoslovakia. They possessed specific genes for leaf rust resistance (e.g., *Lr3*), as well as for stem rust resistance (e.g., *Sr5, SrTmp*). Of special importance for the breeding at that time was the translocation 1BL.1RS with resistance genes *Lr26, Sr31, Yr9* and *Pm8*, particularly cvs Kavkaz and Aurora, as well as German lines obtained from Dr. Hoeser, Weihenstephan. Above mentioned cultivars and lines were used in the Czech wheat breeding. Another source of rust resistance was the French cv. Moisson (gene *Sr29)* or Mexican cv. Siete Cerros (gene *Sr11*). In the last period wheat breeding was influenced by recently registered West European cultivars. They contributed by several resistance genes to cultivars registered in the Czech Republic, e.g., genes on the translocation from *Aegilops ventricosa* (*Lr37, Sr38* and *Yr17*). Yellow rust resistance of older Czech cultivars was based on specific resistance genes (e.g., *Yr1, YrHeIV,*

Yr3a+Yr4a, *YrCV*) and on field resistance. After yellow rust epidemics in the sixties field resistance to this rust was required for registration of a new cultivar. Powdery mildew resistance of commercial cultivars was mostly based on *Pm2+Pm6* and *Pm4b*; many older cultivars possessed *Pm8* and *Pm5*. Effectiveness of powdery mildew resistance genes usually had short duration; genes *Pm2+Pm6* and *Pm4b* were effective longer.

Breeding for resistance to several other diseases has been based mostly on sources of foreign origin tested in the Research Institute of Crop Production, Prague - Ruzyně (RICP) or at other institutions. Cv. Arina was used in the breeding for leaf blotch resistance (*Mycosphaerella graminicola* /Fuckel/ Schröter, anamorph *Septoria tritici* Rob. ex Desm.) The same cultivar displayed good resistance to tan spot and fusarium head blight. Registered cultivars Senta and Siria showed a relatively high resistance to the glume blotch (*Phaeosphora nodorum* /E.Müller, anamorph *Staganospora nodorum* /Berk./ Cast. and Germ./) Eyespot resistance of wheat cultivars registered in the Czech Republic is mostly based on VPM 1 or Cappelle Desprez resistances.

Research

Surveys of rust and powdery mildew resistance genes in registered cultivars were published in several contributions (Bartoš et al. 1999, 2001, 2002, Huszár et al. 2001). Other sources for resistance breeding were lines developed in cooperation of breeders and researchers. E.g., resistance from *Triticum timopheevi* was transferred to hexaploid wheat at the Plant Breeding Station Úhřetice in cooperation with the RICP (Zadražil, Bartoš 1964). Genes *Lr9, Lr19* and *Lr24* were transferred from CIMMYT spring lines into lines derived from commercial cultivars (Stuchlíková 1993). From a line with *Lr19* an advanced line „Lutea“ was developed in Slovakia and tested in the State Variety Trials. Successful transfer of resistance genes was carried out from *Triticum monococcum* in the RICP (Valkoun et al. 1986 a, 1986 b). Three stem rust resistance genes, one leaf rust resistance gene and one powdery mildew resistance gene have been transferred to hexaploid wheats. One of stem rust resistance genes proved to be identical with the earlier described gene Sr35. A line possesssing the gene for powdery mildew resistance derived from *T. monococcum* was applied in the resistance breeding of the registered mildew highly resistant cv. Vlasta (Šíp et al. 1999). Cv. Vlasta has remained resistant since registration in 1999.

Research in the RICP has contributed to the determination of genes for resistance to rusts particularly in East European cultivars. Publications on „rye resistance“ (T1BL.1RS) from that workplace belonged to the first contributions on this important source of resistance (Bartoš, Bareš 1971). A review on chromosome 1R in wheat breeding was published later (Bartoš 1993). By classical genetic analysis a suppressor of the gene *Pm8* was found (Hanušová et al. 1992, 1996). In cooperation with Dr.J.Košner monosomic analyses were also applied in the studies of resistance genes. By this method location of rust resistance genes and also genes with negative effect on resistance were postulated (Košner, Bartoš 1982 a, 1982 b, 1983 a, 1983 b, 1984, 1989, 1995). A leaf rust resistance gene in cv. Siria was located on chromosome 1A and therefore the presence of *Lr10*, described on that chromosome, was postulated (Košner et al. 1998). The presence of *Lr10* in cvs Siria and Alka was verified by a molecular marker (Blažková et al. 2002). A molecular marker was also used for the determination of *Lr37* (Ambrozková et al. 2002, Bartoš et al. 2004). In cooperation with Slovak colleagues' application of molecular markers for *Lr24* and a protein marker for *Lr19* in pyramiding resistance genes was applied (Šliková et al. 2004). Sources of resistance to virus diseases are tested in field trials. Even the best of registered cultivars in tests display only a moderate resistance to BYDV. Sources of resistance from CIMMYT were used in crosses carried out for the transfer of resistance (Vacke et al. 1996, Vacke J., Cibulka R. 2000). Also two advanced lines SG-S-604/96 and SG-S-26/98 from the Plant Breeding

Station Selgen, Stupice were used as sources of resistance. An attempt to develop lines possessing common and dwarf bunt resistance was performed with resistant Swedish cvs. Tjelvar and Stava (Blažková et al.1997).

2. Wheat Aneuploid Research at the RICP

Wheat aneuploid techniques, based on the complete series of aneuploids isolated by Prof. E. Sears in the 1950´s, was started at the RICP in 1969. The work aimed at two main directions: localization of the genes on individual chromosomes by monosomic analyses, and the study of effects of some important genes using intervarietal substitution lines obtained by aneuploid techniques.

Development of monosomic series

One of the initial steps was to produce a monosomic series in a Czech spring cv. Zlatka (T. *aestivum* L. var. *lutescens,* then a popular local cultivar, originated from the cross: [Janetzkis Früh x Marquis] x Heines Koga), using crossings with the monosomic series of Chinese Spring (Sears USA via PBI Cambridge, UK, Professor C. Law), and was completed in the 1980´s.

Zlatka cultivar was subjected to a detailed cytogenetic study to reveal desynaptic disorders and translocations, based on analysis of the F_1 generation of crosses with the monosomic series of Chinese Spring. A low frequency of atypical configurations (17,9%) was revealed, particularly in the offspring of chromosomes 7A and 6A, and 2 reciprocal translocations between 5A/6D and 3D/5D chromosomes, distinct from Chinese Spring (Košner , Bareš 1979)

Monosomic analysis with Chinese Spring monosomics detected effects on quantitative traits of chromosomes 4B, 5A, 5B and 5D of Zlatka (Košner 1987).

The Zlatka monosomic series has been used in numerous monosomic analyses to locate genes of interest, mostly those coding for disease resistance as mentioned above, and in obtaining substitution lines, oriented mainly to the genes controlling flowering time in wheat:

The study of genes of growth habit, photoperiodic response and earliness per se

The delay of heading time within the series of reciprocal substitution lines for homoeologous group 5 chromosomes (*vrn* genes) between two winter wheat cultivars Mironovskaya 808 and Bezostaya 1 suggested the presence of multiple alleles at the *vrn* loci. Subsequent analysis of phenology and yield components confirmed different action of the alleles present at the individual loci *vrn-A1*, *vrn-B1* and *vrn-D1* (Košner, Pánková 1998, 2001). The frost tolerance within the set of substitution lines depended on individual chromosomes substituted (Prášil et al., 2003).

The set of substitution lines with a changed winter growth habit into a spring growth habit due to substitutions of homoeologous group 5 chromosomes carrying dominant genes *Vrn-1* to the genetic backgrounds of winter cultivars Zdar, Vala and Košutka with contrasting photoperiodic sensitivity showed that individual substitutions of 5A, 5B or 5D chromosomes affected differently the flowering time, growth stages (Pánková, Košner 2004) and yield components (Pánková, Košner unpublished), and frost tolerance of wheat (Prášil et al. 2005).

Substitutions for homoeologous group 2 chromosomes with the genes *Ppd-D1*, *Ppd-B1* and *Ppd-A1* were obtained in a winter background of cv. Zdar and in a spring background of cv. Zlatka.

Analyses on the growth habit and earliness of European wheat cultivars have been published, revealing that the spring varieties that had the similar photoperiodic response to varieties with winter habit were earlier in heading. The varieties coming from lower geographical latitudes were usually earlier and less photoperiod sensitive than those varieties coming from the higher latitudes, which could result from the selection by breeders (Košner, Žůrková 1996, Košner, Pánková 1997). Cluster analysis of heading under different sowing dates within a set of winter wheat cultivars enabled their grouping by similar profiles of vernalization and, possibly, photoperiod response (Košner, Pánková 2002 a).

Monosomic analysis of a local alternative landrace Česká Přesívka showed the presence of a gene (s) influencing earliness on chromosome 3B.The substitutions of chromosome 3B of Česká Přesívka (CP3B) into the backgrounds of spring and winter wheat cultivars changed their heading time, which was independent of vernalization but responding to some extent to photoperiod (Košner, Pánková 2002 b). The gene (s) is being mapped using recombinant substitution lines (see the Poster Session, EWAC 2005; Pánková et al.).

Molecular marker checks of substitutions revealed recently the loss of some expected substitutions and rearrangements, thus continuation of a detailed study of the flowering time genes will only be possible using the lines with confirmed chromosome substitutions.

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Pleiotropic effects of *Ppd-A1*, *Ppd-B1* and *Ppd-D1* genes in recombinant lines of common wheat in Poland

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Day length is one of the most important factors affecting the rate of wheat development. (Worland et al. 1993). Genetic control of day length insensitivity is determined by many genes present on different chromosomes. Genes *Ppd-D1* (formerly *Ppd1*), *Ppd-B1* (formerly *Ppd2*) and *Ppd-A1* (formerly *Ppd3*) localized on chromosomes of the second homoeologous

group (2D, 2B and 2A, respectively) have the major influence on this trait expression (Welsh et al. 1973; Law et al. 1978; Scarth, Law 1984; Snape et al. 2001). Moreover, genes localized on other chromosomes from the first and sixth homeologous group (Law 1998) and on 3D (Miura, Worland 1994), 4B (Halloran, Boydell 1967) also affect the date of heading.

In order to evaluate precisely the effects of genes *Ppd-A1, Ppd-B1, Ppd-D1*, the series of homozygous recombinant lines were created in John Innes Center, England (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). Particularly wide research with *Ppd-D1* lines was made. 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 recombinant lines of common wheat cv. Mercia.

Materials and methods

Recombinant lines created by the John Innes Centre in cv. Mercia with *Ppd-A1, Ppd-B1* and *Ppd-D1* genes and control lines (*ppd*) were used in this investigation. These lines were supplied by Anthony Worland. Experiment were carried out over three seasons (2001/02, 2002/03, 2003/04) at the Experimental Farm in Czeslawice. The experiment was conducted using a randomised block design. Every year kernels were sown into five-row plots 1m long using 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. Main shoot data were obtained from a random sample of ten leading tillers in each plot. Plant height, number of spikelets/spike, number of grains per ear, weight of grains in ear, 1,000 grain weight and spikelet fertility were analyzed. The results obtained were statistically analyzed individually for each year. The ANOVA programme was used, applying the F-Snedecor test.

Results and discussion

During three seasons, recombinant lines with *Ppd-D1* gene had greater accelerated ear emergence than recombinant lines with *Ppd-B1* and *Ppd-A1* genes. The recombinant lines with *Ppd-D1* had accelerated ear emergence about 5 to 4 days during the three years experiments (it depended on the year). The recombinant lines with *Ppd-A1* had accelerated ear emergence about 3 to 2 days and the recombinant lines with *Ppd-B1* had accelerated ear emergence only about one day in comparison to the control lines (Tab.1). The differences were significant between analyzed recombinant lines and their control (cultivar Mercia). Worland et al. (1998) analyzed recombinant lines of Cappelle-Desprez to which allele *Ppd-D1* was substituted from Mara and Ciano varieties. Authors proved that genes *Ppd-D1* originating from Ciano variety affected more the acceleration of the heading date, though differences were small. Many authors found the same dependence on a basis of experiments performed upon recombinant, substitution and monosomic lines, as well as varieties with genes *Ppd-D1* as compared to forms with alleles *ppd-D1* (sensitive to day length) (Worland, Law 1986; Petrović, Worland 1988; Worland et al. 1988; Worland et al. 1990; Börner et al. 1993; Kowalczyk et al 2003).

During three seasons, all recombinant lines with *Ppd* genes were shorter than their control lines. Recombinant lines containing genes *Ppd-D1* were significantly shorter as compared to the control during three years of investigation (Table 1). Height reduction was from 3.4 cm in 2002 to 4.7 cm in 2003 and 2004. No significant differences of plant height were found between studied recombinant lines with *Ppd-B1* genes and control varieties (Table 1). In 2003 recombinant lines carrying *Ppd-A1* genes were significantly shorter than their control. Börner et al. (1993) studied in Germany lines Cappelle-Desprez (Mara 2D) finding that gene

Ppd-D1 influenced plant height reduction. Miazga et al. (1995) and Kowalczyk at al. (2003) studied Polish recombinant lines with *Ppd-D1* gene in the genetic bacground of different varieties of common wheat. The authors showed that *Ppd-D1* influenced plant height reduction.

When analyzing number of spikelets of the main spike of recombinant lines in comparison to the control, significant differences were not found. Values of these traits in all lines were very similar. Recombinant lines with *Ppd-B1* genes set more kernels in the spike as compared to the control and to the lines with *Ppd-A1* and *Pp-D1* genes. No significant differences were found between analyzed recombinant lines and their control varieties in the third year of study (Table 1). Worland et al. (1998) tested lines of Cappelle-Desprez with substituted genes *Ppd-D1* from Mara and Ciano varieties in England and Germany. Authors proved that lines of Cappelle-Desprez (Mara 2D) in both countries set more kernels in spike. However, in recombinant lines Cappelle-Desprez (Ciano 2D), they found higher (in England) and lower (in Germany) values of the trait than in control forms.

Analyzed recombinant lines with *Ppd* genes were characterized with similar weight of kernels in the spike as compared to the control. In 2002 the smallest value of this trait was recorded in control lines (1.96 g) but significant differences in comparison to lines with *Ppd* genes were not recorded. In experiments performed in 2002-2004 with recombinant lines of different *Ppd* genes, it was shown that 1000-kernel weight depended mainly on year and to the lesser extent on the line. Higher values of the trait were found in 2003. Worland et al. (1998) proved unfavorable pleiotropic effects of genes *Ppd-D1* originating from Mara and Ciano varieties on value of the trait in England. In Germany, lines Cappelle-Desprez (Mara 2D) with genes *Ppd-D1* had lower, and Cappelle-Desprez (Ciano 2D) higher value of the trait than in control forms.

Recombinant lines with different *Ppd* genes had similar spikelet fertility to their control lines in all years. No significant differences were found between analyzed recombinant lines and their control varieties in the third year of study (Table 1). Lower spikelet fertility was recorded in all lines in 2002. Worland et al. (1998) analyzed pleiotropic effects of genes *Ppd-D1* in recombinant lines Cappelle-Desprez (Mara 2D) and Cappelle-Desprez (Ciano 2D). Authors proved that studied lines with genes *Ppd-D1* in England were characterized with higher spikelet fertility. In Germany, lines Cappelle-Desprez (Mara 2D) had higher, and Cappelle-Desprez (Ciano 2D) slightly lower value of that trait than control forms.

Conclusions

1. Recombinant lines of Mercia with the *Ppd-D1* gene had accelerated ear emergence more than recombinant lines with *Ppd-A1* and *Ppd-B1* genes.
2. Plants of recombinant lines with different *Ppd* genes were shorter than their control lines, but significant reduction of plant height as compared to control forms was found in recombinant lines Mercia containing gene *Ppd-D1*.
3. On a base of a six-year study it was found that the number of spikelets in spike, number and weight of kernels in spike as well as spikelet fertility depended mainly on the year. No signifficant differences for these traits were recorded between lines with *Ppd-A1, Ppd-B1* and *Ppd-D1* genes in comparison to their control.

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Table 1. Pleiotropic effects of *Ppd-A1, Ppd-B1* and *Ppd-D1* genes in 'Mercia' recombinant lines on yield components in Poland (Czeslawice 2002-2004)

Mercia recombinant lines	Ear emergence (days from May 1)			Plant height (cm)			Number of spikelets in ear			Number of grains per ear			Weight of grains per ear (g)			Fertility of spikelets			Weight of 1.000 grains (g)		
	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004	2002	2003	2004
Ppd-A1	19.1*	27.2*	30.4*	84.9	85.2*	86.8	19.8	17.2	17.4	43.6	44.7	48.3	2.02	2.24	2.33	2.20	2.60	2.77	46.5	50.0	48.2
Ppd-B1	20.7*	28.3*	31.9*	85.6	87.2	87.0	20.5	17.9	18.1	46.3	48.8	49.6	2.11	2.37	2.45	2.25	2.71	2.74	45.5	48.7	49.5
Ppd-D1	17.6*	25.5*	28.1*	84.1*	85.3*	84.6*	18.7	17.2	17.6	42.0	47.8	48.5	2.01	2.45	2.39	2.24	2.77	2.75	47.9	51.4	48,9
Control	21.8	30.4	33.0	87.4	90.0	89.3	19.6	17.1	17.2	43.8	45.9	48.1	1.96	2.44	2.38	2.20	2.68	2.78	45.6	50.3	49.4

* - significant differences between line and their control at p = 0.05

Analysis of Ukrainian wheat varieties by using diagnostic marker for *Yrns-B1*

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Winter wheat is the most important grain crop in the Ukraine. The average yield is 3.2 t/ha, with 3.65 t/ha in the steppe-forest zone and 3.1 t/ha in the steppe zones. The most frequent diseases that cause considerable loss of wheat grain yield in Ukraine are leaf rust, mildew, fusarium of head and grain or fusarium of root rot.

Yellow rust (*Puccinia striiformis* f. sp. *tritici*) is also a serious disease of wheat, that can strongly influence grain yield. This rust can affect the early stages of plant development. Major infection can result in stunted and weakened plants, leading to yield losses as high as 50% due to shrivelled grain and damaged tillers (Roelfs et al 1992).

Rust resistance can be divided into specific resistance and non-specific types. Specific resistance is usually under mono- or oligo-genetic control. It is effective at the juvenile and adult stage but rapidly overcome in the field. Non-specific adult or quantitative resistance is mostly under polygenetic control and effective at the adult stage. It is generally considered to be durable.

Studies of Khlestkina et al. (2002) revealed that microsatellite marker *Xgwm533* is suitable as a diagnostic marker for *Yrns-B1*, a gene for non-specific adult plant disease resistance against stripe rust (*Puccinia striformis*). The allele 117 bp was described to be characteristic for the resistance.

Earlier Anderson et al. (2001) have positioned a major QTL for resistance to Fusarium head blight - *Qfhs.ndsu-3BS* between two microsatellites loci *Xgwm493* and *Xgwm533*. In 2003 Spielmeyer et al. (2003) have shown that the 120 bp allele amplified by microsatellite marker *Xgwm533* is diagnostic for the presence of the *Sr2* gene in wheat germplasm from Australia, Mexico, USA, Canada, Kenya and India. The *Sr2* gene has provided durable, broad – spectrum rust resistance effective against all isolates of *Puccinia graminis* worldwide for more than 50 years. By McIntosh et al. (1995) this gene has been described as one of the most important disease resistance genes deployed in modern plant breeding.

The aim of our work was to analyze variability at locus *Xgwm533* in Ukrainian germplasm and to reveal the allele distribution at this locus among Ukrainian wheat varieties in order to understand whether there is some selection effect on the *Xgwm533* locus during the breeding process in Ukraine from 1912 until 2002 and whether there is a correlation between the alleles of *Xgwm533* and the resistance to yellow rust in Ukrainian wheats.

Materials and Methods

The wheat varieties cultivated and used in breeding programs in Ukraine in the period of 1912-2002 were chosen from collections of the Plant Breeding and Genetics Institute (Odessa), Mironovskiy Institute of Wheat, 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) Table 1.

For each variety, DNA was isolated from 20 individual seeds in accordance with Plaschke et al. (1995). Then pools of DNA from five seeds for each variety were developed and analyzed.

Primers for *Xgwm533*, PCR amplification and an analysis of PCR products were performed using ALF-express sequencer as described by Röder et al (1998). Sizes of alleles of microsatellite loci were defined by comparison with internal size standards and reference varieties (Chinese Spring, Aztec, Soisson) and calculated with Fragment Analyzer v.1.02 (Amersham Biosciences) computer program.

Table 1: The origin and year of registration of tested wheat varieties

Variety	Year of registration	Place of origin*
Krymka (HTRI7987; HTRI4503; PBGI)	1912	Khersonskaya gub., Odessa
Banatka (HTRI141; HTRI142; HTRI144)	1912	Khersonskaya gub.
Kooperatorka (HTRI5174; PBGI)	1912-1929	Odessa
Ukrainka 0246	1923	Mironovka
Zemka	1929	Odessa
Ukrainka	1929	Mironovka
Odesskaya 3	1938	Odessa
Lutescens 17	1941	Cherkasy
Eritrospermum 15	1941	Cherkasy
Hostianum 237	1929	Saratov, Russia
Lesostepka 75	1945	Bela Tserkov
Artemovka	1945	Donetsk
Odesskaya 12	1947	Odessa
Odesskaya 16	1947	Odessa
Belotserkovskaya 198	1948	Bela Tserkov
Zenitka	1952	Kharkov
Veselopodolyanskaya 499	1954	Poltava
Stepova	1959	Odessa
Bezostaya 1	1959	Krasnodar, Russia
Kavkaz	1967	Krasnodar, Russia
Avrora	1967	Krasnodar, Russia
Odesskaya 51	1968	Odessa
Priboy	1968	Odessa
Polukarlik 1	1974	Krasnodar, Russia
Ilichevka	1974	Mironovka
Kiyanka	1981	Kiev
Donskaya semi-dwarf	1985	Rostov, Russia
Odesskaya 132	1989	Odessa
Odesskaya 133	1989	Odessa
Donskaya 46	1990	Rostov, Russia
Odesskaya 162	1991	Odessa
Khersonskaya 86	1991	Kherson
Lutescens 7	1991	Podolsk,Ternopol, Kiev
Skifyanka	1993	Krasnodar, Russia
Yuna	1993	Krasnodar, Russia
Fedorovka	1994	Odessa
Mironovskaya 27	1994	Mironovka
Mironovskaya 28	1994	Mironovka
Zbruch	1994	Podolsk,Ternopol, Kiev

Table 1: Continued

Polesskaya 90	1994	Kiev
Vympel	1995	Odessa
Kievskaya ostistaya	1995	Kiev
Donchanka 3	1995	Rostov, Russia
Odesskaya 265	1996	Odessa
Odesskaya 267	1997	Odessa
Porada	1997	Odessa
Veselka	1997	Bela Tserkov
Kolomak 3	1997	Poltava
Kolomak 5	1997	Poltava
Ivanovskaya ostistaya	1997	Kiev
Mironovskaya 33	1998	Mironovka
Tsyganka	1998	Kiev
Nikoniya	1999	Odessa
Belocerkovskaya semi-dwarf	1999	Bela Tserkov
Sirena	2000	Odessa
Lelya	2000	Odessa
Prima	2000	Odessa
Zastava	2000	Odessa
Mironovskaya 65	2000	Mironovka
Ukrainka poltavskaya	2000	Poltava
Selyanka	2001	Odessa
Luzanovka odesskaya	2001	Odessa
Znaxidka	2001	Odessa
Yatran' 60	2001	Kiev
Olesya	2001	Bela Tserkov
Zustrich	2002	Odessa

*-Varieties that have been developed in Russia and used in Ukraine marked Russia, all other varieties have been developed in Ukraine

Results and Discussion

Genetic variability at locus *Xgwm533* was found in the gene pool of the investigated wheat accessions. The observed allele sizes were 98, 105, 115, 117(118), 119(120), 127, 131, 133, 135, 140, 143, 145, 160 and 163 bp of which the117 (118) bp allele had the highest frequency of 0.53. This allele, which in the literature is determined as diagnostic for *Yrns-B1* is prevailing (68.2 %) in genotypes of modern wheat varieties of the Plant Breeding and Genetics Institute (Odessa). This allele was also detected in local populations of wheat varieties Banatka and Krymka. Many wheat varieties that have been developed and used during the period 1912-1975, showed compound profiles (2-3 alleles). This is mostly due to heterogeneity of Ukrainian wheat varieties of this period.

The distribution of alleles at locus *Xgwm533* observed for Ukrainian wheats did not correspond to a normal distribution (Fig. 1). We assume that the X*gwm533* locus was under selection pressure in breeding programs in Ukraine. During 1912-1929 the allele distribution at locus *Xgwm533* was widely spread. However, at the end of the 1950s the 117 bp allele became more frequent compared to the others (Fig. 2). In addition we tested the allele distribution at other microsatellite loci on chromosome 3BS. The microsatellite analysis was

performed for *Xgwm285, Xwmc540, Xwmc231, Xwmc1, Xgwm376, Xgpw3248, Xgpw3254, Xgpw4207* (unpublished data). We have not revealed similar changes in allele distribution for these loci during the investigated period. As an example, for the locus *Xgpw3248*, allele distribution is shown in Figure 3. There are no apparent changes of allele frequencies over time.

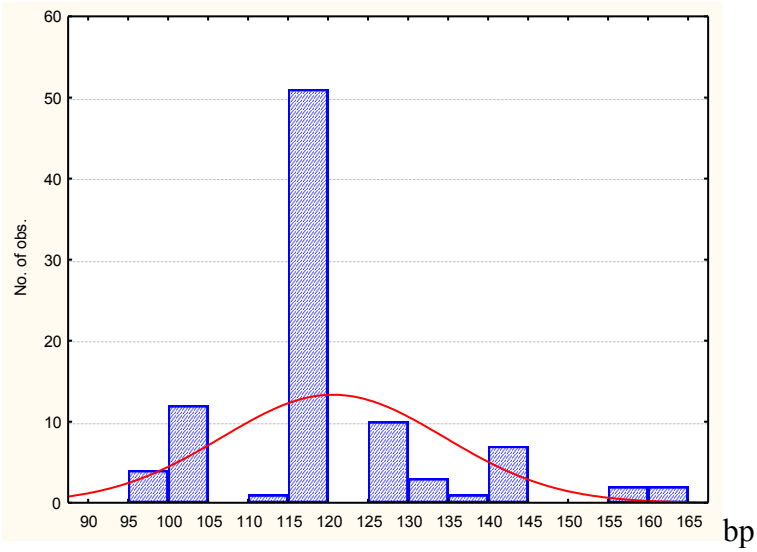

Fig. 1: Diagram of allele distribution at locus *Xgwm533* among Ukrainian wheat varieties

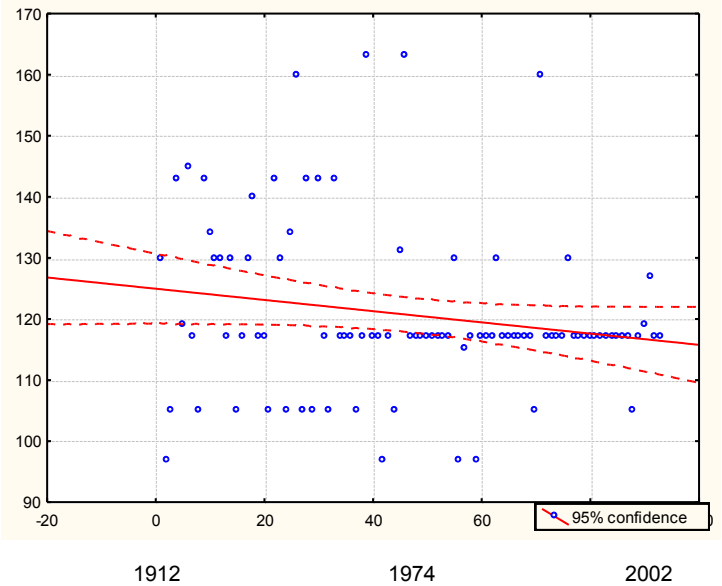

Fig. 2: Scatterplot of allele distribution at locus *Xgwm533* among Ukrainian wheat varieties during the period between 1912 and 2002

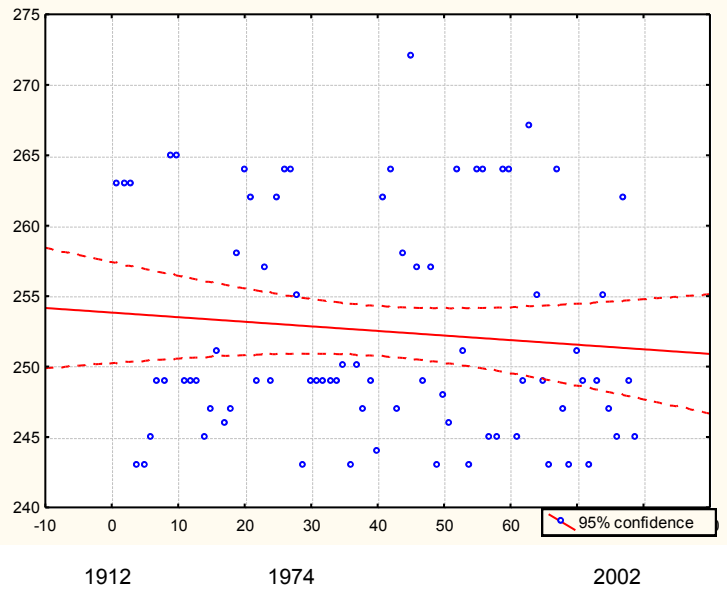

Fig. 3: Scatterplot of allele distribution at locus *Xgpw3248* among Ukrainian wheat varieties during the period between 1912 and 2002

A weak correlation between allele 117 bp and resistance to *Puccinia striiformis* was observed for some of the wheat varieties phenotyped. We will perform additional phytopathology tests.

Conclusions

We have detected changes in the allele distribution at locus *Xgwm533* among Ukrainian wheat varieties and wheat varieties that have been used in the breeding process in Ukraine at the period of 1912-2002. As a result of these changes the *Xgwm533*-117bp allele became most frequent for genotypes of modern Ukrainian wheats. We did not find any similar changes in allele frequency for a set of other microsatellites that have been located on chromosome 3BS.

There is a lot of information about linkage of the *Xgwm533* microsatellite marker with genes determining resistance to different diseases. For example, the most prominent QTL for type II resistance to Fusarium head blight is located on 3BS and was positioned between *Xgwm533* and *Xgwm493* by Anderson et al. (2001). Near the locus *Xgwm533* major QTLs determining resistance to FHB have been mapped in two populations 'Ning7840' x 'Wheaton' and 'Ning7840' x 'IL89-7978' (Zhou et al. 2003) and in a population of DH-lines received from the 'Wuhman-1' x 'Marigna' cross (Somers et al. 2003). Spielmeyer et al. (2003) found that *Xgwm533* was linked to the stem rust resistance gene *Sr2*.

According to classical genetic and molecular data genes for disease resistance frequently occur in clusters on particular chromosomes (Islam et al., 1989). Disease resistance genes are often located on a chromosome in a complex region containing different pathogen race specificities (Hartl et al., 1995; Michelmore and Meyers 1998). For example, leaf rust resistance gene *Lr26* is completely linkage to *Yr9,* the stem rust resistance gene *Sr31,* and the powdery mildew resistance gene *Pm8,* which are located on chromosome 1B (McIntosh et al., 1998).

The 3BS chromosome region where *Xgwm533* is located is carrying genes determining resistance to several diseases. This may be the reason why this region was under selection pressure in Ukrainian breeding programs.

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QTLs for salt tolerance in three different barley mapping populations

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Introduction

Soil salinity is one of the crucial factors limiting crop production. Progression of salinisation of agriculturally arable land is mainly connected with mismanagement of water in irrigation systems, in particular under arid and semiarid climate conditions and global changes of water flow in the landscape. Selection of salt tolerant genotypes is necessary to ensure yield and to reclaim salt affected soils. The development of molecular marker(s) could facilitate the selection process. Phenotyping of mapping populations under salt stress conditions and calculation of QTLs are suitable instruments to detect markers that are responsible for tolerance/sensitivity. However, a quantitative inherited trait like salt tolerance requires a range of adaptations, with a whole host of genes interacting with each other to produce the visible phenotype.

Materials and Methods

108 barley accessions from the Genebank Gatersleben (Germany) were tested at the germination stage under salt stress conditions. The plant material consists of spring barley

accessions from Pakistan (19), Tunisia (33), Libya (33), and winter barley accessions from Afghanistan (23), because these countries were known for problems with soil salinity.

Three different concentrations of sodium chloride solution (1.5%, 2.0% and 2.5%) and distilled water as a control were used to assess the salt tolerance. Ten seeds of each line and variant were tested on filter paper in plastic boxes. Seeds were transferred to climate chambers with a constant temperature of 20°C and a 12 hours light and 12 hours dark photoperiod. After ten days the plant material was scored with a modified scheme according to Mano et al. (1996) (Fig.1). In addition 92 DH lines of the Oregon-Wolfe-Barley (OWB), 72 DH lines of the Igri-Franka and 72 DH lines of the Steptoe-Morex mapping populations were tested in the same manner as described for the Genebank material, but with two replications. The scoring data were used to calculate QTLs with the QGENE programme of Nelson (1997).

Fig. 1: Scoring scheme for salt tolerance at the germination stage

Results

There are different levels regarding to salt tolerance within the Genebank accessions (Fig. 2).

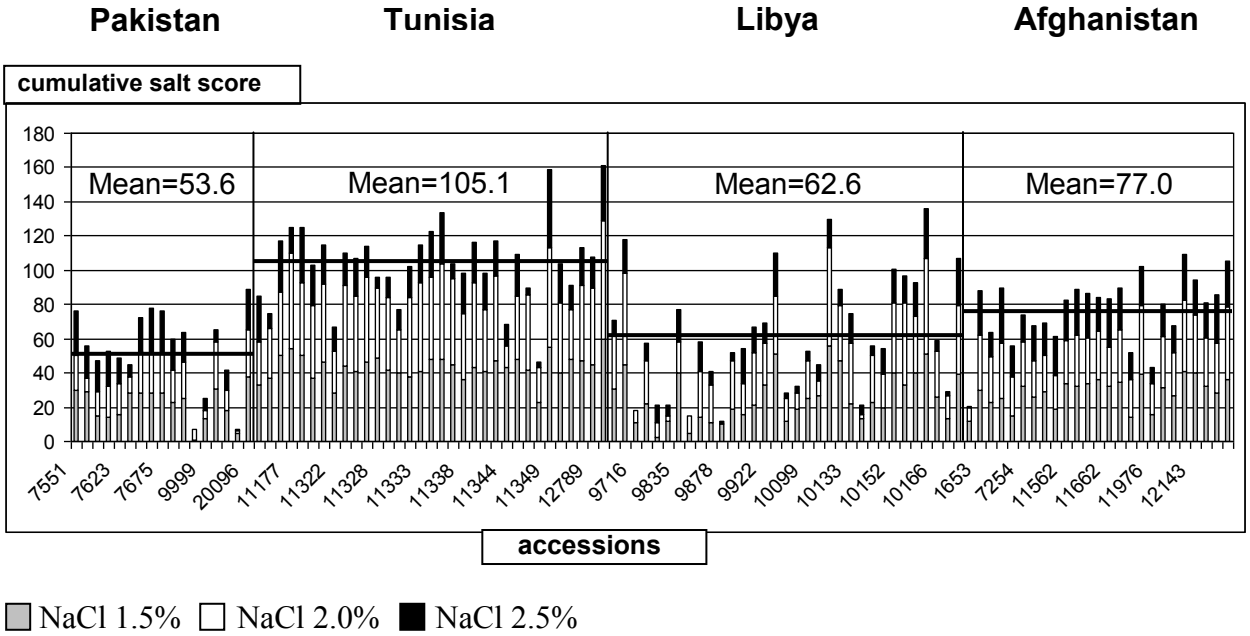


Fig. 2: Salt tolerance of barley accessions of the Gatersleben Genebank collection from four different countries at the germination stage

Barley accessions from Tunisia were characterised by a better growth under salt stress conditions than accessions from the other countries. The winter barley accessions from Afghanistan showed fewer differences in the stress response than accessions from Libya or Pakistan.

The diagrams of the salt score of the three tested mapping populations (Figure 3) present the mean of two replications. Within the OWB mapping population 24% of the lines performed better than the best parent REC and 49% of the lines were worse than the more sensitive parent DOM. No line of the Igri-Franka mapping population showed a better growth under salt stress conditions than the best parent Franka. Due to the little amount of seeds the parents of the Steptoe-Morex mapping population were not tested and therefore they are not included in the diagram. The Steptoe-Morex population showed the best growth under salt stress in comparison to the OWB and Igri-Franka mapping populations (Fig. 4).

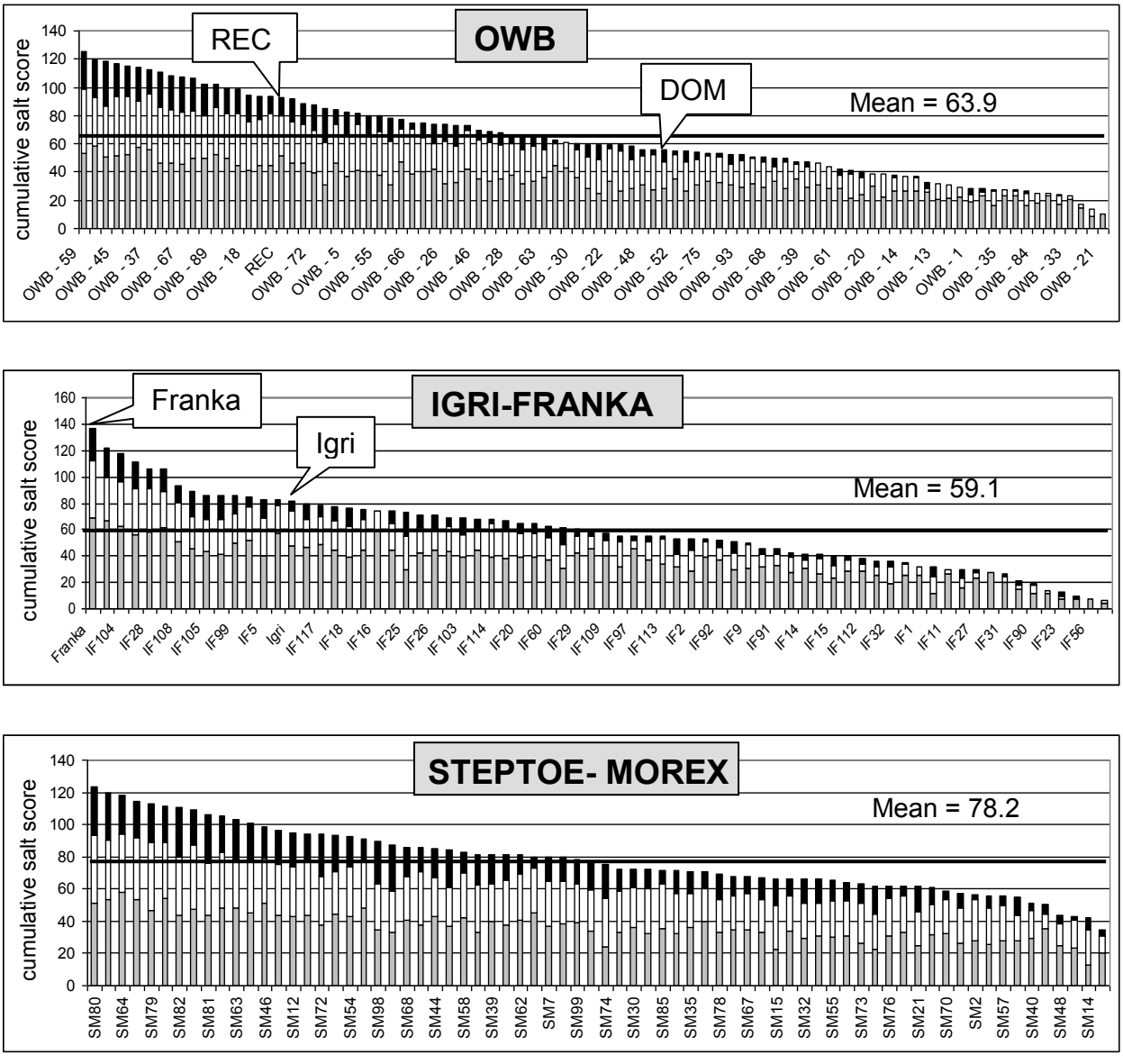
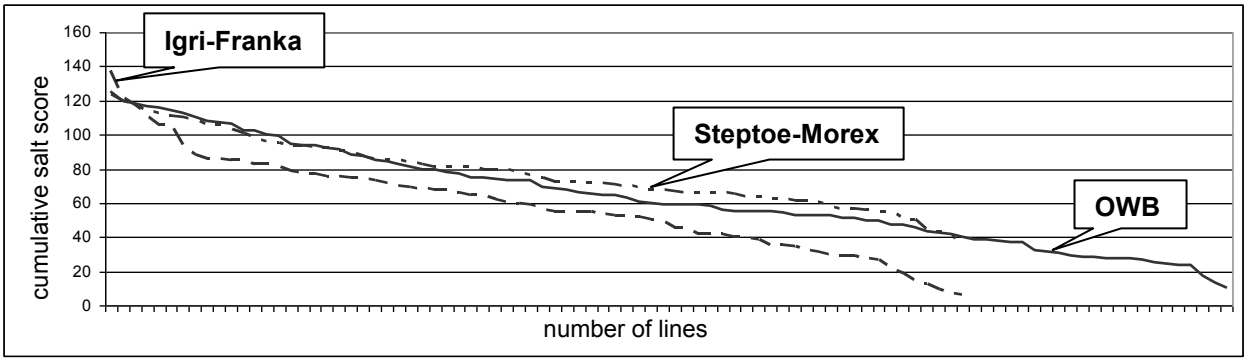


Fig. 3: Salt tolerance of OWB, Igri-Franka and Steptoe-Morex mapping populations at the germination stage



– – – –Igri-Franka, — · · — Steptoe-Morex, ——— OWB mapping population

Fig. 4: Comparison of the three different barley mapping populations with respect to salt tolerance

Main QTLs for the growth under salt stress conditions were found on linkage group 5H and 7H for the OWB, on 3H for the Igri-Franka and on 5H for the Steptoe-Morex mapping populations. Both QTLs for the OWB on 5H and 7H and the QTL for the Steptoe-Morex mapping population were located in the centromere region. The QTL for the Igri-Franka population was located on the short arm of linkage group 3H.

Discussion

Linkage group 5 of *Triticeae* possesses clusters of QTLs and major loci controlling plant adaptation to the environment (Cattivelli et al. 2002). In both spring barley mapping populations OWB and Steptoe-Morex investigated here, QTLs related to growth under salt stress conditions were found on linkage group 5H. In addition for the OWB population a main QTL on linkage group 7H was detected. In OWB the detected QTLs became more distinct with increasing salt concentration (Weidner and Börner, 2005). Mano and Takeda (1997) found the most effective QTLs for salt tolerance at different loci on chromosome 5H in two barley mapping populations. In Steptoe-Morex QTLs were located near the centromere region, which confirms our findings for the same population and in line with our results for OWB. The third mapping population investigated in the present study is based on a cross between the winter barley Igri and the spring barley Franka. The main QTL on linkage group 3H is in line with other findings of the winter barley mapping population W766 (data not published).

According to Mano and Takeda (1997) additional QTLs with minor effects for germination speed under salt stress conditions were detected on chromosomes 3H and 7H for Steptoe-Morex and Harrington-TR306, respectively. According to Munns (2002), salt and water stress tolerance during a time frame from germination to a few days into the seedling stage, depends upon hormonal regulation. Expressed by a good growth regardless of the real impact of plant stress it seems to correspond with good plant vigour during the germination stage. The theory of a special kind of plant vigour may be based on hormonal regulation is supported by another experiment about pre-harvest sprouting (Lohwasser et al. 2006, present Newsletter) investigating the OWB mapping population. The stress conditions differed from salt stress, but scoring criteria was the growth of the plantlets after seven days and the main QTL was located in the same region as for salt stress.

Good plant vigour is a prerequisite to escape from unfavourable environment conditions. Further investigations are necessary to detect the genes which protect the plants at later developmental stages against salt stress because of high salt concentrations inside the plant.

Finally it should be mentioned that all three mapping populations are saturated with expressed sequence tag (EST) based markers (Kota et al. 2001, Thiel et al. 2003, Varshney et al., unpubl. data). Besides determining the position within the genome one may detect genes with known function, controlling the traits of interest.

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Cereals with better heavy metal tolerance and nutritional value: physical and genetic mapping of copper tolerance and shoot micronutrient content in wheat

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Introduction

In small quantities, copper is an essential micronutrient for all living organisms, but in larger quantities it is toxic, inhibiting the growth and development of wheat plants (Tari *et al*., 2002). On soils polluted with heavy metals, a decline in yield can be achieved either by reducing the heavy metal content of the soil or by developing varieties tolerant to heavy metals. Tolerant plants that accumulate heavy metals in the shoots could be of significance in the cheap phytoremediation of polluted soils. Some 50 % of all foodstuffs are made from the grain yield of cereals. However, the grain of common wheat contains far below the optimum quantity of the essential elements Fe and Zn, and also little Cu and Mn (Salunkhe and Desphande, 1991). Thus, although the heavy metal content of plant parts intended for consumption must be kept at a low level in plants grown on polluted soils, on non-polluted soils it may be necessary to increase the mineral element concentration of the grain for certain

elements (Fe, Mn, Cu, Zn). The present work was thus aimed at determining chromosomes and quantitative trait loci (QTLs) involved in the copper tolerance of wheat. It was also hoped to identify loci influencing shoot Cu, Fe, Mn and Zn concentrations and to investigate ways of utilising copper-tolerant wheat in practice.

Materials and Methods

In order to identify copper-sensitive and -tolerant wheat varieties and to compare the copper tolerance of wheat and related species, tests were made on the copper tolerance of 27 genotypes belonging to the *Triticieae* subtribe. 'Chinese Spring'('Cappelle Desprez') substitution lines were used to determine the chromosomal localisation of genes responsible for copper tolerance. QTLs influencing copper tolerance were determined by analysing three mapping populations [the 'ITMI' wheat mapping population, 'Chinese Spring(Cheyenne5A)' × 'CS(*Tspelta*5A)' and 'CS' × 'CS(Cheyenne5B)'] and 'CS'–*Aegilops tauschii* chromosome 3D introgression lines. The physical mapping of genes influencing copper tolerance was achieved by analysing deletion lines for the long arm of chromosomes 5A and 5D of 'CS'.

Tests to compare the copper tolerance of wild and cultivated wheat species were carried out in hydroponics, while for the other analyses the plants were grown in soil. In the hydroponics system the plants were grown in Hoagland nutrient solution with Cu concentrations of 10^{-7} M (control) and 10^{-4} M (Cu treatment). In the soil system, no copper was added to the control, while concentrations of 1000, 1250 and 1500 mg/kg Cu were used for the treatments. After germination the plants were grown to the 2- to 3-week stage in a phytotron chamber or in a greenhouse, after which measurements were made on the root and shoot lengths and on the dry mass. A Tolerance Index (TI), consisting of the ratio of the given parameter under treated and control conditions, was used to determine the degree of tolerance. In the greenhouse varieties found in earlier experiments to be sensitive or tolerant were also grown to maturity for the determination of yield levels. Each experiment was carried out in three replications, with at least 4–6 plants per genotype in each replication.

Fluorescence induction measurements were carried out using a PAM-2000 instrument (Waltz, Effeltrich, Germany), and the F_v/F_m parameter was used to calculate the Tolerance Index. The Cu, Fe, Mn and Zn concentrations were determined by atomic absorption spectrophotometry.

The genotypes of recombinant lines for a single chromosome from the populations 'CS(Ch5A)' × 'CS(*Tspelta*5A)' and 'CS' × 'CS(Ch5B)', and 5AL and 5DL deletion lines of 'CS' were analysed using microsatellite (SSR) markers and the method of Röder (1998).

The MAPMAKER (Lander *et al*., 1987) and the JoinMap 3.0 (Van Ooijen and Voorrips, 2001) programs were used to prepare linkage maps. The first step in QTL analysis was to determine the distribution of phenotypic data, after which the linkage between individual marker alleles and a given phenotype was determined using the single marker ANOVA method. This was followed by interval mapping with the regression and maximum likelihood methods, using QTLCafe (Seaton, ed.) and MapQTL 5 software (Van Ooijen, 2003).

Results and Discussion

On the basis of Cu-induced growth inhibition or of the reduction in the fluorescence induction parameter (F_v/F_m), the most tolerant of the 27 genotypes within the *Triticieae* subtribe were the rye species, while the most sensitive were found among the diploid A genome species. No exceptionally tolerant genotypes were found among the *Aegilops* species. However, most of the hexaploid genotypes examined could be regarded as tolerant, the most tolerant of all being 'Chinese Spring', though 'Bánkúti 1201' proved to have pronounced sensitivity to the

damage caused by excess copper. In later tests, ‘Chinese Spring’ continued to be the most tolerant genotype, while ‘Bánkúti 1201’ was used as the sensitive control variety.

A soil testing system was elaborated in which solid pulverised $CuSO_4 \cdot 5\ H_2O$ was mixed into the soil. Over a three-year period, a concentration of 1250 mg/kg proved to be the most informative, as it revealed differences in tolerance between the genotypes by the age of two weeks, but plants grown to maturity were still capable of producing grain, depending on the genotype. The results obtained with the soil testing system for six genotypes exhibited a close correlation with those obtained in hydroponics experiments.

Testing of the ‘Chinese Spring’ (recipient) / ‘Cappelle Desprez’ (donor) substitution series showed that chromosomes 7D, 5A, 3D and 5D had the greatest effect on copper tolerance, while chromosomes 5B and 6B had a small but significant influence on the copper tolerance of the recipient parent. The role of homoeologous group 5 was confirmed by tests on wheat/rye substitutions. In the course of QTL and deletion analysis, a major QTLs for Cu tolerance were identified on the long arm of chromosome 5A (*Xgwm982*, LOD=3.18, Phen. var. 57,6 %) and 5D (*Xcdo412b*, LOD=3.19, Phen. var. 28.9 %), while loci with minor effects were found on chromosomes 1AL, 2DS, 4AL, 5BL and 7DS. Tests on ‘CS’–*Aegilops tauschii* introgression lines also revealed a locus influencing Cu tolerance on chromosome 3DS (nearest marker: *Xgwm161*). These results, together with those reported by other teams (Ganeva *et al*., 2003); all confirm the polygenic control of Cu tolerance.

Loci influencing the heavy metal contents in the shoot were identified in an ‘Opata85’ × ‘Synthetic’ (‘ITMI’) wheat mapping population: QTLs influencing shoot Mn and Zn concentrations were localised on chromosomes 3BL (*Xpsr903*, LOD=2.43, Phen. var. 23.6 %) and 3AL (*Xmwg30*, LOD=2.0, Phen. var. 28.5 %). Under Cu-treated conditions the Zn concentration was controlled by a locus on chromosome 7A (*Xcdo545b*, LOD=1.91, Phen. var. 23.2 %). A QTL influencing the Fe concentration of Cu-treated plants was found in the centromere region of chromosome 3B (*Xfba091a*, LOD=2.57, Phen. var. 32.0 %), while a locus influencing the Cu concentration was identified on chromosome 1BL (*Xksui27a*, LOD=3.01, Phen. var. 31.7 %). The locus found on chromosome 5AL (*Xbcd1355*, LOD=2.13, Phen. var. 25.0 %) influenced shoot Cu accumulation. The analysis of the element contents in recombinant lines of the ‘Opata85’ × ‘Synthetic’ (‘ITMI’) mapping population demonstrated that tolerant lines accumulated lower quantities of copper in their shoots from Cu-polluted soil. This indicates that Cu tolerance in wheat is based on the restriction of heavy metal uptake or of its translocation from the roots to the shoots (avoidance or exclusion strategy).

Analyses on species related to wheat and on the ‘ITMI’ wheat mapping population revealed that genotypes that accumulate large quantities of copper in their shoots have poor tolerance and low biomass yield, so they are not suitable for phytoremediation. However, tests on tolerant and sensitive wheat genotypes grown to maturity on Cu-treated soil demonstrated that Cu-tolerant wheat, unlike the sensitive genotypes, is capable of producing an acceptable yield even on soil with a copper concentration of 1500 mg/kg. This indicates that yield losses could be reduced on copper-polluted soils by growing tolerant wheat varieties.

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Cytological and microsatellite mapping of the genes determining liguleless phenotype and phenol colour reaction of kernels in durum wheat

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Double-ditelosomics (dDT; 2n=26 + 4t) in durum wheat are useful to map the genes once the chromsosome arm location is known and to identify chromosomes in other aneuploids. The ditelo-monotelo-disomics (DtMt; 2n=26 + 3t) can be also used for determining arm location and gene to centromere distances. Nearly complete sets of aneuploid stocks in the back ground of two durum wheat varieties, "Langdon" and "LD222", were available for the research. The objectives of the present study were to identify the distance from the centromere of the genes determining liguleless phenotype and phenol colour reaction of kernels using durum aneuploid stocks of homoeologous group 2 chromosomes. We also applied polymorphic microsatellite markers for molecular mapping of the gene for liguleless phenotype in durum wheat.

1. Telosomic mapping of the genes determining phenol colour reaction of kernels (*Tc1* and *Tc2*)

Polyphenol oxidases catalyze the oxidation of endogenous phenolic acids, and produce short chain polymers that decrease noodle whiteness. A major genetic effect on polyphenol oxidase activity has been reported to be located on chromosomes 2A and 2D (Zeven, 1972; Wrigley & McIntosh, 1975; Souza *et al*., 1998; Jimenez & Dubcovsky, 1999; Simeone *et al.*, 2002) in hexaploid wheat. Wrigley & McIntosh (1975) found that the gene influencing phenol colour reaction of grain was located on the long arm of chromosome 2A. In tetraploid wheat, Jimenez & Dubcovsky (1999) also found that the gene affecting polyphenol oxidase activity was located on chromosome 2A. Nair & Tomar (2001) found that the genes conferring phenol colour reaction on grain and on glumes were not linked, and at least two genes determined phenol colour reaction of grains in tetraploid wheat. However, there do not appear to have been any reports which have reported the location of the gene affecting this trait on chromosome 2B.

Double-ditelosomic (dDt) 2A (2n=26 + $2t_{2AL}$ + $2t_{2AS}$; 13" + t_{2AL}" + t_{2AS}") and dDt 2B (2n=26 + $2t_{2BL}$ + $2t_{2BS}$; 13" + t_{2BL}" + t_{2BS}") were used to estimate the genetic distance of the genes from the centromere. In the test cross progenies, Langdon// dDt 2A/ LDN-DIC DS 2A and Langdon// dDt 2B/ LDN-GB DS 2B, the chromosome numbers of plants segregated into either 2n=28 or 2n=27 + 2t (73:66 for chromosome 2A and 71:67 for chromosome 2B, respectively) and the ratios fitted the expected 1:1 ratios. Segregation for phenol colour

reaction of kernels of both populations was 68:71 for chromosome 2A and 58:70 for chromosome 2B) and also fitted the expected 1:1 ratios. However, the segregations for phenol colour reaction and chromosome number were not independent and both *Tc1* and *Tc2* showed linkages to the respective centromeres, 46.8±0.92 cM and 40.7±0.95 cM, respectively.

Ditelo-monotelosomic (DtMt) 2AL (2n=26 + $2t_{2AL}$ + t_{2AS}; 13" + t_{2AL}" + t_{2AS}'), DtMt 2AS (2n=26 + $2t_{2AS}$+ t_{2AL}; 13" + t_{2AS}" + t_{2AL}'), DtMt 2BL (2n=26 + $2t_{2BL}$+ t_{2BS}; 13" + t_{2BL}" + t_{2BS}') and DtMt 2BS (2n=27 + $2t_{2BS}$+ t_{2BL}; 13" + 1' + t_{2BS}"+ t_{2BL}') of "Langdon" (LDN) were used for chromosome arm location of the genes. The use of DtMt stocks to determine the chromosomal arm location of genes depends on identification of the progenies with aberrant segregation. The cross with the parent carrying the dominant gene for phenol colour reaction of kernels should have an excess of the dominant phenotype because the F_1 plant receives only the chromosomes with the dominant allele. For example, if *Tc1* was located on 2AL, the cross between the DtMt 2AS and LDN-DIC DS 2A would produce a monotelodisomic (13" + t1") F_1 plant with a 2A chromosome from LDN-DIC DS 2A and a chromosome arm 2AS from DtMt 2AS. This monotelodisomic plant would produce gametes (either male or female) carrying either the entire chromosome 2A having *Tc1* or the telosome chromosome 2AS which would be transmitted through the female, but not through the male gametes. Hence, all euploid (2n=28) plants will show a strong phenol colour reaction of kernels. In the F_2 of the crosses DtMt 2AS/ LDN-DIC DS 2A and DtMt 2BS/ LDN-GB DS 2B, all euploid (2n=28) F_2 plants had strong phenol colour reactions. Therefore *Tc1* and *Tc2* are located on 2AL and 2BL, respectively. The cross between the DtMt 2AL and LDN-DIC DS 2A should produce a dimonotelosomic (13" + t1") F_1 plant with a 2A chromosome from LDN-DIC DS 2A and a chromosome arm 2AL from the DtMt 2AL. As *Tc1* is located on 2AL, in the monotelsodisomic F_1 plant, the gametes with the entire chromosome 2A will have either *Tc1* or *tc1*, in a ratio depending on the recombination frequency. The gametes with chromosome 2AL will have either *Tc1* or *tc1*, also depending on recombination frequency. The ratio *Tc1*: *tc1* will be (1-p):p, where p is recombination frequency. The telosome will be transmitted through the female gamete, but not through the male gamete. Hence, phenol colour reaction of kernels of all euploid (2n=28) plants should segregate as $(1-p^2):p^2$. Euploid (2n=28) plants from F_2 populations of DtMt 2AL/ LDN-DIC DS 2A and DtMt 2BL/ LDN-GB DS 2B segregated in the ratio, 143:24 and 99:14, respectively. These fitted in the ratios of $(1-p^2):p^2$. for These findings also support the conclusion that *Tc1* and *Tc2* are located on chromosomes 2AL and 2BL, respectively. The present results corroborate the findings of Jimenez & Dubcovsky (1999) and Souza *et al.* (1998) showing that polyphenol oxidase activity was conferred by genes located distally on the long arms of homoeologous group 2 chromosomes.

2. Telosomic and microsatellite mapping of the gene determining liguleless phenotype (*lg1*)

The durum wheat leaf consists of three parts: the lamina, the sheath, and the ligular region. Single plants, which lack ligules and auricles on all leaves, were occasionally found in bulk populations of Cyprus durum wheat accessions and were easily recognized at every stage of plant growth. The liguleless variants have upright leaf laminas that wrap around the culm. The liguleless character may influence light interception by the canopy, and may reduce light capture by the leaves, so adversely affecting growth and grain yield, though this does not appear to have been assessed. It is known that the liguleless character is controlled by complementary recessive genes (lg_1 on chromosome 2B and lg_2 on chromosome 2D) in hexaploid wheat (McIntosh & Baker, 1968) and by a single recessive gene in tetraploid wheat (Ausemus *et al.*, 1946). Bagnara & Rossi (1972) induced a liguleless mutation in durum wheat and found that it was controlled by a single recessive gene. However, the genes for liguleless phenotype in *Triticum aestivum* have not been mapped, because complementary recessive genes control the liguleless phenotype in this species.

The F_2 populations of liguleless k17769/*Triticum dicoccoides* and k17769/*T. dicoccum* segregated in the ratio, 15 liguled: 1 liguleless. However, the F_2 population of k17769/*T. durum* and k17769/*T. turgidum* segregated in a 3:1 ratio. F_2 hybrids of k17769/LDN-DIC DS 2A segregated in 15:1 ratio and F_2 hybrids of k17769/LDN-GB DS 2B segregated in 3:1 ratios. This indicated that the gene, lg_3 was located on chromosome 2A. The supposed genotypes are Lg_1Lg_1 Lg_3Lg_3 for *T. dicoccoides* and *T. dicocuum*, Lg_1Lg_1 lg_3 lg_3 for *T. durum* and *T. turgidum*, and lg_1 lg_1 lg_3 lg_3 for the liguleless line, k17769. To map the genes for the liguleless phenotype in tetraploid wheat, a double-ditelosomic (dDt) 2B was crossed as male to k17784. The F_1 plants (13"+ t1t"') as male were crossed to a liguleless line, k17784. B_1F_1 plants of k17784// k17784/dDt 2B were grown and classified for chromosome number and liguleless phenotype. The segregations for chromosome numbers of plants (79:86) and ligulelessness (89:76) fitted in 1:1 ratios. lg_1 was distally located on chromosome 2B and linked to the centromere. The map distance was estimated as 60.4±0.9 cM.

As the lg_1 gene was recessive, all euploid (2n=28) plants in the F_2 of DtMt 2BL/k17784 is expected to segregate into $[1- (1-p)^2]$ liglue: $(1-p)^2$ liguleless. The ratio of $[1- (1-p)^2] : (1-p)^2$ is difficult to statistically distinguish from the ratio of 3 ligluled: 1 ligluleless, when the gene located on the distal of the arm. Hence, we applied polymorphic microsatellite markers for molecular mapping of the gene for liguleless phenotype. Of 88 BC_1 plants from k17784//k17784/LDN-GB DS 2B, 41 individuals had a ligule (Lg_1lg_1) and 47 were liguleless (lg_1lg_1). The segregation ratio of liguleless phenotype was 1:1 ($\chi^2 = 0.409$; $0.5<p<0.75$). For phenol colour reaction of the kernel, 40 individuals had a strong reaction (Tc_2tc_2) and 48 individuals had a weak reaction (tc_2tc_2) ($\chi^2 = 0.7273$; $0.25<p<0.5$). The segregation ratio of phenol colour reaction was also 1:1. Twenty microsatellite markers, mapped on long arm of chromosome 2B (Röder *et al.*, 1998 and unpublished mapping data) were used to identify polymorphism between k17784 and LDN-GB DS 2B. Five polymorphic markers, which detect single loci, were used to map lg_1 and Tc_2. The segregation ratios of the microsatellite markers confirmed the expected 1:1 ratios. Map distances among loci were calculated using Map Manager QTX ($p<0.01$). The genetic map for the locations of lg_1 and Tc_2 is shown in Fig. 2. *Xgwm382* is distally located in long arm of chromosome 2B (Röder *et al.* 1998). The genes lg_1 and Tc_2 were linked with and distal to the marker *Xgwm619,* which was integrated into a genetic linkage map of durum wheat as a marker distally located in long arm of chromosome 2B (Korzun *et al.*, 1999). The gene lg_1 was 9.1 cM distal to Tc_2. Thus the order of the loci from the centromere was *Xgwm382*, *Xgwm619*, Tc_2 and lg_1. Except for *al* of rye (Korzun *et al.,* 1997), the distal location of lg_1 shown in this study was in accord with the findings for *lg* of rice (Causse *et al.*, 1994), for lg_1 of maize (Ahn & Tanksley, 1993), for *li* of barley (Pratchett & Laurie, 1994), and for *lg-1* of sorghum (Zwick *et al.*, 1998).

3. Aneuploid stock of durum "LD222" with liguleless phenotype

Two recessive genes principally control the liguleless character in tetraploid wheat. The F_2 progenies of k17769 (liguleless mutant)/*Triticum dicoccoides* and k17769/*T. dicoccum* segregated in a 15:1 ratio, whereas the F_2 progenies of k17769/*T. durum* and k17769/*T. turgidum* segregated in a 3:1 ratio. Segregation of F_2 progenies between k17769 and chromosome substitution lines for homoeologous group 2 chromosomes of "Langdon" suggested that the liguleless genotype had occurred by mutation at the lg_3 locus on chromosome 2A, and then by mutation at the lg_1 locus on chromosome 2B, in the process of domestication of tetraploid wheat. The supposed genotype of *T. durum* is Lg_1Lg_1 lg_3 lg_3. A genetic stock having the genotype, lg_1lg_1 Lg_3Lg_3, is currently not available to locate lg_3 and to assess the linkage of Tc_1 - lg_3 on chromosome 2A. To locate lg_3, we developed ANW 12A (LD222*7/k17769), a near-isogenic line of durum "LD222" with ligulelees phenotype. ANW 12A was crossed to dDT 2A of "LD222". Liguleless dDT 2A plants (lg_1lg_1 lg_3lg_3) were

isolated from F_2 plants. DtMt 2AL (lg_1lg_1 lg_3lg_3) and DtMt 2AS (lg_1lg_1 lg_3) will be isolated from the crosses, DtMt 2AL/ANW 12A and DtMt 2AS/ANW 12A, respectively.

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Detection of QTLs for grain protein content by backcross inbred lines in durum wheat

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The lack of sufficient genetic variation for useful traits within the cultivated wheats has limited the ability of plant breeders to improve grain yield and grain quality. A good source of genetic variation has been found in wild wheats and related species. The wild tetraploid wheat *Triticum turgidum* ssp. *dicoccoides* shows particular promise as a donor of useful genetic

variation for several traits, including disease resistance, drought tolerance, grain protein quality and quality. However, wild germplasm contains both favourable traits and many commercially unacceptable characters. Backcrossing has been extensively used in self-pollinating crops to transfer simply inherited characters to cultivars which are deficient only in the characters being transferred. The application of backcrossing to the improvement of quantitative characters has been limited mainly because of the low heritability of these characters and the difficulty of transferring, simultaneously, relatively large number of genes. In contrast to more traditional procedures of wheat breeding, the backcross inbred line method, first described by Wehrhahn and Allard (1965), produces backcross inbred lines (BILs) that can be tested in replicated trials over environments prior of selection. The development of molecular markers and molecular maps, and the recent strategies for molecular breeding, referred to as advanced backcross QTL (AB-QTL) analysis (Tanksley and Nelson, 1996), have shown that the backcrossing method has considerable potential for the genetic analyses of quantitative traits and as a method of breeding higher yielding and quality crop varieties.

This study discusses the introgression of high grain protein content alleles from ssp. *dicoccoides* into more adapted and agronomically acceptable durum wheat germplasm by means of the BIL method, and the identification of molecular markers to be used in marker-assisted selection.

1. The durum wheat – var. *dicoccoides* backcross inbred lines

A population of 92 BILs was developed using a procedure similar to that of Wehrhahn and Allard (1965). The semi-dwarf and high yielding cultivar Latino of durum wheat was used as a recurrent parent, and the accession MG29896 of *Triticum turgidum* var. *dicoccoides* was used as donor parent because of its high grain protein content and the acceptable seed size. Three successive backcrosses were made to cv. Latino followed by four generation of self-pollination. After the first backcross, 120 random BC_1 plants were chosen to initiate a backcross program. No selection was applied in any generation, and plants were chosen randomly for additional backcrossing or selfing. However, some plants were lost in the subsequent generations and a total of 92 BC_3F_5 lines were obtained and multiplied to have sufficient seeds for use in replicated trials.

On the basis of phenotype evaluation a subset of 28 BILs was selected for molecular marker characterisation. One hundred and twelve microsatellites, 7 biochemical and 5 morphological markers were used to reveal *dicoccoides* chromosome segments retained into the 28 BILs (Gadaleta et al. 2003). The percentage of *dicoccoides* genome introgressed into Latino ranged between 0.9% and 26.3% with an average of 6.3%. This value is very close to the expected one (6,25%), thus indicating that the *dicoccoides* genome could be easily introgressed in the cultivated genome background. The number of introgressed *dicoccoides* segments ranged between 1 and 14 and the number of terminal segments was similar to interstitial ones. The lines containing the lowest number of *dicoccoides* fragments are particularly appropriate to produce, by a low number of additional backcrosses, a series of near-isogenic lines (NILs) having the complete *dicoccoides* genome distributed as single fragments.

2. Variation for grain protein content and grain yield components in the BILs

The complete series of backcross inbred lines and the recurrent parent cv. Latino were evaluated for grain quality (protein content, gliadin and glutenin components), grain yield components (grain yield per area, 1000-grain weight, test weight, heading date, plant height) and for diseases resistance (powdery mildew) in replicated trials, with plants grown under normal field conditions, in southern Italy at two locations over two years (Blanco et al.

2003).Variation among backcross inbred lines was observed for all traits. Significant line x environment interactions suggested that genotype x environment effects may be important for every trait. The performance of the BILs was variable across environments. Since locations differ in environmental conditions, it is likely the some QTL-alleles interact with the environment resulting in the variable performance of the BILs across environments. Since a significant negative correlation was observed between protein content and grain yield, precautions were necessary to avoid reduced grain yield when protein percentage was increased by selection. Six superior BILs were selected for increased protein content, while retaining the desirable agronomic characteristics of the recurrent parent and without other undesirable characteristics of the donor parent, such as fragile spikes, hard glumes, late maturity, tallness.

3. Detection of QTLs for grain protein content (GPC)

After 3 backcross generations to the recurrent parent, the BIL population will consist of near-isogenic lines which are identical each other and to the recurrent parent except for single or a few small donor segments. Markers located outside a segment with a target gene will have an identical pattern between the backcross lines and the recurrent parent, while markers located inside the segment may have one or more polymorphisms. Some markers, however, may show an identical amplification pattern in the backcross lines and the donor parent. These markers are not associated to QTLs and then they are "false positives", that is they detect introgressed segments not involved in the expression of protein content loci. Indeed, the marker analysis on the six selected high GPC lines detected the introgression of several segments of the var. *dicoccoides* located on 12 different chromosomes and no clear evidence of marker-QTL association was obtained. One of the high protein content line (3BIL-85) was then backcrossed to Latino and the generations F_2, F_3 and F_4 were evaluated for protein content and grain yield per spike in four different field trials. On the basis of the F_3 data, 10 high and 10 low grain protein content progenies with similar grain yield per spike were selected for making two DNA bulks of extreme phenotypes to be screened according to the bulked segregant analysis (Michelmore et al. 1991). 25 primer pairs of microsatellite markers and 10 primer combinations of AFLP markers were used to amplify bulked and parental DNA simultaneously. A total of six polymorphic markers between the parents was also found to be polymorphic in the two bulks. Such markers were considered potentially linked to QTL for protein content and then screened on the complete population of 144 F_3 progenies. The putative linkage of these markers with protein content QTLs was confirmed by regression analysis of each marker locus on the F_2, F_3 and F_4 populations. Three QTLs with major effects on GPC were detected on chromosome arms 2AS, 6AS and 7BL, identified by the markers *Xcfa2164*, *XP39M37*$_{(250)}$ and *Xgwm577*, respectively (Blanco et al. 2005). The phenotypic effect of each QTL was variable across locations, thus indicating that individual QTLs seem to be sensitive to environmental factors.

Multiple regression analysis indicated that the three QTLs explained all the genetic variance of the trait. The high GPC parental line 3BIL-85 was not significantly different from the recurrent parent Latino for grain yield per spike (GYS), but the phenotypic correlation coefficient between GPC and GYS had negative values (from -0.02 to -0.28) in each trial, although it was statistically significant only in the F_3 progeny trial. No co-located QTL for GYS was detected, thus excluding the hypothesis that the putative QTLs for GPC were indirect QTLs for low grain yield. The negative protein-yield response could be due to: a) co-location of grain yield per spike QTLs with reduced phenotypic effects not detectable by the experimental design or statistical procedures, or to b) opposite pleiotropic gene effects due to the major bio-energetic requirements for synthesis of protein then carbohydrates.

4. Conclusions

The relatively low heritability of quantitative traits makes it difficult to select for useful alleles from wild germplasm based on phenotypic evaluation of single plants and/or in single environments. The production of backcrossing lines is a laborious and sometimes expensive procedure, but the availability of BILs for quantitative traits facilitates identification of the genes involved in the inheritance of continuous variation, as BILs can be evaluated in replicated trials to identify those which deviate significantly from the recurrent parent. The BIL population should consist of highly homozygous lines genotypically identical to the recurrent parent (93.75%) and having a mean of 6.25% of genome donor segments. Significant variation among BILs for protein content, grain yield and related traits were observed in field plot trials over four environments.

A total of nine BILs had significantly higher protein content than the recurrent parent cv. Latino, some of them associated to poor grain yield. Six superior BILs were selected for increased protein content, while retaining the desirable agronomic characteristics of the recurrent parent and without other undesirable characteristics of the donor parent.

In conclusion, genes for increased grain protein content were transferred to a desirable agronomic background and suggested that improvement for protein percentage, a quantitatively inherited trait, could be attained using the BIL method. To facilitate marker-assisted selection for high grain protein content without significant losses in grain yield, each BIL was analysed with microsatellite and AFLP markers and six markers were found to be associated with three different protein content QTLs.

Mapping loci by backcross inbred lines should enable the obtention of near-isogenic lines (NILs) in which the individual effects of each QTL can be examined in detail without confounding variation due to other putative QTLs. These near-isogenic lines could also be useful to conduct physiological studies aimed at investigating the potential mechanisms leading to high protein content, to fine-map the position of each QTL, and eventually to map-based cloning.

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Diversity of HMW-Glutenins in European landraces and obsolete cultivars and its geographical distribution

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Many modern cultivars, in wheat and in other crops as well, are often rather similar and have relatively narrow genetic base. Landraces, which have arisen through a combination of a natural selection and selection performed by farmers (Belay et. al., 1995) have some valuable characters which can contribute to present breeding (Keller et. al., 1991). Landraces and obsolete cultivars represent very valuable part of the genepool (Zou, Yang, 1995, Vojdani et. al., 1993) because they comprise most of the intra- specific genetic diversity of crops and can be utilized as a source of some valuable characters, e.g. protein content and quality or winterhardiness (Dotlacil et. al., 2000).

Beside comparative studies of morphological and agronomic characters, diversity of cultivars can be estimated by means of biochemical techniques and DNA analyses and consequent data analyses (Zeven, van Hintum, 1992; Zeven, Schachl, 1989). Genetic diversity of landraces and old cultivars can be caused, beside breeding, also by their origin (climate, geographical and other conditions of the regions of origin)-van Hintum and Elings, 1991. Wang and Guo (1992) found similarity in gluten alleles in accordance with geographical origin. Ehdaie and Waines (1989) estimated significant genetic variability in grain weight per spike and in productive tillering.

Grain quality in wheat is determined mainly by content and quality of storage proteins in kernels. These proteins are represented by monomeric gliadins and polymeric glutenins which comprise several types of subunits and are usually divided as HMW (High Molecular Weight) and LMW (Low Molecular Weight) Glutenin subunits (GS_S); HMW-GSs genes are localized at the *Glu –1* loci on long arms of 1A, 1B and 1D chromosomes (Shewry and Tatham, 1997). Their allelic variants are associated with differences in grain quality (Payne, 1987, Manley et al., 1992, Weegels et al., 1996). Due to this specific nature, HMW-GS_S genes can be utilized as markers of some important characters as well as for characterization of cultivars and lines in wheat (Cerný and Sasek, 1996; Sasek et al., 1995; Kraic et al., 1995).

Material and Methods

Two sets of winter wheat landraces and obsolete cultivars (122 in set I. and 101 in set II.) originating from European countries (see Table 1) with modern cultivars as a check (Sparta, Samanta and Ilona in set I.; Samanta and Sarka in set II.) were studied in three- years field experiments (set I. 1995-98, set II. 1998-2001). Trials were sown on micro-plots (1,5 m²) in Praha - Ruzyně using standard growing practices when omitting application of growth regulators and fertilizers during vegetative growth. Earliness and plant height were recorded in field conditions. At maturity, 30 stems with spikes were taken from each plot to analyze spike morphology, productivity and harvest index. Also crude protein content in seeds was analyzed using Kjeltec Auto System II (method by Kjeldahl).

Simultaneously, HMW- Glutenin patterns in experimental set I. were characterized in 100 individual halved grains by means of standard SDS- PAGE technique for wheat (Kraic et al., 1995). We evaluated the glutenin patterns by means of densitometry (Image Master DTS) and

implemented the classification by Payne and Lawrence (1983) for identification of HMW *Glu*- alleles.

The 3- years´ field trials were analyzed by means of statistical software UNISTAT.

Results and Discussion

It is obvious that climatic and soil conditions of regions from which cultivars originate can influence their characters. This is especially expressed in landraces, where environmental conditions had a significant impact on their development (Belay et. al., 1995). Therefore, simple classification of cultivars according to the country of origin has been used to estimate the impact of geographic origin on selected characters. Cultivars from the territory of former Czechoslovakia are classified as one group (Czech and Slovak cultivars), as well as cultivars from the territory of former Soviet Union (now Russia and Ukraine). In addition to the two above mentioned groups, cultivars from other 11 European countries were studied and; results are summarized in Table 1.

Early heading and maturity were found especially in cultivars from Hungary and Bulgaria, on the contrary the late heading and maturity were characteristic for cultivars originating from West and North Europe (especially from the Netherlands, Great Britain, Denmark, Germany and Sweden). Plant height and spike length differed between particular groups; however, it was difficult to find some relations to the geographic origin of cultivars. Hardly any conclusions can be done for the harvest index, as well, due to the high heterogeneity of cultivars and differences between both evaluated sets. As concerns the spike productivity, relatively low grain weight per spike has been found in cultivars from France, Switzerland and Austria (also due to the higher share of old landraces). On the contrary cultivars from Great Britain, Germany, Sweden, Poland and Denmark seem to have higher grain weight per spike. Higher number of grains per spikelet was found in cultivars originating from Great Britain (1.90), Denmark (1.76 and 1.93 respectively) and from some other West-European countries. As concerns grain weight and crude protein content, it was not possible to find some differences based on the geographic origin.

Clustering according to the country of origin thus provided only very rough characterization in a few characters. It seems that within the evaluated landraces and cultivars two groups with some specific characters responding to warmer arid and colder humid climates can be distinguished :

1. Cultivars from (South)- East Europe (Bulgaria, Hungary, partially also Russia and Ukraine), where earliness and lower spike productivity are characteristic.
2. Cultivars from (North)- West Europe (Great Britain, Sweden, Denmark, the Netherlands and partially other countries), with higher spike productivity and a bit longer vegetation.

The results proved that donors of valuable characters can be found among landraces and obsolete cultivars. Inspite of the negative relations between spike productvity and protein content, some high-protein cultivars with acceptable level of spike productivity (and/or earlines) could be found in the both sets (Dotlacil et al., 2000).

Another attempt to characterize genetic diversity of cultivars of different origin has been made by using *Glu*-subunits heterogeneity. Among 122 cultivars studied within the set I., two different gluten subunits were found on chromosome 1A (or the active allele was missing), 10 different *Glu*-subunits and/or their combinations on 1B chromosome and 3 different allelic combinations on 1D chromosome. Incidence of particular HMW *Glu*- alleles and their combinations is shown in Table 2. Within 224 identified *Glu*- lines the allele 1 was the most frequent one at the locus 1A (48.7%), nevertheless, the absence of HMW- *Glu* -subunit (0) in 1A locus was also very common (45,5 %). Relatively rare was allele 2*, which has been

found in 6.7% examined cultivars. Similarly, only three different allelic combinations were identified at the locus 1D, among them alleles 2+ 12 were most common (63% lines) followed by 5+10 combination with a much lower frequency (33%). Allelic combination 3+12 was observed only in 5 cultivars (3.6%). Similar results for 1A and 1D chromosomes were referred by Sasek et al., (1995) and Gregova et al., (1999). Much more diversity has been recorded in the 1B locus where 10 different alleles and /or their combinations could be identified, among them alleles 7+9 (39.7% *Glu*- lines), 7+8 (22.3 % *Glu*- lines), 6+8 (16.1 % *Glu*- lines) and allele 20 (present in 12.1 % G*lu*- lines) can be considered as common and broadly spread. Alleles 8, 6, 9, 7 and allelic combinations 13+16 and 17+ 18 were rare.

In the subsequent step of evaluation of cultivars only using composition of *Glu*- alleles in principal *Glu* – lines has been applied. In this way each culticar was represented by only one predominant *Glu*-lines and the number of analyzed lines has been reduced to 122. However, this reduction did not lead to the elimination of rare alleles within the analyzed set and also relative frequencies of all alleles in the both files (n_1 = 122; n_2 = 224) were in good conformity. This conformity has been tested by $\chi 2$ test and approved as significant at the level P= 0.87 for chromosome 1A, P= 0.99 for 1B and P= 0.93 for 1D.

Table 1: Mean values of selected characters in groups of cultivars assessed by the country of origin (* $P < 0.05$)

Country of origin	Number of acc. in exp. sets		Plant height (cm)		Spike length (cm)		Grain weight per spike (g)		Thousand grain weight (g)		Number of grains per spikelet		Harvest index	
	Set I	set II	Set I	Set II	Set I	Set II	Set I	Set II	Set I	Set II	set I	Set II	Set I	Set II
Czech/Slovak	19	2	108	85	9.1	8.8	1.35	1.95	41.1	47.9	1.80	2.52	0.42	0.51
Austria	9	9	118	120	9.5	8.0	1.27	1.30	40.6	42.3	1.73	1.84	0.39	0.40
Bulgaria	3	5	111	119	9.5	9.0	0.95	1,55	41.5	45,3	1.46	1.88	0.40	0.40
Switzerland	10	5	112	124	9.3	8.3	1.16	1.24	37.3	43.9	1.72	1.63	0.38	0.35
Denmark	8	3	114	113	9.0	8.5	1.25	1.53	36.3	39.8	1.76	1.93	0.37	0.40
France	13	16	113	121	9.4	9.0	1.11	1.38	40.7	45.2	1.57	1.86	0.36	0.40
Great Britain	5	-	113	-	7.8	-	1.48	-	38.5	-	1.90	-	0.39	-
Germany	16	31	116	120	8.9	8.5	1.29	1.64	40.1	45.1	1.70	1.91	0.39	0.40
Hungary	10	-	113	-	8.6	-	1.04	-	39.6	-	1.73	-	0.40	-
Netherlands	3	7	115	111	9.0	7.3	1.11	1.51	36.7	43.6	1.64	2.01	0.35	0.39
Poland	5	6	120	121	9.7	8.3	1.47	1.46	41.7	43.6	1.82	1.86	0.39	0.39
Russia/Ukr.	17	5	115	122	9.2	8.8	1.13	1.46	39.2	46.5	1.63	1.92	0.39	0.42
Sweden	5	5	116	108	8.6	7.8	1.34	1.62	38.6	42.1	1.72	1.89	0.38	0.40
Other	-	7	-	113	-	8.6	-	1.34	-	44.4	-	1.86	-	0.41
F-test			2.5*	7.6*	2.8*	2.8*	6.4*	3.1*	2.6*	3.1*	3.5*	2.5*	4.6*	3.2*
LSD (Sheffe. 95%)			5	4	0.4	0.4	0.07	0.09	2.1	2.2	0.18	0.19	0.01	0.02

Table 2: The frequency of HMW - *Glu* alleles identified in the set of 122 landraces and old winter wheat cultivars; n_1 = 122 cultivars (dominant *Glu*- lines within cultivars) and n_2 = 224 Glu-lines with presence over 5 % i cultivar

1A					1B					1D				
	n_1	%	n_2	%		n_1	%	n_2	%		n_1	%	n_2	%
0	57	47.2	100	44.6	7+9	45	36.6	88	39.7	2+12	79	64.2	142	63
1	58	46.3	109	48.7	7+8	28	22.7	51	22.3	5+10	39	31.7	74	33
2*	8	6.5	15	6.7	6+8	21	17.1	36	16.1	3+12	5	4.1	8	3.6
					20	16	13	27	12.1					
					17+18	3	2.4	5	2.2					
					13+16	3	2.4	4	1.8					
					7	3	2.4	7	3.1					
					9	2	1.6	3	1.3					
					8	1	0.8	1	0.4					
					6	1	0.8	2	0.9					

Table 3: Geographic distribution of HMW-Glu alleles in identified *HMW-Glu*- lines (224) derived from 122 winter wheat landraces and obsolete cultivars

		1 A			1 B									1 D		
Country of origin	Number of lines	0	1	2	7+8	6+8	7+9	17+18	13+16	7	8	9	6	20	2+12	3+12	5+10
AUT	16	5	10	1	8	1	6	0	1	0	0	0	0	0	12	0	4
BGR	11	4	6	1	1	0	9	0	1	0	0	0	0	0	4	0	7
CHE	13	6	7	0	5	3	0	0	2	0	0	0	0	3	10	2	1
CZE	29	16	11	2	4	1	21	1	0	0	0	0	0	2	14	3	12
DNK	16	9	6	1	0	5	6	0	0	0	0	0	0	5	12	0	4
EST	3	0	3	0	0	0	3	0	0	0	0	0	0	0	0	0	3
FRA	21	10	10	1	6	5	3	0	0	2	0	0	1	4	18	0	3
GBR	6	5	1	0	0	1	0	0	0	0	0	0	0	5	6	0	0
GEO	5	1	4	0	0	0	4	0	0	1	0	0	0	0	2	1	2
GER	23	14	9	0	8	6	4	0	0	0	0	0	0	5	18	0	5
HUN	22	10	7	5	4	0	15	2	0	0	1	0	0	0	10	2	10
NLD	3	0	3	0	1	2	0	0	0	0	0	0	0	0	2	0	1
POL	13	4	9	0	2	4	4	2	0	1	0	0	0	0	8	0	5
RUS	6	1	4	1	1	0	3	0	0	1	0	0	1	0	2	0	4
SWE	15	10	5	0	3	6	1	0	0	0	0	2	0	3	13	0	2
UKR	20	3	14	3	8	0	9	0	0	2	0	1	0	0	9	0	11

Geographical distribution of HMW *Glu*-alleles (Tab. 3) implies that most alleles with frequent incidence (as alleles 1 at 1A; 7+9, 7+8 and 6+8 at 1B; 2+12 and 5+10 at 1D) were also widely spread geographically. On the contrary, absence of some such alleles could be considered as specific for several countries (e.g. absence of *Glu*-alleles 7+8 at 1B in cultivars from Denmark, Estonia, Great Britain, Georgia) or absence of *Glu*-alleles 6+8 at the same locus in cultivars from Russia, Ukraine, Hungary, Georgia, Estonia and Bulgaria). Absence of *Glu*-alleles 7+9 at 1B was characteristic for cultivars coming from the Netherlands, Great Britain and Switzerland. Rather specific geographic origin was found by relatively frequent allele 20, which has been identified in cultivars originating from Denmark, Switzerland and

Estonia. As concerns rare GS_S, allele 2* at 1A locus was found in cultivars from Hungary (5), Ukraine (3), Czech Republic (2) and in single cultivars from Austria, Bulgaria, Denmark, France and Russia. Similarly, GS_S 3+12 at 1D chromosome were recorded only in cultivars close to Central Europe (Czech Republic – 3, Hungary – 2, Switzerland –2). Nevertheless, this allelic combination has also been found in one cultivar from Georgia. Among relatively rare allelic combinations at 1B, GS_S 17+18 were found in 5 cultivars from Poland, Hungary and former Czechoslovakia.

Even though Gluten in patterns themselves cannot characterize geographic origin of cultivars they can provide some evidence on incidence of particular Glu- alleles in different European regions. We attempted to calculate incidencies of Glu- alleles in characterized Glu lines originating from West, North- West, Central and East Europe (see Tab. 4). In spite of a schematic approach, incomplete coverage of regions and relatively low and unbalanced number of evaluated lines, some general tendencies can be found. For 1A chromosome, absence of *HMW –Glu* subunit was very frequent in West- and Nort-West Europe and strongly decreased in Middle and especially East of Europe. Allele 1 has higher incidence in East Eorope (67 %) as well as the rather rare allele 2, which occurred also in Middle Europe (12 % and 13 % of evaluated lines respectively). Clear tendency of decreasing incidence of 2+12 alleles from west (82%) to east (17%) and opposite tendency of 5+10 pattern (from 15 % to 81 % in East Europe) is noticeable for 1D chromosome, whereas pattern 3+12 remains rare in all regions (the highest 6 % occurrence is in Middle Europe). Incidence of *HMW Glu* subunits at 1B can be hardly distinguished for some rare alleles (6, 7, 8, 9, 13+16) and for alleles 7+8. Clearly increasing incidence from west (16 %) to east (60 %) has been found for alleles 7+9; the inverse trend has been expressed for allele 20 (which was identified in 28 % of lines from Western Europe and quite missing in Eastern European cultivars) and alleles 6+8. Allelic combination 17+18 has been found merely in Middle Europe.

Table 4: Distribution of HMW-Glu alleles in Western (CHE, FRA, GBR, GER, NLD), North – Western (DNK, EST, SWE), Middle (AUT, CZE, HUN, POL) and Eastern (RUS, UKR, BGR, GEO) European regions (% of evaluated lines originating from particular regions)

European region	Numer of lines	1 A			1 B										1 D		
		0	1	2	7 +8	6 +8	7 +9	17 +18	13 +16	7	8	9	6	20	2 +12	3 +12	5 +10
Western Europe	66	53	46	2	30	26	16	0	3	3	0	0	2	28	82	3	15
North-Western Europe	34	56	41	3	9	32	29	0	0	0	0	6	0	24	74	0	27
Middle Europe	80	31	46	13	23	8	58	6,3	1,3	1,3	1	0	0	3	55	6	39
Eastern Europe	42	21	67	12	24	0	60	0	2,3	9,5	0	2	2	0	17	2	81

Summary

Two sets (122 and 101 cultivars, respectively) of European winter wheat landraces and obsolete cultivars were evaluated in 3-years field trials. Clustering according to the country of origin enabled only very rough distinctions in a few characters (earliness, spike productivity).

Cultivars in the set 1 (n=122) were analyzed by means of SDS-PAGE and HMW *Glu* - subunits were identified. Relatively rare were allele's 2* at 1A and 3+ 12 at 1D as well as

alleles 8, 6, 9, 7, 13+16 and 17+ 18 at 1B. Allele 20 at 1B has been identified only in cultivars from DNK, CHE and EST. Allele 2¸ at 1A locus was found mainly in cultivars from East, South - East and Central Europe. Also allelic combination 17+18 at 1B has been found merely in Middle Europe. Glutenin patterns themselves were not sufficient for geographic characterization of cultivars, however, significant changes in incidence of particular alleles in different European regions were observed. At 1A chromosome, absence of *HMW –Glu* subunit was very frequent in West- and North-West Europe and strongly decreased in Middle and especially East Europe whereas allele 1 has higher incidence in East Europe. Tendency of decreasing incidence of 2+12 alleles from west (82%) to east (17%) and opposite tendency of 5+10 alleles is noticeable for 1D chromosome. At 1B chromosome, increasing incidence from west (16 %) to east (60 %) has been found for alleles 7+9; an inverse trend has been found in allele 20.

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Genetic analysis of orange wheat blossom midge resistance in wheat using chromosome substitution lines and isogenic lines

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Orange wheat blossom midge (owbm), (*Sitodiplosis mosellana*), is a pest which has shown increased incidence and a spreading distribution in the UK in recent years. The pest has been causing widespread damage and significant yield losses, and caused much insecticide use in 2004 and 2005, with its inherent dangers and damage to the environment. The pest continues to be a major and repeated threat, although risks vary between season, locality and individual crop. Because of the sporadic nature of infestation, genetic control is much better than chemical control, and experiments using precise genetics stocks were carried out to try to identify genes for resistance.

Damage by the pest is mainly due to larval infestation of the immature grain where they cause floret sterility or damage to the developing grain. Resistance mechanisms can be hypothesized to be of three types- escape, resistance, and tolerance. Escape is when the timing of the life cycle enables the plant to go past stage of growth when it is susceptible to infestation before adult flight. For example, this can be adjusted by using genes to accelerate or delay the flowering time. Resistance is where the plant kills or reduces the growth of the larvae, and this has been demonstrated in both UK and Canadian varieties (Thomas et al. 2001). Tolerance is where the larvae infest the grain, but yield losses are minimised, although this has yet to be conclusively demonstrated for this pest.

Three years of trials were carried over the growing seasons 2001-2002, 2002-2003, 2003-2004. All experiments were sown at the Morley Research Station, Norfolk, UK and were treated with standardized programmes of herbicides and fungicides, except no insecticides. Each experiment used a randomised drilled plot design with 2 replications. All individual plots were scored for ear emergence, and then anthesis time (growth stage 61). Three weeks after anthesis 10 random ears were collected, a group of 5 ears from 2 different locations within the plot. These were taken back to the laboratory and frozen until they could be assessed for midge infestation. Each ear was looked at individually and scored for the presence and number of wheat orange blossom midge by examining each individual floret separately and noting the number of midge larvae in each floret. Data were recorded as the number of larvae per 100 grain and as % of grains infested.

1. Evaluating genes for escape using isogenic lines for photoperiod insensitivity

To evaluate the effect of changing the timing of flowering on infestation, a set of isogenic lines differing for alleles at the loci controlling photoperiod response, in the genetic backgrounds of the winter wheat cultivars Cappelle-Desprez and Mercia were evalauated. These consisted of lines of Cappelle-Desprez carrying an allele at the locus *Ppd1 (Ppd-D1)* on chromosome 2D conferring photoperiod insensitivity from the Italian variety Mara, and an allele conferring photoperiod insensitivity at the *Ppd2 (Ppd-B1)* locus on chromosome 2B from the variety Chinese Spring. The Mercia lines carried an allele at the locus *Ppd1 (Ppd-D1)* on chromosome 2D conferring photoperiod insensitivity from the Mexican variety, Ciano 67, an allele conferring photoperiod insensitivity at the *Ppd2 (Ppd-B1)* locus on chromosome 2B from the variety Chinese Spring, and an allele for photoperiod insensitivity from the Indian variety C591 at the *Ppd3 (Ppd-A1)* locus on chromosome 2A.

The infestation levels on the photoperiod insensitive, and hence early flowering lines, grown in 2002 and 2003 were compared to their control parents, Cappelle-Desprez and Mercia. In 2002, Mercia had an infestation level much lower than Cappelle-Desprez, but the isogenic lines of the two varieties behaved similarly in having greatly reduced, and in the case of Mercia (*Ppd1*), a negligible infestation level. In these years, earlier flowering before temperatures were conducive for midge flight was clearly an efficient mechanism of escape. Even in 2003, when infestation levels were much higher, earlier flowering was a mechanism for reducing damage.

The *Ppd* alleles differ in the potency of inducing earlier flowering where *Ppd1* is earlier than *Ppd2*, which in turn, is earlier than *Ppd3*. In 2002, this also reflected the differential response to midge infestation where both the *Ppd1* and *Ppd 2* lines have low infestation levels, but *Ppd1* is still less than *Ppd2*. However, in 2003, this trend is not consistent, and whereas the Cappelle *Ppd1* has a level lower than the *Ppd2* line, for the Mercia isogenics the trend is reversed. This clearly indicates that although earlier flowering is a mechanism of escape, it is not independent of other variables, such as temperature, which can induce midge flight and hence infestation at times other than within the normal range of UK varieties. However, it would generally be expected that possessing photoperiod insensitivity and hence earlier flowering, is a mechanism that plant breeders can apply to reduce midge damage.

2. Identifying genes for resistance using chromosome substitution lines

To investigate the chromosomal locations of possible genes for resistance, a set of single chromosome substitution lines of the donor variety, Bezostaya, into the recipient variety Cappelle-Desprez were grown (Law and Worland 1967). Because marker analysis had shown that certain of these were not genetically correct, not all of the possible set of 21 possible substitution lines (1A to 7D) were evaluated, and lines 4B and 5B were absent. All available lines were grown in 2001/02, 2002/03, but only substitution lines not differing significantly in flowering time but showing significant differences from the recurrent parent were grown in 2003/04, namely lines 1D, 3A and 7B, and Bezostaya was also not represented in this trial.

As with the *Ppd* isogenic lines, levels of infestation increased significantly over the three years of trials, being lowest in 2002 and greatest in 2004 (Figure 1). As levels of infestation increased, differences between lines also decreased. In both years of joint testing, Bezostaya was infested significantly less than Cappelle-Desprez, but also had a significantly earlier flowering time, so this is probably due to escape, rather than resistance/tolerance *per se*. The substitution lines also showed a similar pattern of increasing infestation over years, and infestation levels increased from between 5-25 larvae per 100 grain in 2002, to 16-55 larvae per 100 grain in 2003. However, within all years, there was a significant difference between the lines, indicating significant genetical variation in levels of infestation. The relative differences between lines were also generally maintained between 2002 and 2003, indicating that they are true genetical effects.

The most significant effect, however, in both 2002 and 2003 was the correlation between levels of infestation and flowering time. This was particularly the case for Cap (Bez 2D) which showed negligible infestation in 2002 due to its earlier flowering time, which is due to it carrying the *Ppd1* (*Ppd-D1)* gene for photoperiod insensitivity (which also accounts for the earlier flowering time of Bezostaya).

In 2002, one line, Cap (Bez 1A) was infested to a significantly greater extent than Cappelle-Desprez, indicating genes for greater attractiveness to the pest, although this was not repeated in 2003, where only Cap (Bez 1B) showed greater infestation levels. However, in 2002 there were four lines that were less infested than Cappelle-Desprez, indicating chromosomes from Bezostaya carrying genes for resistance/tolerance, namely 7B, 3A, 1D and 2D. All of these

also showed lower infestation levels in 2003, although only significant for 7B. In addition, chromosomes 3B, 6A and 7A showed lower 2003 levels, although not significantly different in 2002. 7B also showed lower infestation levels in 2004 and although also earlier flowering than Cappelle-Desprez in all years, this could not account for the effect alone, since in 2004 there was an early flight of midges which coincided with this line reaching anthesis. This early flight was followed by a second flight of midges two weeks later, which affected the rest of the lines. Nevertheless, 7B showed less % grain attacked compared to the larvae found, and this could be an indication of resistance or tolerance of this line.

3. Evaluating a molecular marker diagnostic for resistance

A molecular genetic marker has been reported by a Group in Winnipeg, Canada, as being diagnostic for a major resistance gene, *Sm1*, in Canadian varieties (Thomas et al. 2004). To evaluate whether the Elsoms Seeds resistant varieties Welford and Carlton carry this gene, and whether this molecular test is diagnostic for resistance in European wheats, molecular marker analysis was carried out by PCR using primers supplied by the Canadian Group (data and methods provided in confidence). Using primer information, and the optimised protocol obtained from the Canadian Group, marker analysis was carried out on a range of varieties which the trials in the project had shown to differ in levels of infestation over the different years of trial.

In UK material, there appeared to be no correlation between possessing the *Sm1* diagnostic band and resistance. Varieties contrasting widely in susceptible, such as Tanker and Welford, both possess the band, and few varieties, only Charger, Rialto and Savannah in this sample, lack the band. This may indicate that although this band is diagnostic in a restricted gene pool where the specific cross is known to segregate both for *Sm1* and the band, it is not of general use in European germplasm.

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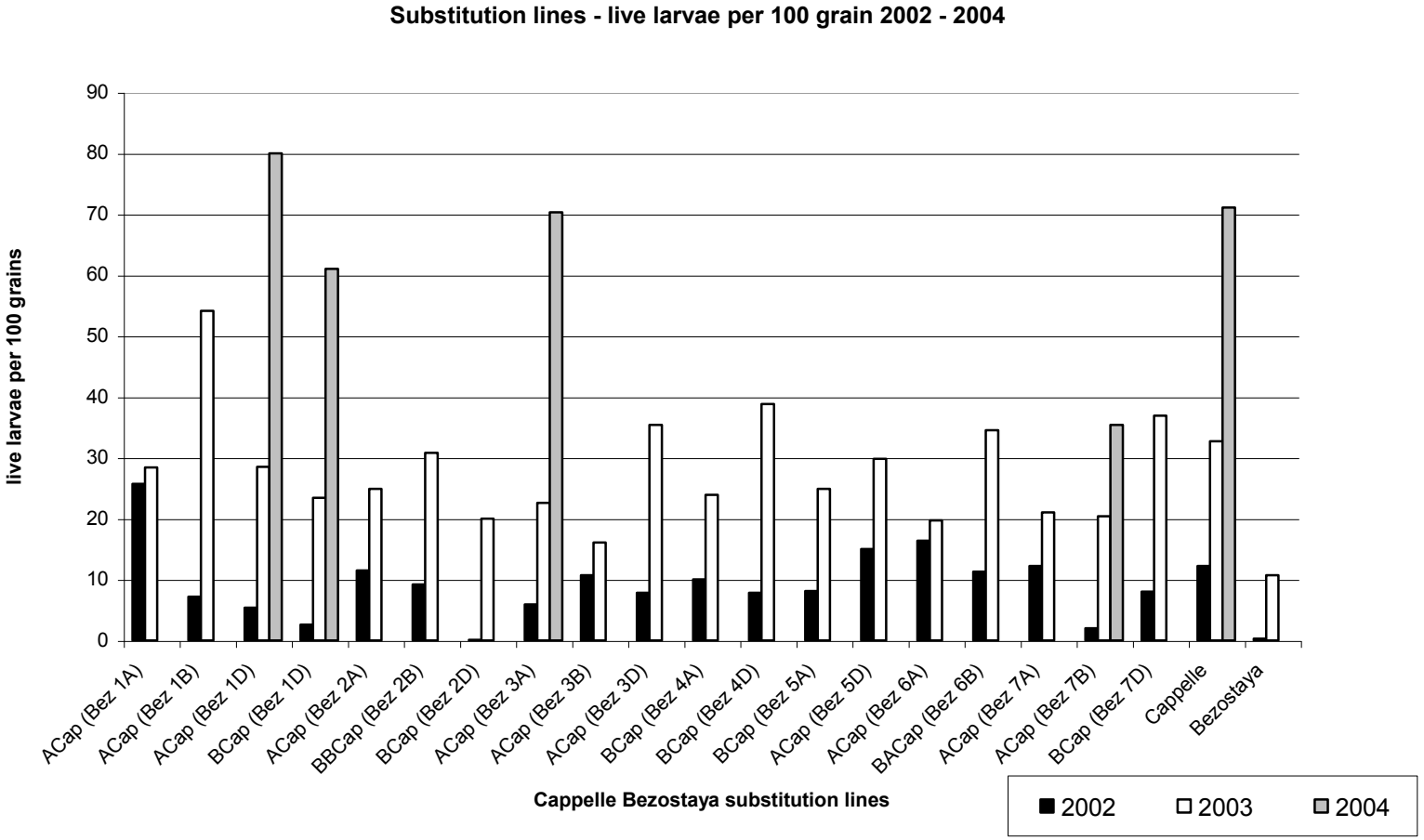

Figure 1: Levels of orange blossom midge infestation measured as the number of live larvae/100 grain sampled on the Cappelle-Desprez (Cap) Bezostaya (Bez) single chromosome substitution lines, for three years of trials

Resistance to powdery mildew in tetraploid wheats

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Powdery mildew is a common disease induced by the biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici*, which causes considerable damage to wheat cultivations, particularly in areas with temperate climates. As a rule, the way to control powdery mildew is the cultivation of resistant varieties. However, due to the pathotypes continuous evolution, the resistance against a specific strain of the pathogen usually become ineffective within a short period and new sources of resistance are required. Up to now, more than 30 loci conferring resistance to powdery mildew were identified in the wheat gene pool and some of these loci have been associated to molecular markers. *Triticum turgidum* var. *dicoccoides* and var. *dicoccum*, are good sources of powdery mildew resistance genes. Previous germplasm screening identified var. *dicoccoides* accession MG29896 and var. *dicoccum* accession MG5323 as very resistant to different isolates of powdery mildew in greenhouse and in field conditions to the natural populations. The aim of the present work was to study the genetic control of powdery mildew resistance in the two different accessions and to identify molecular markers linked to powdery mildew resistant genes.

1. Powdery mildew resistance analysis in *Triticum turgidum* var. *dicoccoides*

A backcross inbred line (5BIL-29-BC5F5) derived from crossing the powdery mildew resistant accession MG29896 (*Triticum turgidum* var. *dicoccoides*) and the susceptible durum wheat cultivar, Latino (*Triticum turgidum* var. *durum*) showed no symptoms during all the development stages in field conditions. The F_2 population derived from the cross Latino × 5BIL-29 was used in the present study. F_2 individuals were assayed in field conditions using the 0-9 scale of Saari & Prescott (1975). The F_3 progenies derived from 120 randomly chosen F_2 plants were analysed for field resistance by randomised complete block design with three replications. The Bulked Segregant Analysis was performed by using SSR and AFPL molecular markers. DNA of 16 F_3 progenies, 8 homozygous resistant and 8 homozygous susceptible, were pooled to obtain a resistant and a susceptible bulk. A set of durum wheat cv Langdon-D genome disomic substitution lines (Joppa & Williams, 1983) was used for the chromosome location of molecular markers. Linkage analysis was conducted with MAPMAKER 3.0.

The analysis of powdery mildew reaction in the F_2 population showed a ratio not significantly different from 3:1, expected for the segregation of a single dominant resistance locus. The evaluation of 120 F_3 progenies exhibited 24 progenies resistant, 69 progenies segregating and 27 completely susceptible. These data fitted in the 1:2:1 segregation ratio thus confirming the presence of a single dominant resistance locus.

A total of 86 AFLP primer combinations (Pst/Mse) were screened in the parental lines and the 2 bulks to identify markers associated with the powdery mildew resistance gene. 9700 discrete fragments of DNA, ranging in size from 80 to 900 bp, were amplified with an average of 120 DNA fragments per primer combination. One hundred and ninety bands were polymorphic between the two parental lines, and 20 bands showed polymorphism between susceptible and resistant bulks. Only 5 AFLP markers appeared to be linked to the resistance locus and their primer combinations were analysed on the 120 F_3 progenies. A 3:1 segregation ratio was observed for each marker. The five AFLP were found to be linked to the resistance gene, the nearest AFLP marker to the resistance gene was at 1.3 cM.

The 5 molecular markers associated to mildew resistance locus were localised on the 5B chromosome by Langdon-D genome disomic substitution lines.

In order to find further molecular markers linked to the resistance locus, 35 SSR markers localised on chromosome 5B (Röder et al. 1998) were tested. Twenty eight microsatellites were polymorphic between Latino and MG29896, but only the marker *Xgwm408-5BL* was polymorphic between Latino and 5BIL-29. The marker was then analysed in F_3 progenies. Data of molecular markers (AFLP, SSR) and resistance segregation were analysed with MAPMAKER software. Liu et al. (2002) localised *Pm30* locus originating from wild emmer on the chromosome arm 5BS, strictly linked to *Xgwm159*. Comparing the map position, it was possible to deduce that the resistance locus present in this segregant population was different from *Pm30*. The wild emmer-derived powdery mildew resistance gene, temporally called *PmMG*, appears to be a new gene localized on 5BL chromosome arm, which can be used to diversify the resistance resources for wheat powdery mildew.

2. Powdery mildew resistance analysis in *Triticum turgidum* var. *dicoccum*

The accession MG5323 was crossed to *Triticum turgidum* var. *durum* cv. Latino, susceptible to powdery mildew. A set of 120 recombinant inbred lines (RILs) was produced by single seed descent. RILs and parental lines were tested for resistance to natural mildew population, at the three leaf stage under controlled conditions in greenhouse, using a randomised complete block design with 2 replications. Plant resistance assays were performed, using a modified 0-4 scale (Saari and Prescott, 1975) where the level of infection reflects the percentage of leaf surface infected. Eight resistant and eight susceptible RI lines were selected for making two DNA bulks of extreme phenotypes to be screened, according to the Bulked Segregant Analysis (BSA), with microsatellites markers.

The analysis of powdery mildew reaction in the greenhouse showed 99 susceptible lines (score 2-4) and 21 resistant (score 0-1). The chi-square test fitted to a 3 : 1 segregation ratio in the RIL population, thus suggesting the presence of two recessive genes in the var. *dicoccum*. Two hundred microsatellites markers were tested between the two parents, showing a polymorphism of 50 %. Polymorphic markers were analysed between the two bulks and four markers were found to be polymorphic. Such markers were considered potentially linked to mildew resistance and then screened on the complete set of RILs. The putative linkage of these markers with powdery mildew resistance was assayed by regression analysis of each marker locus on RI lines. The regression analysis showed a highly significant association of markers *Xwmc25-2B Xwmc243-2B* and *Xwmc257-2B* located on the short arm of chromosome 2B.

Conclusions

Triticum turgidum var. *dicoccum* and *Triticum turgidum* var. *dicoccoides* were found good sources of powdery mildew resistance genes. The genetic analysis of this study pointed out the presence of one dominant gene in var. *dicoccoides* accession MG29896 and two recessive genes controlling powdery mildew resistance in the var. *dicoccum* accession MG5323. The wild emmer-derived MG29896 powdery mildew resistance gene, temporally called *PmMG*, is a new dominant gene, which can be used in the constitution of wheat varieties resistant to this pathogen. Moreover molecular markers, linked to these different resistant genes, were identified and they could be useful in marker-assisted selection programs.

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Determination of variety resistance of wheat to common bunt (*Tilletia tritici* Bjerk., *T. laevis* Kühn)

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Introduction

Common bunt has been associated with wheat cultivation since the beginning of recorded history. It has been present from the earliest agricultural experiences of man. In the past the same terms were used to refer to a variety of diseases and injuries. In 1755 Tillet used common bunt in some of his early demonstrations of the cause and contagious nature of parasitic plant diseases. Anyway, superstition and the supernatural followed common bunt even down to the middle of the nineteenth century.

After an introduction of effective chemical treatments the problem seemed to be solved once and for all. Still, common bunt and dwarf bunt repeatedly occur for various reasons, for example in small-scale farming, in organic farming, or when seed is resowed without a chemical treatment, or when small doses of fungicides or fungicides with lower efficiency are applied.

Common bunt is caused by *Tilletia tritici (*Bjerk.) Wint., syn. *T. caries* (DC.) Tul. and *T. laevis* Kühn syn. *T. foetida* (Wallr.) Liro, dwarf bunt is caused by *T. controversa* Kühn. Typical bunt symptoms are a strong odour caused by trimethylamine, abnormal appearance of spikes, flecking on leaves, abnormally high numbers of tillers, dwarfing. The most convincing evidence is the presence of bunt balls. Bunt balls are mature sori that consist almost entirely of teliospores and are covered by the thin, modified ovary wall.

Common bunt and dwarf bunt still cause significant losses when seed dressing is improperly used. Besides yield reduction also quality is affected. One diseased spike can contaminate an enormous amount of grain. Food and fodder industries refuse contaminated grain and occurence of bunt balls can cause problems in export.

Efficient control methods are chemical seed treatment and resistant varieties. Biological control and various techniques of organic seed treatment were described to decrease bunt occurrence. Also utilization of fast wheat growth at the time of infection through cultural practices can make infection lower, e.g., spring wheat cultivation, or to avoid too deep and too late sowing of winter wheat.

To control common bunt and dwarf bunt in our country at present it is possible only to recommend properly chemically treated seed, for example on the seed dresser of the type

Rotostat. When dwarf bunt occurs Dividend 030 FS (difenoconazole) or Sibutol 398 FS (bitertanole, fuberidazole) should be employed.

Material and Methods

Inoculation was carried out by shaking the seed with teliospores (10 mg spores /10 g seeds) in a glass flask for 1 min. Plot size varied in diverse experiments from 0,4 to 1,2 m^2 (2-6 rows 1 m long). Infection was expressed in % of diseased ears. Seed of registered varieties originated from the Central Institute for Supervising and Testing in Agriculture, Czech Republic, seed of foreign sources from Gene Bank Departement of the RICP, Prague-Ruzyne and from B.J.Goates (USDA-ARS, Aberdeen, USA), who also provided us with differential lines carrying 15 different *Bt* genes. The most widespread varieties were selected according to publication of the Czech Central Institute for Supervising and Testing in Agriculture: Overview of varieties 2003, Cereals. The selection was based on the data on seed growing area from the year 2002. Varieties with seed growing area 2% and higher were included. In 1988 and 1989 a sample of bunt originating from RICP-Ruzyne of unknown virulence was used. Inoculation mixture of different bunt proveniences used in 1998/1999 was expected to have virulence to the genes *Bt1, Bt2, Bt4, Bt6, Bt7* and *Bt10*. In inoculum for experiments in 1995/1996 and 2004, virulence to the genes *Bt1, Bt2* and *Bt7* was supposed. For race differentiation lines/varieties carrying genes *Bt1-Bt13* were used, differential varieties Doubbi (*Bt14*) and Carleton (*Bt15*) were not employed because of unreliable infection of spring wheat in our conditions. A limit of 10% of diseased ears was used to differentiate susceptible and resistant reactions.

Results and Discussion

This contribution summarizes results of several years of field infection tests with *T. tritici* (Bjerk.) Wint., syn. *T. caries* (DC.) Tul. and *T. laevis* Kühn, syn. *T. foetida* (Wallr.) Liro.

Out of the most widely grown registered varieties in the Czech Republic (Table 1) var. Niagara had the lowest infection (22.1% of ears infested), most severely infested was var. Corsaire (82.8%). Varieties Sulamit and Nela displayed lower infection in comparison with the average level of infection. Of the less frequently grown registered varieties (Table 2), var. Bill had a lower infection (12%) and var. Globus the lowest one (1%). Based on our two-year trials formerly registered varieties Vala and Roxana belonged to the most resistant. In the first two years infection of var. Roxana did not reach 6% while infection of the susceptible variety was over 50%. In 1996 the infection of var. Roxana reached almost 40% but also the most susceptible variety had a much higher infection level (80%) in that trial.

Reactions to inoculum composed of *T. tritici* and *T. laevis* of selected winter wheat varieties registered in the Czech Republic and advanced lines. Individual infection levels in 6 replications were adapted for the application of ANOVA. For this reason data in Fig.1 are not identical with those in Tables 1 and 2. Varieties Globus, Bill and Niagara showed the lowest infection. According to ANOVA, variety Globus significantly differed from all the other tested entries. Varieties Globus and Bill come from the German company Nordsaat Saatzucht. Borum (2001 cit. after Fischer et al. 2002) reported relative bunt resistance of the var. Bill.

Reactions of sources of resistance originating mostly from North America were verified in 1996 and 1999 (Table 3) and 26 foreign resistant varieties were chosen. The most resistant varieties with levels of infection 0-9.9% are listed in Table 3. Although there are many sources of resistance effective to bunt, use of most of them is difficult because of their agronomic traits which do not suit to European conditions (Blažková, Bartoš 1997, 2002).

The Swedish winter wheat var. Tjelvar, resistant to common bunt and dwarf bunt, was used for transfer of resistance. It was crossed with the susceptible Czech vars. Hana and Regina. Selected progenies of the crosses were tested in F_4 and F_5. Homozygous resistant lines were chosen that are earlier than cv.Tjelvar, with shorter straw and without translocation 1BL.1RS that adversely affects baking quality (Blažková et al. 1997).

Table 1: Reactions of the most widespread winter wheat varieties registered in the Czech Republic to *T. tritici* and *T. laevis* (* *T. laevis* – Ruzyně 1995; ** Přehledy odrůd 2003, Obilniny – ÚKZÚZ

Seed growing area** %	Variety	% of infected ears 2004 Race mixture
11	Sulamit	35.6
7	Nela	33.6*
6	Ebi	57.5
6	Drifter	54.4
5	Contra	50.8
4	Niagara	22.1
4	Alana	47.7
4	Versailles	62.1
4	Corsaire	82.8
3	Banquet	43.2
3	Batis	80.2
3	Šárka	not tested
2	Samanta	48.5*
2	Windsor	72.2
2	Vlasta	not tested

Table 2: Reaction of winter wheat varieties registered in CZ to *T. tritici* and *T. laevis*

Varieties with low level of infection	Year	Infection (%)
Roxana	1988	3,0
	1989	5.4
	1996	39.3
Vala	1988	5.5
	1989	14,3
Bill	2004	11.9
Niagara	2004	22.1
Globus	2004	1.0

Varieties with high level of infection	Year	Infection (%)
Sabina	1988	57.1
Iris	1989	61.6
Goldendrop	1996	80.3
Corsaire	2004	82.8

Table 3: Sources of resistance to mixture of *T. tritici* and *T. laevis*

1995/1996	
Variety	Infection (%)
Amigo	0
Crest	0
Franklin	0
Stava	0
Tjelvar	0
Cardon	0.9
Wasatch	1.2
Hildebrands Weissweizen	1.5
Šechurdinovka	4.9
Nebred	5.4
Dobrovická přesívka	9.1
Maximum level of infection 1995/1996	
Goldendrop	80.3

1998/1999	
Cultivar	Infection (%)
Blizzard	0
Bonneville	0
Crest	0
Franklin	0
Hansel	0
KW 9403	0
KW 9410	0
Lewjain	0
Manning	0
Meridian	0
Promontory	0
Sprague	0
Ute	0
Winnridge	0
Weston	1.5
Maximum level of infection 1998/1999	
Regina	54.3

Table 4: Differences in virulence of common bunt samples of diverse origin

Variety	Infection (%)	
	Sample origin	
	I.	II.
Nela	33.7	0
Bussard	58.1	2.4
Euris	42.5	4.6
Maximum level of infection		
Ina	82.2	95.4
	III.	IV.
Tambor	29.2	2.6
Maximum level of infection		
Euris	36.8	69.1

I. = *T. laevis*, Ruzyně, CZ, (L-5)
II. = *T. tritici*, Kroměříž, CZ, (T-1)
III. = *T. laevis*, Bulgaria
IV. = *T. laevis*, Kroměříž, CZ, (T-5)

Experiments with commercial varieties infected with different provenences of bunt showed specific differences in virulence of bunt on several varieties (Table 4). Provenience of *T. tritici* from Kroměříž attacked only slightly (0-5%) varieties Nela, Bussard and Euris and similarly var. Tambor was only very slightly infected by *T. laevis* that originated from Kroměříž too. First three varieties mentioned were susceptible to the provenience *T. laevis* from Ruzyně (infection 34-58%), var. Tambor to *T. laevis* from Bulgaria (infection 29%). Koch and Spiess (2000) described var. Tambor as a relatively bunt resistant variety.

A wider set of bunt samples (22) originating from 10 countries were tested on 13 differential varieties used internationally to determine pathogenic races. Most of the samples were able to overcome genes *Bt1*, *Bt2* and *Bt7*. Virulence to genes *Bt3*, *Bt5, Bt8, Bt11, Bt12* and *Bt13* was not detected (Table 5).

Resistance can be overcome by selective increase of incidence of virulent races or by development of new combinations of genes for virulence in the bunt population. This is why there is always need for new sources of resistance for breeding bunt resistan varieties.

Table 5: The most common pathotypes of *T. tritici* a *T. laevis* (1999-2000, 22 samples from 10 countries tested; V – virulence)

Bt genes	Origin of the most frequent samples		Total virulence of all samples
	Romania	CZ	
	Hungary	Latvia	
	Latvia	Switzerland	
1	V		V
2	V	V	V
3			
4			V
5			
6			V
7	V	V	V
8			
9			V
10			V
11			
12			
13			
Percentage of samples	45	27	-

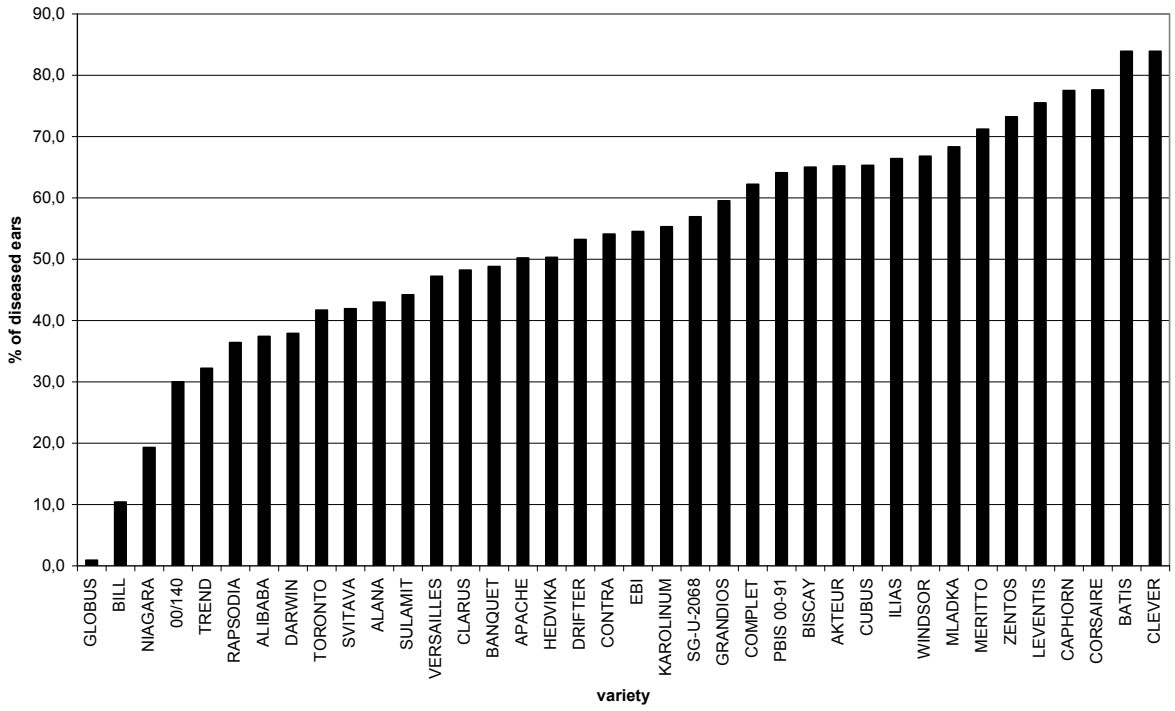

Fig.1: Reaction of selected winter wheat varieties and advanced lines to bunt (Field trial 2004)

Summary

Of the most common varieties registered in the Czech Republic var. Niagara had the lowest percentage of diseased ears (22.1%), the highest var. Corsaire (82.8%) in infection tests with a mixture of common bunt samples. Of the recently registered varieties, Globus (1% ears infected) and Bill (11,9%) were least attacked varieties. Reaction of sources of resistance coming mostly from North America was verified and 26 resistant foreign varieties were chosen. Differences in virulence of some Czech bunt samples were detected. For example var. Nela, Bussard and Euris were resistant to sample of *T. tritici* from Kroměříž, but susceptible to sample of *T. laevis* from Ruzyně. In the test of 22 samples from 10 countries virulence to genes *Bt1, Bt2* and *Bt7* was most frequently detected. Without infection stayed differential varieties carrying genes *Bt3, Bt5, Bt8, Bt11, Bt12* and *Bt13.*

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Collection, distribution, phenotyping and genotyping directed towards utilization of existing wheat genetic stocks to enhance tolerance/resistance of wheat cultivars to abiotic and bioticsStresses with emphasis on drought: A commissioned project of the Generation Challenge Program

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Since 1936, a wealth of genetic stocks has been developed in tetraploid (*Triticum turgidum* L.) and hexaploid (*T. aestivum* L.). These genetic stocks include material containing intervarietal and interspecific translocations, chromosome and chromosome arm additions and deletions, chromosome and alien substitution addition lines, mono- and polysomic series, recombinant doubled haploid populations, mapping populations, NILs, point and other mutations, and synthetics. Hexaploid wheat is allopolyploid in origin, and the homoeology existing between its three component genomes allows for a range of aneuploidy (i.e. additions, substitutions, deletions, etc.) to be tolerated.

These genetic stocks were often developed for specific purposes, e.g. as new sources of disease resistance genes, but have seldom been systematically screened for other value-added traits of interest. A few genes introduced from other species, e.g. rye (*Secale cereale* L.), have had a tremendous impact on wheat improvement. It can be assumed that a systematic screening of available genetic stocks will reveal useful genetic variation for many value-added traits of immediate interest to breeders. An important advantage is that any gene characterized in any of the existing or newly created wheat genetic stocks can be transferred to improved wheat cultivars without requiring the utilization of any biolistic or *Agrobacterium* transformation techniques. And finally, it is anticipated that the distribution of these genetic stocks worldwide will result in many research projects, in particular in the area of gene identification (including across species/genera), marker development, and association mapping, which will greatly increase our knowledge of wheat genetics and breeding efficiency in the future.

It is intended that, to the fullest extent feasible, all genetic stocks within this project will be placed within the Multilateral System of Access and Benefit Sharing to be established under the International Treaty of Plant Genetic Resources for Food and Agriculture ITPGRFA), and that they may be freely distributed under the standard ITPGRFA material transfer agreement (MTA) (http://www.fao.org/ag/cgrfa/itpgr.htm). It is also intended that, to the fullest extent feasible, results obtained from using these genetic stocks will be considered to be public goods which can be used without restrictions by others. Due to a number of ambiguities in the ITPGRFA that have not yet been resolved, it is not feasible to anticipate all potential implications of the ITPGRFA at this time. Accordingly, to the extent that any germplasm used in this project is protected by intellectual property rights, and to the extent that placing such germplasm within the Multilateral System would be inconsistent with such rights, the parties will endeavor to devise a system for working with and distributing such germplasm that both respects those intellectual property rights and achieves the public goods aims of this project as outlined above.

Background

The introduction of genetic variability from (distantly or closely) related wheat relatives, or alien species, has often been specifically aimed at the introduction of simply inherited value-added traits, mostly genes for disease resistance (for a complete list see McIntosh et al., 2003;

Catalogue of Gene Symbols for Wheat), and their impact on wheat improvement has been remarkable. Considering, however, all the alien derived stocks that have been developed over decades, their utilization in wheat research and improvement has been limited. A few had a tremendous impact. For example, the number of wheat/rye translocations that were used by wheat breeders is actually only a few in number. The wheat/rye translocation (1BL.1RS) occurring in more than 300 cultivars worldwide can be traced to one German source and all 1AL.1RS translocations, once widely present in bread wheat cultivars grown in the Great Plains of the US, trace to just one source, "Amigo" (Schlegel, 1997 a,b; Rabinovich, 1998). Other rye translocations carry genes for copper efficiency and Hessian fly resistance. Chromosome 1R and 7R enhance zinc efficiency in wheat rye addition lines (Cakmak et al., 1997). Rye and most Triticale are nearly immune to Zinc deficient soils. Zinc deficiency is the most widespread micro-nutrient deficiency depressing cereal yield and quality world wide.

While successes in enhancing qualitative traits using genetic stocks has been limited in the sense of the diversity of the genetic basis, the utilization of genetic stocks for quantitative trait improvement and their utilization in wheat breeding programs have been even more limited. Singh (1998) showed an increase in grain yield of about 5% in the irrigated trials through the introgression of a wheat/*Agropyron* 7DL.7Ag translocation. This translocation carrying the *Lr19* gene (from *Ag. elongatum*) was transferred into wheat in the early 1960's (Sharma and Knott, 1966). It took more than 25 years for anyone to notice that the 7D.7Ag translocation carried not only a gene for resistance to leaf rust resistance, but also gene(s) that had a positive effect on such a quantitative trait as grain yield. It took even longer to notice that the widespread wheat-rye translocations in wheat, 1RS.1AL and 1RS.1BL, significantly increase root biomass.

These are just a few examples illustrating the great potential, genetic stocks may have for the immediate improvement of wheat production. A major limitation of their exploitation has always been that genetic stocks were often developed for specific purposes and have not been systematically screened for other traits of interest to breeders, farmers or the processing industry. A further limitation has been that often the agronomic wheat background of these genetic stocks is not attractive to wheat breeders in their original form or the specific background genotype.

Water shortage has been identified as the single most important constraint for crop production. Production of low cash-value crops like wheat will most likely concentrate more and more in rainfed areas or wheat will only receive limited supplementary irrigation. In these production systems, temporary or extended periods of drought are common. Therefore, breeding wheat cultivars with enhanced drought tolerance is paramount for many wheat improvement programs worldwide, including that of CIMMYT.

Objectives

This project proposes the establishment of a consortium of key institutions worldwide that will provide the genetic stocks for use in gene discovery through comparative analysis. The consortium aims to:

- Gather existing relevant wheat genetic stocks in one centralized facility in the CIMMYT Genebank, and
- Make these available for agronomic and molecular characterization that will lay the foundation for association genetics across diverse wheat germplasm.
- Multiply and on request distribute seed of these stocks either from CIMMYT or from a cooperating/donor institute
- Initiate phenotyping

The individual centers contributing genetic stocks would function as cooperating locations, because they have the extensive cytogenetic expertise and experience to maintain and check these periodically. The effort in maintaining the integrity of unstable wheat aneuploids and substitutions should not be underestimated. Therefore, these cytogenetic stocks will be maintained and distributed by the donor/originator institution.

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Posters

Chromosome banding analysis in *Aegilops geniculata* and its potential use for wheat improvement

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The tetraploid goat grass *Ae. geniculata* (2n=28, genome formula $M^gM^gU^gU^g$) provides a wide range of useful genes that can be transferred into bread wheat. It is particularly interesting as a source of genes for earliness, high grain protein content (Bochev, 1988) and resistance to various diseases and pests (Dimitrov, 2003).

One of the aims of our study was to characterize the interspecific chromosome banding polymorphisms in order to establish the basic banded karyotype of *Ae. geniculata* and develop cytological markers for alien chromosome identification. For differential staining of chromosomes, the N-banding method (Gill et al., 1991) was followed. Further we focused our efforts on studying a collection of 20 *Ae. geniculata* accessions of different origin for resistance/tolerance to leaf rust and Cu-toxicity, which are important yield-limiting factors in some areas in Bulgaria.

Several *Ae. geniculata* chromosomes were transferred into wheat 'Chinese Spring' genome following standard hybridization-backcross scheme involving a selected accession which exhibited leaf rust resistance and good tolerance to excess Cu. The effects of *Ae. geniculata* chromosomes on plant development, morphology and yield components were assessed in a four-year field trial. Resistant/tolerant lines were developed. Leaf rust resistance was evaluated at the seedling stage after inoculation with two races of *Puccinia recondita* f.sp. *tritici* (77 and 167) which dominate in the pathogen population in Bulgaria. Tests for Cu-toxicity tolerance were carried out after 8-day treatment of seedlings with 1 and 10 μM $CuSO_4$. The tolerance was assessed by calculating the tolerance index (TI) as a ratio between average values of root and shoot growth parameters in stressed plants and the corresponding values in the control plants.

Chromosome N-banding analysis

All 14 pairs of *Ae. geniculata* chromosomes were stained successfully by the N-banding method. The analysis revealed interspecific polymorphism in the heterochromatin structure of chromosomes. Chromosome-specific N-bands were established that allowed discrimination of all *Ae. geniculata* chromosomes. As a result, the standard N-banded karyotype of *Ae. geniculata* was constructed and a generalised idiogram was proposed. The standard karyotype was used to detect and identify the alien chromosomes in the developed wheat-*Ae. geniculata* lines.

Leaf rust resistance and copper toxicity tolerance

The phytopathological tests on seedlings showed differential response among 20 *Ae. geniculata* accessions and confirmed the potential of the species as a donor of leaf rust

resistance. Nine accessions were resistant to both races (167 and 77) of *P. recondita*. Six accessions showed mixed reaction or susceptibility to the more virulent race 77 and resistance to race 167.

The seedling response to high Cu concentrations differed among *Ae. geniculata* accessions. There was a 22 % reduction in the root length, and a 36 % reduction in the root fresh mass at 1 µM, on average over all accessions. At 10 µM the root growth was considerably inhibited (65 % reduction). The shoot length and fresh mass were insignificantly reduced or even slightly stimulated at 1 µM. However, Cu ions had inhibitory effect on shoot growth at 10 µM.

Alien chromosome effects

- The presence of *Ae. geniculata* chromosomes in the wheat genome had mainly negative effects on yield components and final plant height, which were related to the increased dosage of homoeoalleles. Chromosomes $5U^g$ (as addition) and $3U^g$ (as substitution for wheat chromosome 3D) had the most pronounced negative effect on the number and mass of grains per plant, and had also the most consistent reducing effect on the final plant height during different trials.
- The alien chromosomes affected plant development mostly by delaying the ear emergence, except for the chromosome $7U^g$, which promoted earlier heading.
- Chromosomes $5U^g$ and $7U^g$ had specific effects on plant phenotype: plants with disomically added chromosome $5U^g$ formed prostrate culms at juvenile stage; plants with added chromosome $7U^g$ had a bend of the stem between the first and second internode, and covered caryopsis (Fig. 1). This provides additional morphological markers for identification of *Ae. geniculata* chromosomes in wheat genome. We compared lines DA5U^g and DA7U^g with wheat-*Ae. umbellulata* 5U and 7U chromosome addition lines, kindly provided by Dr. T.E. Miller, JIC. Chromosome 5U had the same effect as its *Ae. geniculata* homoeologue $5U^g$ on position of culms. The presence of chromosome 7U, however, did not cause covered caryopsis, and its effect on stem was less pronounced (Fig. 1a).

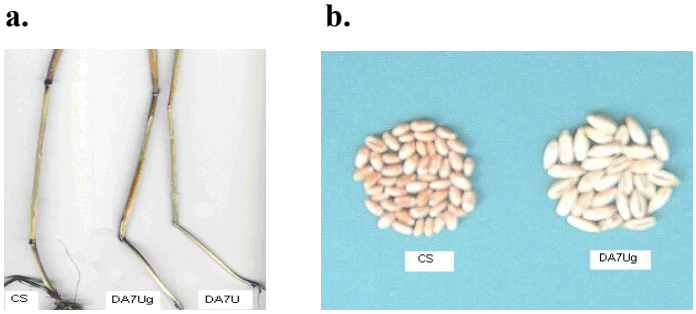

Fig. 1: Morphological markers for chromosome $7U^g$; (a) stem (b) covered caryopsis

- Homoeologous group 3 of *Ae. geniculata* chromosomes have positive effects on plant response to stress. Line DA3M^g was resistant to race 77, suggesting that chromosome $3M^g$ is a carrier of a resistance gene. Line DS3U^g exhibited the lowest root growth inhibition under Cu-stress at seedling stage (root length TI=77 %), which corresponded with low peroxidase response.

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The effects of intervarietal chromosome substitution on specific lipoxygenase activity in wheat *Trititcum aestivum* L.

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Lipoxygenases (Lpx, EC 1.13.11.12) are non-heme iron-containing dioxygenases widely distributed in plants, fungi and animals. They catalyze the addition of molecular oxygen to polyunsaturated fatty acids to produce an unsaturated fatty acid hydroperoxide. Products of Lpx pathway are involved in inducing structural or metabolic changes in the cell. In plants, Lpx has been associated with some processes in number of developmental stages (Siedow 1991). It shows influence on quality parameters of wheat gluten (Shiiba et al. 1991).

The lines with intervarietal substitution for the individual chromosomes in wheat are the convenient genetic material for the study of donor and recipient gene effects on the expression of different traits including the enzyme specific activity. The object of our research was the set of intervarietal substitution lines Saratovskaya 29/Janetskis Probat (S29/JP) including all chromosomes except 1B, 6D and 7A. The lines were developed in the Institute of Cytology and Genetics, Novosibirsk, Russia (Gaidalenok et al. 1995). The grain was grown under field conditions near Novosibirsk.

Lpx activity was assayed using spectrophotometer (Doderer et al. 1992). Specific activity was expressed by ratio of activity units to 1 mg of protein in 1 ml of incubation media. Protein concentration was determined by the Lowry method (Lowry et al. 1951).

Results of Lpx activity measurements in all investigated lines are shown in Fig.1. The donor JP exceeded the recipient S29 by 60% on specific activity of Lpx. In all lines except for 3D chromosome and for chromosomes of the 5 homoeologous group this parameter is significantly higher than in the recipient cultivar.

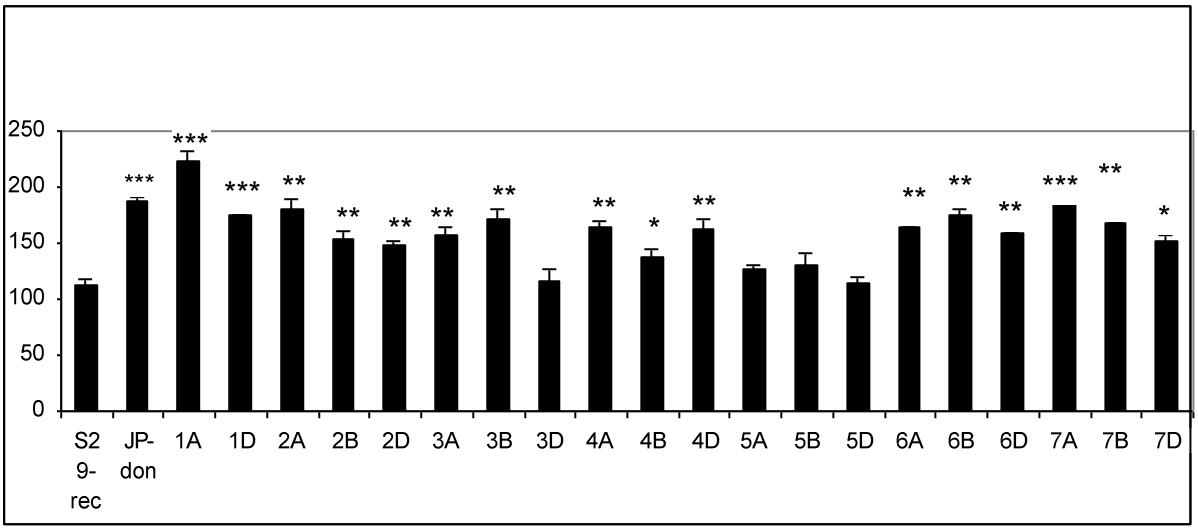

Fig. 1: Specific Lpx activity in substitution lines S29/JP; **, *** - significantly different from S29 at P<0.05, P<0.01, P<0.001, accordingly

It is known that the genes responsible for Lpx synthesis are located on the chromosomes of the homeologous groups 4 and 5 (Hart and Langstone 1977). Judging from the results obtained, it is evident that chromosomes of the different homoeologous groups participate in the control of this character. Earlier we showed that the intervarietal substitution for the homoeologous groups 1 and 6 chromosomes affected the activity level of this enzyme (Trufanov et al. 2001). According to the data obtained, it may be supposed that along with the structural genes for Lpx biosynthesis the regulatory genes controlling its activity level may exist in the wheat genome.

Using the native PAG electrophoresis of the enzyme protein fraction of separate kernels of the parent and lines, the presence of three molecular forms of Lpx was shown with Rf's 0.37 (Lpx-1), 0.32 (Lpx-2) and 0.24 (Lpx-3)). They had different enzyme activity which was evaluated through the level of their specific coloration in polyacrylamide gel slabs (Fig 2).

Chromosomal location of Lpx structural genes is known today, but the regulation of its activity may be under separate genetic control. Taking into account the diverse physiological role of Lpx in plants, further genetic studies of its synthesis and function are extremely important.

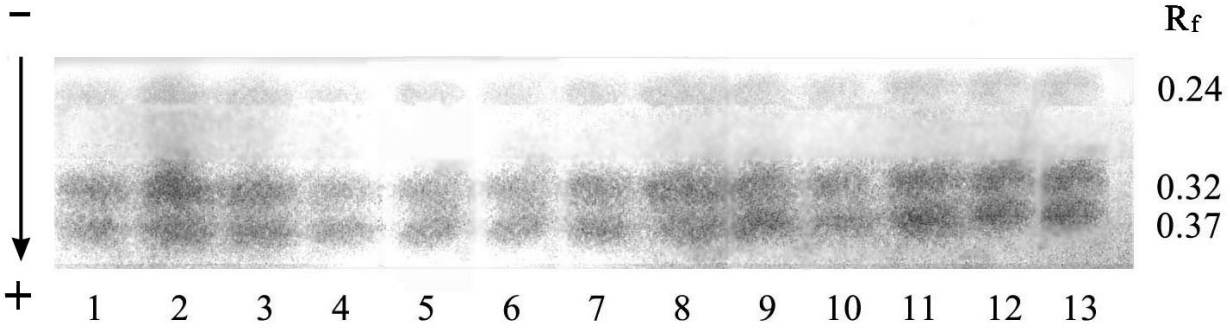

Fig. 2: Molecular forms of Lpx of the recipient S29 (1) and the donor JP (2) and substitution lines S29/JP 1A (3);S29/JP 1D (4); S29/JP 2A (5); S29/JP 2B (6); S29/JP 2D (7); S29/JP 3A (8); S29/JP 3B (9); S29/JP 3D (10); S29/JP 4A (11); S29/JP 4B (12); S29/JP 4D (13)

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Influence of intervarietal chromosome subsitution on biometric parameters of leaves, stem, grain productivity and stomata number in spring wheat

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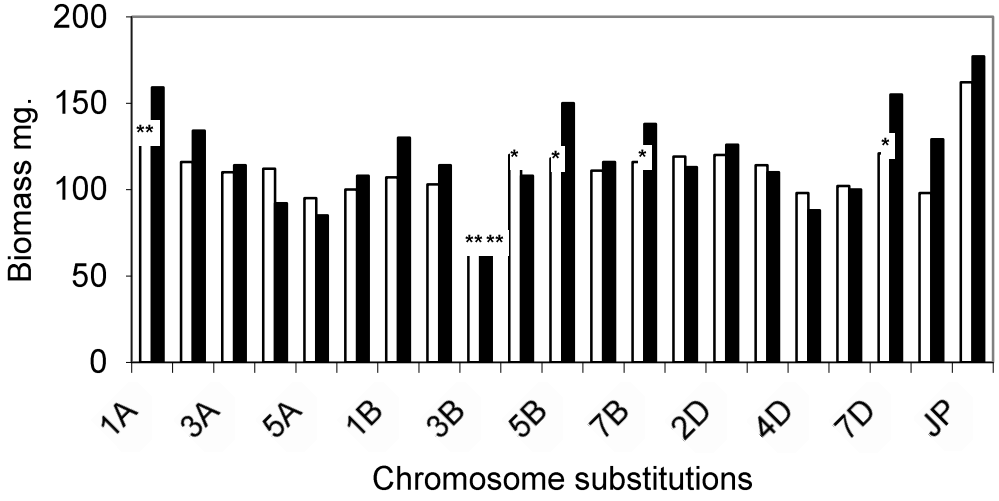
Several quantitative parameters of wheat plant were studied in the full substitution set Saratovskaya 29/Janetzkis Probat (S29/JP) (excluding 6D and 7A chromosomes) developed in the Institute of Cytology and Genetics SB RAS (Gaidalenok et al. 1995). The following parameters were studied: mass of boot and flag leaves, mass of stem, grain productivity and number of stomata on upper and lower sides of leaves per mm^2.

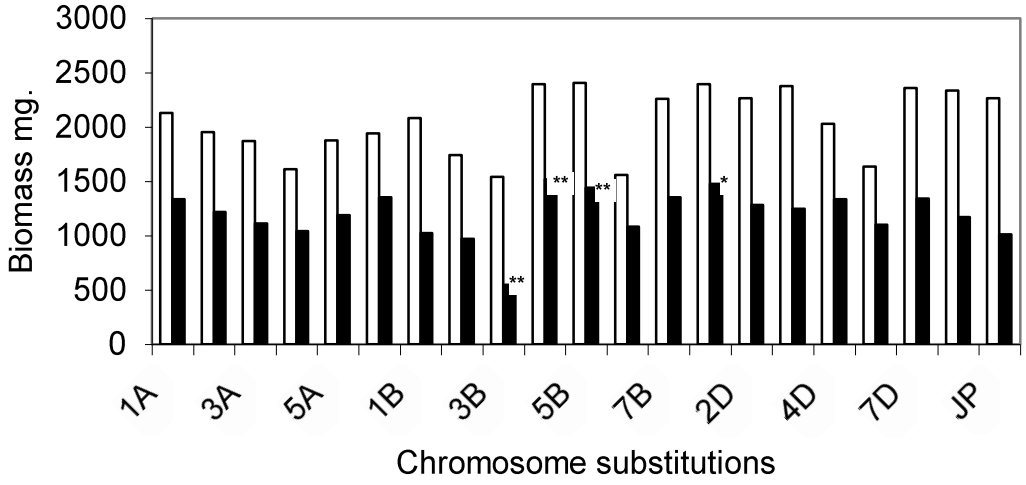
Parental forms and lines were grown under field conditions of East Siberia on loamy soil with addition of humus. Mass of leaves, stem and stomata number were determined during flowering and grain productivity after ripening. Five plants of every genetic sample were taken for measurements. Comparison was made with the recipient S29. Student T–tests were used for statistical processing of data.

In the East Siberia environment no significant differences were found between donor and recipient cultivar. The lowest values of biometric parameters were detected in the line with 3B substitution (Fig.1, 2). Mass of boot leaf consisted 67% of the recipient, mass of stem – 66%. The data obtained have shown that none of the lines exceeded substantially the donor cultivar JP on mass of boot and flag leaves. But the lines with substitutions for chromosomes 1A, 4B, 5B, 7B and 7D showed a significant increase of corresponding means compared to the recipient cultivar S29. In the lines with substitution for chromosomes 4B, 5B and 1D biomass of stem and grain productivity was higher both compared to the donor and recipient cultivars.



*, ** significant at P ≤ 0,05 and ≤ 0,01, respectively

Fig. 1: Mass of boot and flag leaves in substitution lines and parental cultivars



*, ** significant at P ≤ 0,05 and ≤ 0,01, respectively

Fig. 2: Mass of stem and grain in substitution lines and parental cultivars

Stomata apparatus of leaves is directly associated with drought resistance and productivity. However, the genetic control of this character is poorly studied; only stomata resistance genes were localized on chromosome 3A of common wheat (Bobo et al. 1992) and the pecularities of stomata apparatus were studied in monosomic lines of cv. 'Chinese Spring' (Davydov 2001). In the set S29/JP the substitutions for chromosomes 1A, 1B and 1D caused the decrease of stomata number on lower side compared to the recipient (Table 1). The same effect was found in the lines with substitution for chromosomes of 5th homoeologous group. Only in the line S29/JP 3A was the highly significant increase in number of stomata on both sides of the flag leaf found. The data

obtained proves that this quantitative character may be investigated in detail using precise genetic stocks.

Table 1: The number of stomata per mm^2 on upper and lower sides of flag leaf in substitution lines and their parents (S29/JP)

	upper	lower		upper	lower		upper	lower
1A	64,8±3,9	38,2±1,6***	1B	65,2±4,5	42,2±1,8***	1D	53,6±2,2*	35,3±2,5***
2A	62,8±4,1	46,7±2,7	2B	54,6±3,1	42,2±3,9	2D	52,4±1,9**	38,2±2,4***
3A	80,3±5,4***	63,8±2,0***	3B	71,8±2,4	45,6±4,1	3D	74,4±2,0	57,4±2,7
4A	61,4±1,1	55,8±2,1	4B	52,6±2,5	45,8±3,0	4D	57,4±3,0	46,4±2,1
5A	61,6±3,4	42,4±1,4***	5B	52,6±5,1	41,4±1,5***	5D	55,8±3,9*	40,4±1,5*
6A	75,6±3,3*	58,2±1,8	6B	60,8±4,0	42,6±2,8*	7D	68,2±5,4	52,8±0,7
			7B	65,6±1,9	47,0±3,2			
JP	55,4±2,4*	41,6±2,3***						
S29	65,0±2,4	52,8±0,8						

*, **, ***- significant at $P \leq 0,05$, $\leq 0,01$ and $\leq 0, 001$, respectively

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Detection of allelic diversity in semi-dwarfing genes in Bulgarian bread wheat varieties

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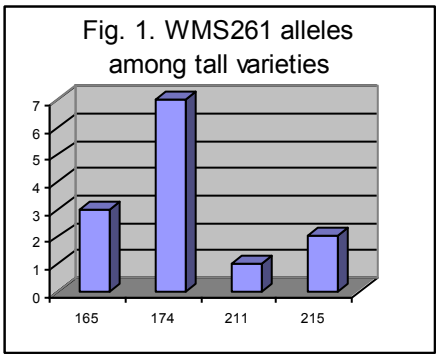
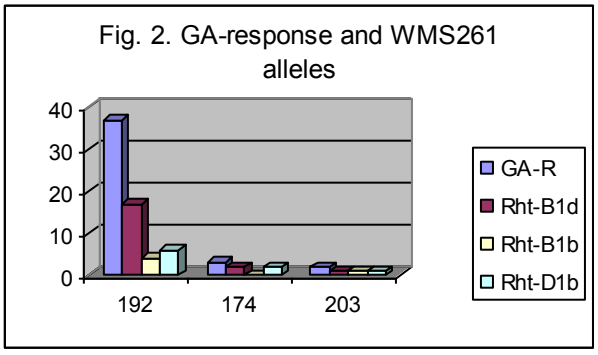
The systematic improvement of wheat varietal structure in Bulgaria was initiated after 1911 in the trial station 'Obraztsov Chiflik' near the town of Rousse by the breeder Prof. Ivan Ivanov. He developed the first more productive varieties through selection from local populations and landraces-involving intervarietal hybridization. Wheat breeding in Bulgaria made considerable progress after 1960s when the number of breeding groups increased and a complex study on a vast number of varieties of different geographic origin started in a search of appropriate parental components for breeding programmes. During this time the introduction of semi-dwarfing and photoperiod-insensitive genes began (Rachinski, 1965;

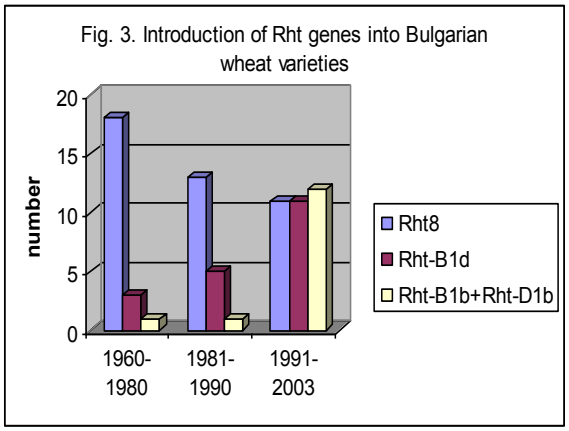
Panayotov *et al*., 1994). Until recently, the knowledge about the type of *Rht* genes in Bulgarian germplasm was scarce. In this study we applied two approaches to identify the *Rht* alleles in 89 varieties released in the period 1925-2003: analysis of allelic variants at the microsatellite locus WMS261, which is a marker of *Rht*8 (Korzun *et al*., 1998) and a gibberellic acid (GA) test according to Gale and Marshal (1975). Based on the comparison of GA-response of Bulgarian varieties with that of the near-isogenic lines of 'Mercia', carrying *Rht*-B1b, *Rht*-B1d and *Rht*-D1b, kindly supplied by Dr. A.J. Worland, JIC, Norwich, we propose a hypothetical distribution of the semi-dwarfing alleles among Bulgarian bread wheat germplasm.

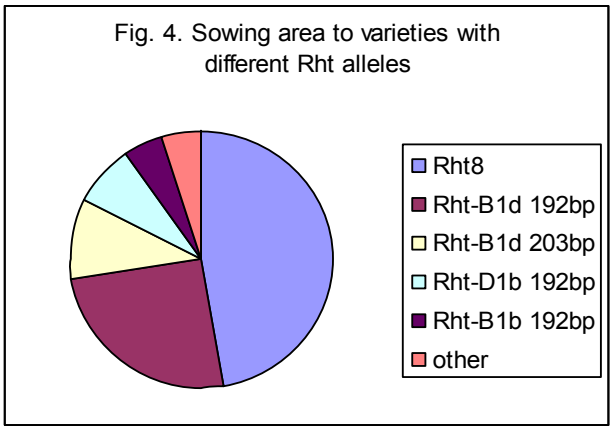
Identification of WMS261 alleles and semi-dwarfing alleles

The first Bulgarian varieties, isolated through selection or hybridization with landraces, carry the rare alleles of 211 bp and 215 bp fragments of the microsatellite locus WMS261 (Fig. 1). The 174 bp allele is prevailing in the group of varieties developed later through hybridization between Bulgarian and European varieties. The variety 'Mentana', a carrier of the 165 bp allele (Worland *et al.,* 1998), is the first Strampelli's variety used by Bulgarian breeders. The 165 bp allele is second in distribution among old varieties. Later, the breeding programmes involve Russian, Italian, Yugoslavian and other foreign semi-dwarfing varieties, due to which other alleles have been introduced into Bulgarian germplasm. Among the commercial varieties released after 1960, three alleles at locus WMS261 are present – 174 bp, 192 bp and 203 bp (Fig. 2).









The varietal structure in Bulgaria and the short-stemmed varieties, which have been grown for long periods over vast sowing areas, support the idea that *Rht*8 and *Rht*-B1d are of the greatest

importance in the South-Eastern European region (Worland and Petrovic, 1988; Worland *et al*., 1998). *Rht*8 was first introduced after 1960 and predominated until 1990 (Fig. 3). During this period the frequency of GA-insensitive varieties is increasing and in 1990s the frequency of varieties carrying *Rht*8, *Rht-B1d* and 'Norin 10'-derived *Rht* genes (*Rht-B1b* and *Rht-D1b*) is almost equal. The introduction of these genes has drastically altered plant height, the reducing effect being highest in varieties carrying *Rht-B1b* and *Rht-B1d*. The prevalence of the 192 bp allele of locus WMS261, 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. Approximately half of the wheat-sowing areas in Bulgaria, mostly in Southern parts, are sown to twelve *Rht*8-varieties (Fig. 4). Varieties carrying the combination of *Rht-B1d* allele and the 192 bp allele of locus WMS261 are ranked second by distribution, mostly in Northern parts of the country, and are characterized by the highest average yield per ha.

Acknowledgements

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Comparative studies of genetic diversity in wheat and barley germplasm collected at different time periods

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Introduction

Allelic diversity in germplasm collections is of great concern for plant breeding and crop improvement programmes. However, human activities like urbanisation, the replacement of traditional agriculture system by modern industrial methods or the introduction of modern high-yielding varieties may present a threat to biological diversity. The *ex situ* conservation of plant genetic resources represents an essential contribution to the conservation of both intra- and inter-specific diversity of crops and their wild relatives. In this context, the storage of

genetic resources in genebanks is of great significance. For example, the genebank of IPK presently holds about 150,000 accessions of different plant species including 28,000 and 21,000 accessions of wheat and barley, respectively (Annual Report- IPK, 2004). To create that genebank collection, more than 140 expeditions were made to different parts of the world, starting in the Twenties of the last century. For instance, E. Mayr collected landraces of cereals in the Austrian Alps during 1922-1932, A. Herrlich and collaborators collected germplasm from the Himalayas (India, Nepal) in the 1930s or H. Stubbe and his associates collected in the Balkans (Albania, Greece) in the early 1940s. Several decades later, some collection missions were repeated, although not always exactly the same areas were covered. That was the case for Nepal (1971), Northern India (1976), Austria (1982, 1983, 1986) or Albania (1993, 1994). Thus, the germplasm collections available in gene banks provide an opportunity to investigate changes in genetic diversity in materials collected from comparable geographic regions during different expeditions.

Materials and methods

In the Gatersleben genebank about 70% of all accessions are landraces which were not influenced by 'modern' plant breeding. They originated from about 140 collection missions World-wide (Gäde 1998). In four selected areas recurrent missions were initiated. From wheat, 18 accessions per area and collection mission were selected for Albania, North India and Nepal. Of the Austrian material 87 and 58 accessions originating from the first and a recurrent mission, respectively, were studied. From barley, 18 accessions per area and collection mission were investigated for Albania and India, whereas 12 accessions per collection mission were analysed for Austria. The distribution of the collection sites within the countries was highly comparable.

Total genomic DNA was extracted from pools of five grains of each accession according to the procedure described by Plaschke et al. (1995).

A total of 27 wheat genomic microsatellite (g-SSR) markers were taken to assess genetic diversity in wheat collections (Röder et al. 1998), whereas barley accessions were analysed using 28 g-SSR (Liu et al. 1996, Ramsay et al. 2000 and Li et al. 2003) and 13 EST-derived or genic microsatellite (e-SSR) markers (Thiel et al. 2003).

Numbers of collection mission specific and shared alleles were counted for each microsatellite locus. Allelic polymorphic information content (PIC) was calculated according to the formula of Anderson et al. (1993): $PIC=1- \sum(Pi)^2$, where Pi is the proportion of the population carrying the i^{th} allele, calculated for each locus. Collection mission means were compared using the parameter free test (*U*-test) of Mann and Whitney (1947).

Results and Discussion

The data obtained for wheat are presented in Figure 1. For the total number of year specific alleles, there was no clear tendency. Whereas this number was slightly higher for the early missions in the material collected in Albania and Nepal, the opposite case was detected for Austria and India. At the single locus level, however, contrasting tendencies were observed. Analogous results were obtained for the PIC values. Applying the *U*-test, no significant differences were detected both in the number of alleles per locus and in the mean PIC values, comparing the material of the repeated collection missions in all four regions analyzed. It was shown that about 50 % of the alleles were in common for both collection periods. The others, however, represented collection mission specific alleles.

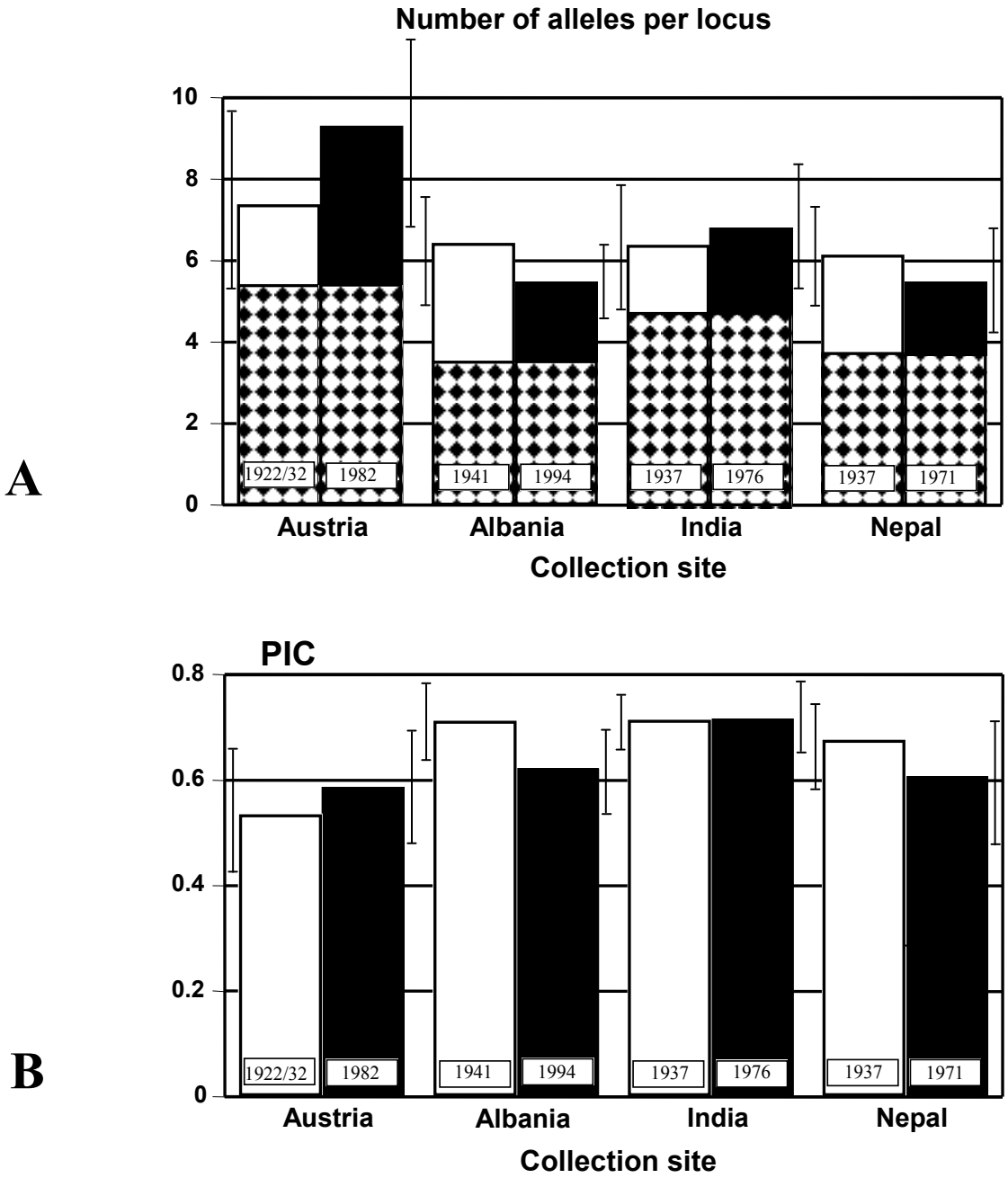

Fig. 1: Average numbers of alleles per locus (A) and average PIC values (B) detected in wheat accessions collected during repeated expeditions in Austria (1922/32 and 1982), Albania (1941 and 1994), India (1937 and 1976) and Nepal (1937 and 1971). Collection mission specific alleles are shown as light (first expedition) or dark (recurrent expedition) columns, common alleles are indicated as mixture.

For barley it was shown that no significant differences in both the total number of alleles per locus and in the PIC values for g-SSR as well as e-SSR markers were detected by comparing the material of the repeated collection missions in India and Austria. However a slight reduction of genetic diversity, statistically significant for g-SSR but not for e-SSR markers, was observed in the materials collected in Albania. It seems that there has been a real loss of genetic diversity in the materials collected in Albania in 1994, compared to 1941. This decrease may be driven by the economic and political isolation of Albania after World War II. The occurrence of 35-42% and 43-55% of the total alleles detected by barley e-SSR and g-SSR markers, respectively, as collection mission specific alleles, suggests an allele flow during the time intervals investigated. Furthermore, the g-SSR markers with an average 7.7 alleles per marker and average PIC value of 0.67 were found to be more superior for diversity

studies in comparison to e-SSR markers that could yield only 4.1 alleles per marker with an average PIC value of 0.47.

Thus, our results are suggestive of an overall stability in the genetic diversity in the wheat and barley genebank accessions collected up to 80 years ago in three diverse geographic regions, and the material that entered the genebank about 40 to 60 years later, originating from the more or less same areas. A range of other studies has examining the genetic diversity of wheat and barley, produced during the last century in various geographical regions or breeding programmes, has also revealed negligible evidence for any quantitative change resulting from breeding activities (Gregova et al., 1997; Donini et al., 2000; Manifesto et al., 2001; Khlestkina et al., 2004, Backes et al. 2003, Koebner et al. (2003).

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Has modern breeding led to a genetic narrowing in European winter wheat?

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Changes in agriculture have been dramatic over the past 50 years. Crop productivity has risen as a result of both improvements in breeding technology and in crop husbandry. There is a commonly held view that the intensification of breeding has led to a reduction in the genetic diversity of the crop (Clunies-Ross 1995), which would imply both an increased risk of genetic vulnerability and an undesirable inflexibility in the face of climate change.

To establish an objective assessment of trends in genetic diversity over time in European winter wheats, we assembled a collection of leading winter wheat varieties released over the period 1950 – 2000. The entries were divided into two 'populations', one ('RL') reflecting wide usage (at least 5% of the acreage, in at least two years in one of the EU member countries/country groupings (GB/IRL, F/B, B/NL/L, D-FRG/A, D-GDR, S/DK/SF) during the period 1945-20000; and the other ('NL') representing the spectrum of varieties in commerce in the UK in the mid 1990's.

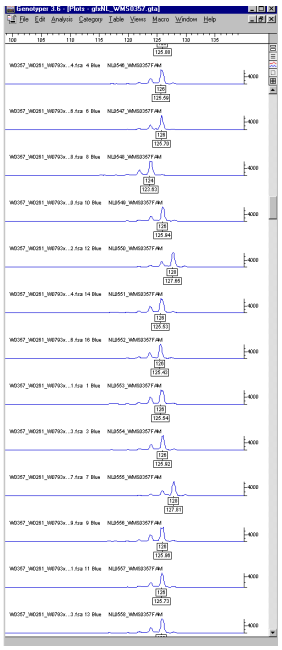


Fig. 1: Fluorescently labelled SSR tracks

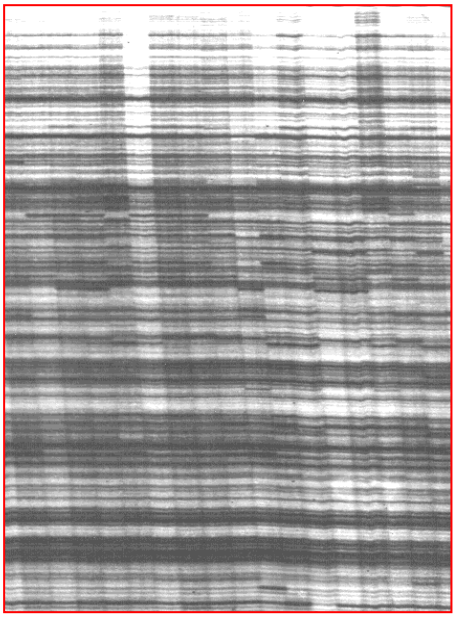


Fig. 2: Radioactively labelled NBS tracks

To collect this information, approaches were made to the regulatory bodies in the member countries, as well as using information from published sources. This resulted in an overall varietal entry number of 510, split as 281 in RL and 229 in NL. A bulked sample of about 30 seeds was ground and used as a source of DNA. Residual ground seed still remains of all entries, immortalising the DNA for future investigations. All materials were multiplied under controlled conditions (cellophane bagging), giving pure line representation of almost the whole collection. Some morphological data was collected from field sowings of the materials,

as this was considered to be highly relevant to the overall picture of diversity acquired by DNA fingerprinting.

Fig. 3

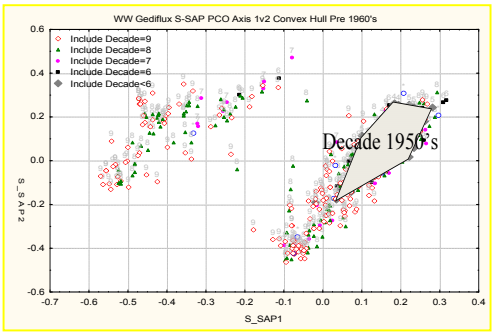


Fig. 4

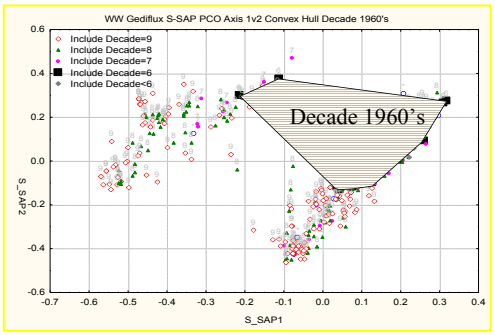


Fig. 5

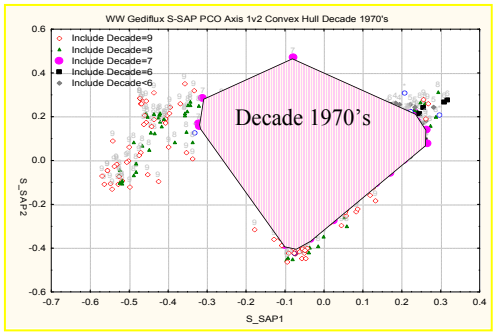


Fig. 6

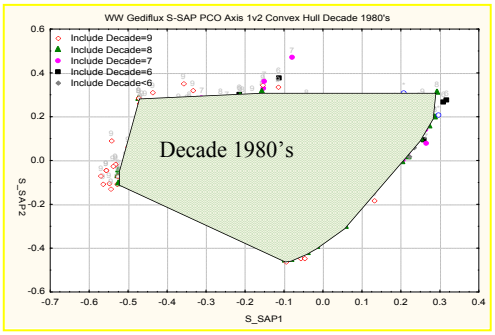
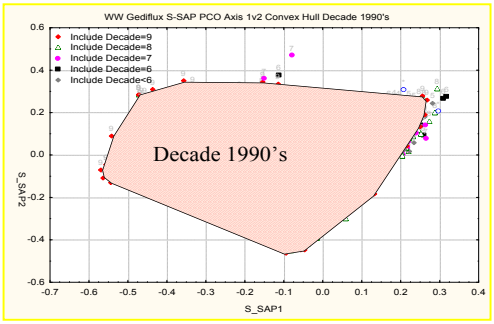


Fig. 7



Figs. 3-7: Convex hulls showing the SSAP diversity over five decades

Three independent DNA profiling platforms – SSR, SSAP and NBS - were applied to the collection. The simple sequence repeat (SSR) component placed one mapped marker on each of the 42 chromosome arms, with genotyping being conducted with fluorescently labelled gwm primers and visualised through ABI platform technology. Single sequence amplified polymorphism (SSAP) profiling targeted genomic sequence flanking the retrotransposon Sukkula 9900 (Leigh et al. 2003), and generated a DNA fingerprint visualised by autoradiogaphy on PAGE separations of TaqI restricted and ligated genomic DNA. This produced genotypic output related to unmapped, non-genic markers. The nucleotide binding site (NBS) technique is a domain-targeted DNA fingerprinting technique which amplifies fragments of a major class of disease resistance genes (Van der Linden et al. 2004). Like SSAP, data was generated from restricted (MseI, HaeIII or PstI) and ligated genomic DNA,

and profiles were visualised by autoradiogaphy on PAGE separation. The final genotypic data set had coverage of about 200 loci.

Some morphological traits were assessed to supplement the genotypic scores. These related mainly to a subset of standard UPOV notes.

The visualisation of diversity trends was based on a series of similarity matrices, which were the basis of a principal coordinate analysis. Diversity at some loci was reduced over the period but showed stability or an increase at others. A clear consistent picture emerged in which in which there was no evidence of any genome-wide reduction in diversity over time.

Unexpectedly, we detected evidence for a widening in genetic diversity during the 1970s and 1980s. As this coincides with the change to semi-dwarf habit, we believe that this expansion of diversity was generated on the introgression of the *Rht1* and *Rht2* 'Green Revolution' genes from CIMMYT. *R* gene diversity increased gradually over the period, reflecting the success of breeders in introgressing novel resistance into their programmes.

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Changes over time in the molecular genetic diversity among Bulgarian bread wheat varieties

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Quantitative estimation of the genetic diversity in a crop germplasm by means of DNA fingerprinting techniques and characterization of diversity trends over time is fundamental for developing science-based breeding strategy. This is important because a decline in genetic variability available for crop improvement might result in a reduction of the crop plasticity to respond adequately to the dynamics of climate, pathogen populations or agricultural practices.

In Bulgaria, systematic improvement activity in wheat started in 1911 by selections from local landrace populations. Since this time, wheat breeding has exploited predominantly the potential of the worldwide genetic resources through inter-varietal hybridization. The introduction of alien genetic variation and the application of experimental mutagenesis have added to the generation of novel diversity. Two major landmarks are the most important: the introduction of semi-dwarfing genes in 1960s aiming at producing high-yielding varieties, and the transition to a breeding strategy directed towards increasing the grain quality after 1990 (Panayotov, 2000).

Here we present the results of a microsatellite-based evaluation of the genetic diversity in 91 winter bread wheat varieties released in Bulgaria up to 2003. Our aim was to answer the question how almost a century-long breeding activity has affected the genetic diversity of wheat germplasm at the molecular level.

Seed samples were kindly supplied by the Institute of Plant Genetic Resources (IPGR, Sadovo, Bulgaria), Institute of Genetics (IG, Sofia, Bulgaria) and RICP (Prague, Czech Republic). The varieties were classified into two groups: old varieties (14), released up to 1960, and modern varieties (77), released in the period 1960-2003. The latter were further grouped according to the decade of variety registration: 1960s (3), 1970s (20), 1980s (19) and 1990s (35). Nineteen wheat microsatellite markers (MW1A001, MW1B002, WMS 3, 18, 46, 95, 155, 165, 190, 261, 357, 389, 408, 437, 458, 513, 577, 631, 680), covering 17 chromosomes and one secalin-specific STS-marker for the rye chromosome arm 1RS were used. In general, a PCR protocol according to Röder et al. (1998) with modifications was followed.

Polymorphism of microsatellite alleles

The material included in this study represent an almost complete spectrum of the bread wheat varieties released in Bulgaria during the 20th century. The microsatellite analysis revealed high levels of DNA polymorphisms. A total of 136 alleles were found at 22 loci. The number of alleles per marker ranged from 2 to 11, with an average of 6.8. For 7 markers, null alleles were detected. The occurrence of rare alleles (frequency <2 %) was observed for 13 markers. The polymorphism information content (PIC) values of the markers ranged from 0.10 to 0.81 with an average of 0.51. These results are very similar to those reported for the South-Eastern European germplasm in a survey of over 500 European wheat varieties (Röder *et al.,* 2002).

Genetic relationships

The selected set of 20 markers discriminated among all 91 varieties (except for two). Three major groups were distinguished. Most of varieties developed in the early years form a distinct cluster (Group 1). Group 2 encompasses the majority of varieties, developed at IPGR. In this group, two sub-clusters are further revealed which include the old and modern IPGR-derived varieties, respectively. Group 3 clusters mostly varieties derived from the Dobrouja Agricultural Institute and few others, released from the IPGR and IG. Minor sub-clusters are observed, which are, in general, in accordance with the genealogical records. The majority of varieties carrying the 1RS.1BL translocation form a distinct sub-cluster.

Changes over time in genetic diversity

The comparison of allele polymorphism in old and modern varieties showed qualitative (loss of alleles and/or appearance of novel alleles) and quantitative (different allele frequency) changes in genetic diversity. The assessment of number of alleles for a given marker over periods of release showed four different patterns. Two markers (WMS95 and 261) displayed a pattern of decreasing number of alleles in varieties released after 1960. Seven markers (WMS18, 190, 408, 437, 513, MW1B002, and SR1R001) showed an increasing number of alleles in varieties developed after 1970 or 1980. For three markers (WMS165, 357 and 458) a shifting pattern of predominance in two or three of their alleles was displayed. For the rest of the markers, alleles detected in old varieties were randomly present in modern ones. The changes in allele number are a result of a loss of some alleles, found in old varieties, as well as of introduction of new alleles into modern ones. The process of allele loss was more intensive in 1960s when only three varieties were registered. A tendency for increasing the number of novel alleles is observed after 1970, this being highest (39) in varieties registered during the last decade.

For 74 % of varieties internal heterogeneity was established with an average level of 10.1 %. The high level of heterogeneity (11.8 %), found in the group of old varieties, remained almost unchanged until 1980s and was reduced to 7.6 % in 1990s. Most of the heterogeneous

varieties, especially those that were non-uniform with more than one marker, were developed earlier and were no longer included in the variety structure of the country, while very few of the commercial varieties are placed in this group.

The genetic diversity was high within both old and modern varieties and averaged 0.64 and 0.65, respectively. No significant differences in the average genetic diversity were recorded regarding the period of variety release (F=0.69), although a tendency for diversity decrease (down to 0.57) was observed in 1970s, after which its value increased gradually to reach 0.71 in 1990s. The genetic diversity values found in Bulgarian bread wheat germplasm over the last century reveal no declining trends in genetic variation and are in good agreement with the conclusions of similar surveys on wheat collections (Donini et al. 2000; Khlestkina et al. 2004).

Acknowledgements

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Influence of environmental conditions on detecting QTLs for the traits pre-harvest sprouting and dormancy in wheat (*Triticum aestivum* L.)

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Introduction

Pre-harvest sprouting (PHS) and dormancy are two important traits for the domestication of modern bread wheat and still problematic for the farmers nowadays. To avoid damage during the harvest it is necessary to localise the genome regions responsible for these traits. In order to find such, regions two wheat mapping populations, the ITMI-population and D-genome introgression lines, the latter developed in Gatersleben, were evaluated for pre-harvest sprouting and dormancy (germinability). Cultivation was performed in the field and in the greenhouse in Gatersleben to discover the influence of environmental conditions on detecting QTLs for these traits.

Material and Methods

A set of 114 recombinant inbred lines of the cut ITMI mapping population was used for the pre-harvest sprouting test. 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 points (1 = resistant, 7 = highly susceptible) was used. Furthermore, a dormancy test was performed. 60 seeds directly harvested and freshly threshed were tested under two different temperature conditions: at 20°C for 7 days and at 10°C for 14 days under a light regime of 12 h light/12 h dark. After counting both germination tests, the percentage of dormant (non-germinated) seeds was calculated followed by the calculation of the dormancy index (DI) (Strand 1965) with the formula:

DI = [2(% dormant seeds at 10°C) + %dormant seed at 20°C] / 3.

For the QTL analysis the available RFLP map created by J. C. Nelson, Cornell University, Ithaca, USA was used. The computer analysis was done with the programme QGENE (Nelson 1997). In addition, 85 "Chinese Spring"-*Aegilops tauschii* introgression lines were also cultivated and evaluated. Genotypic analysis was performed using a set of 80 microsatellite markers previously mapped on the D-genome of wheat (Pestsova et al. 2001).

Results and Discussion

The evaluation of dormancy and pre-harvest sprouting resulted in different data under field and greenhouse conditions. But there was no significant correlation between the evaluated traits and the environmental conditions. However, under field conditions, major QTLs (LOD-score > 3.0) could be localized for pre-harvest sprouting on chromosome 4AL and for dormancy on chromosome 3AL for the ITMI-population (Lohwasser et al. 2005). Under greenhouse conditions a main QTL on chromosome 4AL was found for both traits, for pre-harvest sprouting and dormancy. However, the major QTL on chromosome 3AL could not be detected again (Table 1).

Table 1: Detected QTLs for the ITMI-population.

Character	LOD > 3.0	LOD ≥ 2.0 ≤ 3.0	LOD > 1.5 < 2.0
PHS (field)	4AL	1AL; 1BL; 3DL	1BS;
PHS (greenhouse)	4AL	1AL; 1BL	-
Dormancy (field)	3AL	2BL; 7BL	7DL
Dormancy (greenhouse)	4AL	-	3AL

The D-genome introgression lines were researched under greenhouse conditions at first. A major QTL could be localized for dormancy on chromosome 6DL but no QTL was found for pre-harvest sprouting (Lohwasser et al. 2005). Under field conditions the major QTL on chromosome 6DL could not be identified again. Also for pre-harvest sprouting, it was not possible to find an important genome region (Table 2). Only minor QTLs were found. There seems to be no influence of the D-genome on the traits pre-harvest sprouting and dormancy. Also an influence of environmental conditions could not be researched.

Table 2: Detected QTLs for the introgression lines

Character	LOD > 3.0	LOD ≥ 2.0 ≤ 3.0	LOD > 1.5 < 2.0
PHS (field)	-	5DL; 6DS	-
PHS (greenhouse)	-	-	-
Dormancy (field)	-	1DS	2DS
Dormancy (greenhouse)	6DL	-	6DS

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Screening the Oregon Wolfe Barley population for Aluminium tolerance

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Introduction

Aluminium (AI) toxicity has limited the expansion of the barley crop into important agricultural areas of the world (Minella and Sorrells, 1992). The problem is due to the dissolution of Al at low pH, which is phytotoxic even in micro molar concentrations. In particular, the speciation and ligand substitution kinetics of the Al^{3+} ions in the pH range of 3 to 7 govern its toxicity towards plants (Swaddle et al., 2005). The Al^{3+} ions are highly toxic to plant roots (Kinraide, 1991) resulting in a poorly developed root system and thus susceptibility to drought stress and nutrient deficiencies (Foy, 1988; Kochian, 1995).

While barley is the most sensitive species to Al toxicity among small grain cereals, variation in Al resistance between cultivars does exist (Zhao et al., 2003). This genetic variation can be exploited using conventional crossing procedures, but with the aid of genetic maps, markers and quantitative trait locations (QTL analysis) greater precision can be obtained in selecting desired genotypes (Foster et al., 2000). In this study, the Oregon Wolfe Barley (OWB) mapping population was evaluated for its tolerance to Al toxicity and to locate the genes responsible.

Materials and Methods

The Oregon Wolfe Barley (OWB) mapping population consists of 94 doubled haploid lines developed from the F_1 of a cross between dominant and recessive marker stocks of Dr. R. Wolfe. Phenotypic screening was done by two widely used nutrient solution culture techniques - the Modified Pulse Method and Root Growth method (Hede et al., 2002) with some modifications. The former method evaluates Al tolerance, based on the ability of the seedlings to continue root growth after a short pulse treatment with Al, whereas, the latter evaluates Al tolerance by Root Tolerance Index (RTI) which is root growth in Al compared to the root growth in control. Integrating the phenotypic data with the marker data available, Quantitative Trait Loci (QTL) analysis was done using the QGENE program (Nelson, 1997) to map the genetic loci involved for Al tolerance.

Results and Discussion

Modified Pulse Method

Analysis done using the phenotypic data from this method identified a significant QTL on linkage group 3H with a LOD score value of 2.68, explaining about 13% of phenotypic variation for this trait.

Root Growth Method

Similar results were obtained using the root growth method, which located a QTL on linkage group 3H but with a lower LOD score value of 2.14 showing 10% variation. Interestingly, a significant QTL harboured by linkage group 4H was also mapped with a LOD value of 2.78 which could explain 16% of the whole phenotypic variation.

Previous mapping studies using 'Dayton', one of the most Al tolerant barley cultivars, reports Al tolerance as conferred by a single gene (Minella and Sorrells, 1992), and this gene has been mapped to the long arm of chromosome 4H (Tang et al., 2000). In the present study, in addition to the alleles on 4H, some minor genes have also influenced the trait. These minor gene effects have also been reported by Reid (1971) so also in other cereals such as wheat (Aniol, 1990), maize (Sibov et al., 1999) and rye (Gallego and Benito, 1997). Briefly, the tolerance is dependent on allelic dosage, which in turn depends on Al concentration and pH of the solution.

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The role of disulfide-reductase in determination of technological properties of grain in *Triticum aestivum* L.

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The key role of covalent disulfide bonds in determining the gluten structure and properties is well-established in common wheat (Kaczkowski and Meleszko, 1980; Shewry and Tatham, 1997). In the Laboratory of Technical Biochemistry in Siberian Institute of Plant Physiology and Biochemistry the enzyme GSH-dependent proteindisulfide oxidoreductase (EC 1.8.4.2) was extracted and purified to the electrophoretically homogeneous grade. It catalyzes the dissociation of protein S-S bonds in kernels (Osipova et al., in press). As follows from SDS-PAGE data, the enzyme consists of two subunits with molecular weights 77 and 73 kDa. The molecular weight of the native enzyme is about 167 kDa and this distinguishs it from the known SH/SS metabolism enzymes of the wheat kernel. According to our data, in the developing kernel the enzyme shows maximum of its activity on the 30th day, on the stage of milk maturity.

The ability of wheat disulfide reductase to dissociate S-S bonds of storage proteins in developing grain supposes its participation in the formation of gluten structural matrix and physical properties of dough. For elucidation of disulfide reductase influence on storage protein aggregation, the acetic acid-soluble fraction of these proteins was used including mostly gliadins and, partially, glutenin subunits. Optical solution density was accounted for by the aggregation process time via spectrophotometry. Aggregation index τ_{10}/C (solution turbidity per protein concentration unit for 10 min of the process) was calculated by the formula $\tau_{10} = 2A/C$, where A – optical solution density at λ = 350 nm, C – protein concentration, %. Chopin Alveograph was used for evaluation of dough physical properties.

The results of experiments on aggregation carried out under conditions optimal for disulfide reductase activity pH7,5 are presented in Fig. 1. Aggregation ability of storage proteins in the presence of enzyme was decreased on 25-30% in comparison with the control. Obviously, it is caused by dissociation of S-S bonds in proteins followed by weakening of the gluten matrix rigidity.

Addition of disulfide reductase in the flour of three cultivars with different initial dough parameters resulted in changes of physical parameters of dough (Table 1). In all three cases a significant increase of extensibility was observed by 13-17%. It may be supposed that this effect is a consequence of dissociation of S-S bonds by the enzyme in dough. The increase of extensibility caused the increase of dough strength and decrease of P/L ratio. We suppose that

in bread baking, disulfide reductase may improve the quality of excessively strong, shortly-breaking gluten.

The work is carried out now on the determination of the chromosome localization of structural and regulatory genes for disulfide reductase using the nulli-tetrasomic lines of Chinese Spring.

Table 1: The effect of disulfide reductase (RED) addition[a] to flour on physical properties of dough in different wheat cultivars. (W – dough strength, units of alveograph; P – stiffness, mm; L – extensibility, mm)

Cultivars	Alveograph parameters	Control	With SS RED
Mironovskaya 808	W	188 ± 8	223 ± 6**
	P	60 ± 1	64 ± 4
	L	112 ± 9	131 ± 10*
	P/L	0,54 ± 0,04	0,49 ± 0,1
Irkutskaya winter	W	175 ± 5	226 ± 5**
	P	67 ± 1	64 ± 2
	L	62 ± 3	94 ± 2**
	P/L	1,1 ± 0,03	0,7 ± 0,02**
Omskaya	W	226 ± 34	262 ± 2
	P	147 ± 3	151 ± 4
	L	32 ±2	41 ± 1**
	P/L	4,9 ± 0,3	3,7 ± 0,1*

[a] - 300 mg of enzyme sample containing 5 mg of RED in NaCl was added; 300 mg of NaCl was added to the control

* - significant at $P \leq 0,05$; ** - significant at $P \leq 0,01$.

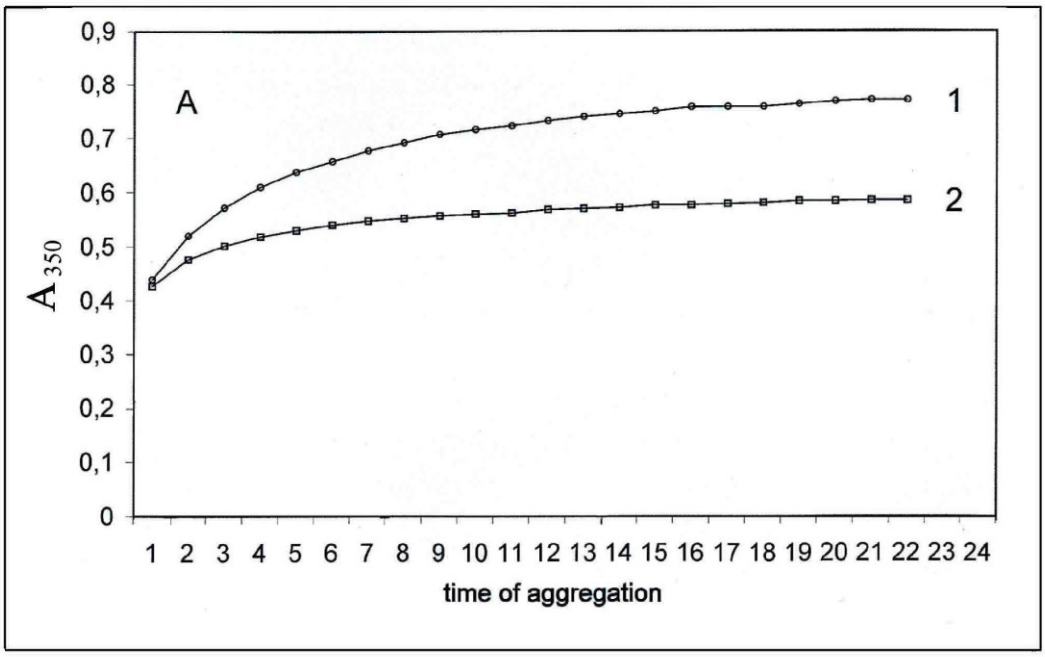

Fig. 1: Wheat SS-RED impact on the aggregation of acetic acid-soluble gluten proteins. B – pH 7.5; 1 – control (without SS-RED), 2 – test (with SS-RED). Average values of 3 independent tests are presented

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The effect of photoperiod and vernalization on flowering time in four wheat lines with the substituted 3B chromosome of Česká Přesívka

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In contrast to the major groups of genes influencing the flowering time of wheat (genes for vernalization and photoperiod responses, *Vrn*, *Ppd*, respectively), the effects of earliness per se genes (*Eps*) are manifested mainly as QTLs that influence the flowering time independently of environmental conditions; they are probably related to differences in leaf or spikelet primordial initiation. *Eps* genes are widespread throughout the wheat genome: their presence has been reported on chromosomes 2B, 3A, 4B, 4D, 6B, 6D, 7B (see: Worland 1996), and indications for about twenty-five *Eps* loci follows from comparative genomics studies (Snape et al. 2001). Yet the mechanism of their function still stays unresolved. A

major *Eps* locus was mapped distal to an RFLP marker locus *Xcdo549* on the short arm of chromosome 3A (Shah et al. 1999).

Substitution of chromosome 3B of a Czech alternative wheat variety Česká Přesívka, (CP3B), into the genetic background of a spring variety Zlatka revealed the action of a gene /s that influence flowering time - the substitution line Zlatka (CP3B) showed a delay in flowering and better over-wintering (Košner 1987). A more detailed analysis of the influence of the 3B chromosome substitution in the genetic backgrounds of two spring (Jara, Sandra) and three winter (Košutka, Vala, Zdar) wheat backgrounds suggested the presence of an *eps* gene that could be homoeologous to the previously known *eps* gene on chromosome 3A. Comparisons between the recipient varieties and the respective substitution lines (SLs) generally revealed no effect on the vernalization response due to the substitution, although there were signs of a genotype x vernalization interaction in the Sandra background. The photoperiod response of both substitution lines in the spring wheat backgrounds compared to the parental cultivars showed a considerable effect on heading of the chromosome 3B substitutions under both short and long days, suggesting the effect of an *eps* gene /s (Košner and Pánková 2002). To describe the phenotype more precisely and to define the point where the flowering time gene acts, two spring and two winter wheat SLs of chromosome 3B of Česká Přesívka were tested under different photoperiod and vernalization regimes. The validity of the substitutions of chromosome 3B had previously been verified using SSR markers.

Materials

Spring wheat varieties / SLs: Zlatka, Sandra / Zlatka (CP3B), Sandra (CP3B)

Winter wheat varieties / SLs: Zdar, Vala / Zdar (CP3B), Vala (CP3B)

Methods

Vernalization treatment: All wheat materials were vernalized (8 weeks; 1 - 3^{o}C), and planted on 20th April 2004 into field plots with unvernalized controls.

Photoperiod treatment: Fully vernalized seeds were planted on 20th April 2004 into field plots (natural long day) and into sheltered plots giving a 10-hrs day length regime.

Days to heading of the plants were evaluated.

Results

Significant differences in time of heading, up to 19 days, caused by the substitution of chromosome CP 3B were revealed between all four SLs compared to their parental varieties grown under short day conditions. Long photoperiod caused the differences to decrease but they were still significant in the winter wheat combinations. In the spring combinations of SLs and parental lines, the long photoperiod minimized the differences between the heading times (Table 1).

Discussion and Conclusions

Short day conditions differentially influenced the heading time of the SLs and their parental varieties in all of the analysed combinations (Table 1). Whilst vernalization minimized these differences in heading time between the SL and their parental varieties in the spring wheat materials (Zlatka, Sandra), a significant difference was still found in the winter wheat materials (Zdar, Vala). These results correspond to the preliminary results about the absence of significant differences between the phenology of the spring SL and their parental varieties

(Zlatka, Sandra) that was also found under glasshouse conditions (long day, 20C), (unpublished data), but differences occur in a winter genetic background.

Table 1: Days to heading of SLs for CP3B and their parental lines under different environmental conditions

8 weeks vernalization, 10 h photoperiod								
	Zlatka	ZL(CP3B)	Sandra	San(CP3B)	Vala	Val(CP3B)	Zdar	ZD(CP3B)
Mean	99,69	108,38	94,00	97,36	103,55	84,27	83,22	102,42
St.dev.	2,1750	4,4058	4,9570	2,4371	1,6348	2,5334	2,5386	1,9752
t stat	5,8098*		2,2872*		21,2000**		18,8111**	

0 weeks vernalization, natural long photoperiod								
	Zlatka	ZL(CP3B)	Sandra	San(CP3B)	Vala	Val(CP3B)	Zdar	ZD(CP3B)
Mean	60,40	60,25	58,81	60,46	-	-	-	-
St.dev.	3,2347	3,3198	1,2150	2,3011	-	-	-	-
t stat	0,8098		1,9606		-		-	

8 weeks vernalization, natural long photoperiod								
	Zlatka	ZL(CP3B)	Sandra	San(CP3B)	Vala	Val(CP3B)	Zdar	ZD(CP3B)
Mean	53,25	52,13	53,05	49,56	58,43	62,17	65,57	63,00
St.dev.	2,0505	4,6600	5,2012	1,5092	2,8791	2,4647	3,6535	2,0917
t stat	0,8098		1,9606*		5,1213*		2,5926*	

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Lipoxygenase activity in intevarietal substitution lines of the homoeologous group 4 and 5 chromosomes of EWAC key substitution sets

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Lipoxygenases (linoleat:oxygen oxidoreductase, EC 1.13.12, Lpx) occur in plants as groups of enzymes catalyzing dioxidation of non-saturated fatty acids by forming superoxide radicals. Structural genes of Lpx are known to be differently expressed in plant development and, apparently, their different isoforms may initiate the synthesis of signaling molecules, involved in inducing structural or metabolic changes in the cell or may participate in the fine regulation of developmental processes (Siedow, 1991). Lpx expression is regulated by jasmine and abscisic acids and also by different forms of stress, such as wounding, water deficiency, or pathogen attack (Porta et al.1999).

The structural genes for Lpx biosynthesis are known to be located in the chromosomes of homoeologous groups 4 and 5 (Hart, Langstone, 1977). In our research specific Lpx activity was studied in the substitution lines of these chromosomes of three sets, Saratovskaya 29/Jantezkis Probat (S29/JP), Chinese Spring/Synthetic (CS/Syn) and Cappelle-Despre 3/Bezostaya 1. Seeds were kindly provided by Andreas Börner, IPK, Gatersleben, Germany. The lines were grown in two replicates in the green-house of Institute of Cytology and Genetics, Novosibirsk.

Lpx activity was assayed spectrophotometrically according to Doderer (Doderer et al. 1992). Specific activity was expressed by ratio of activity units to 1 mg of protein in 1 ml of incubation media. Protein concentration was determined by Lowry method (Lowry et al. 1951). Results are presented as % relative to the recipient.

In the first set it was found that the donor had twice as high a level of Lpx activity. Only in the lines with 4A and 4D substitutions this parameter was found to be higher than in the recipient S29, with 66 and 34%, respectively (Fig.1). In the second set the donor Syn showed only 43% of the specific activity of the recipient CS. The same level of decrease was found in the substitution line CS/Syn 4D (Fig.2). In the third set, the parental cultivars did not significantly differ on Lpx activity. However in the lines with 4B and 4D substitutions specific activity of Lpx turned out to be significantly higher than in both cultivars at 76 and 36%, respectively (Fig.3). Probably, the molecules of Lpx synthesized by the genes of these chromosomes of the donor are more active in the genetic background of the recipient.

The results obtained show that the level of Lpx activity is under complex genetic control. The replacement of the chromosome carrying the structural gene for Lpx biosynthesis caused the changes depending on the genotype of the line. However, in all three cases the changes were detected only in the 4^{th} homoeologous group of chromosomes. It may be connected with more pronounced role of the isoforms coding for the genes determining the total level of Lpx activity and supposes the genetic differences of input of separate Lpx molecules in the total activity level. Taking into account participation of Lpx in the adaptive reactions of plant to different stresses further investigations of the genetic control of its activity level seems to be important.

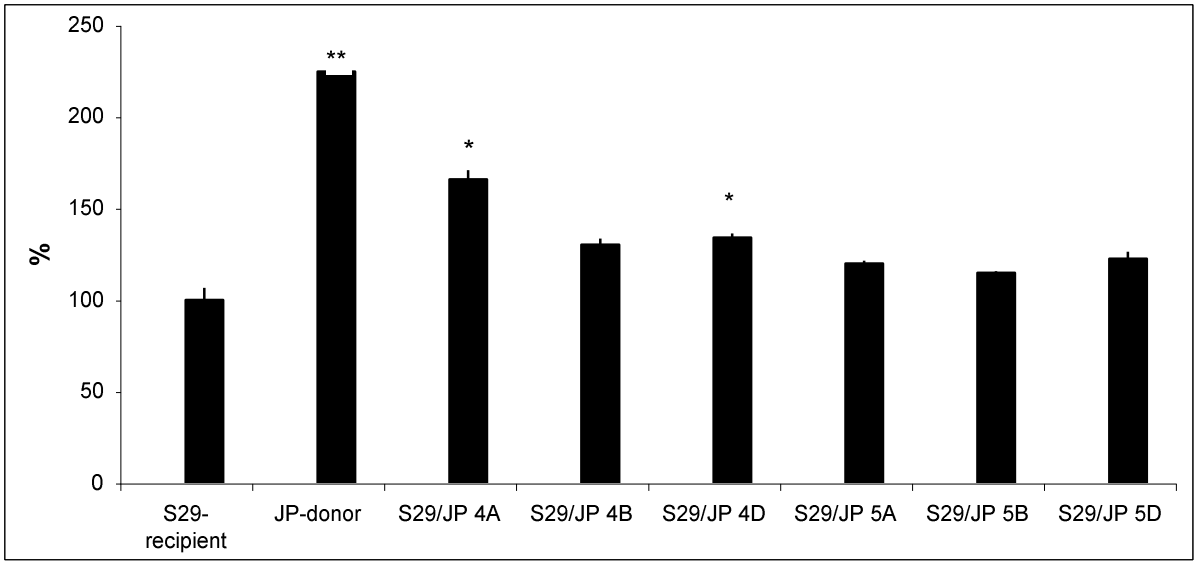

Fig. 1: Specific Lpx activity in the intervarietal substitution lines Saratovskaya 29/Janetzkis Probat (S29/JP) on chromosomes of 4 and 5 homoeologous groups. *, ** - significantly different from S29 at P<0.05, P<0.01, respectively.

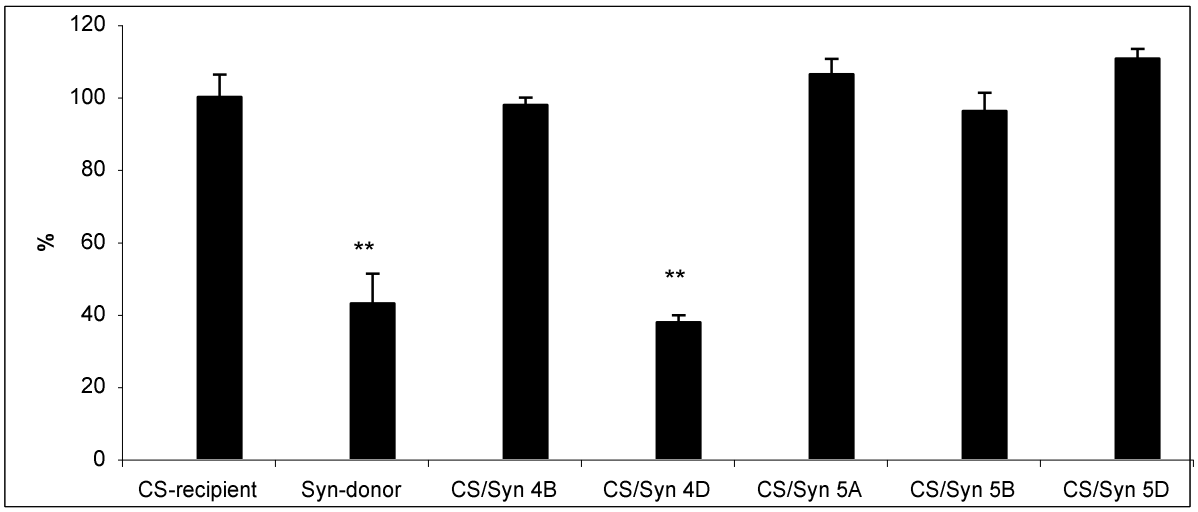

Fig. 2: Specific Lpx activity in the intervarietal substitution lines Chinese Spring / Synthetic (CS/Syn) on chromosomes of 4 and 5 homoeologous groups. ** - significantly different from CS at P<0.01.

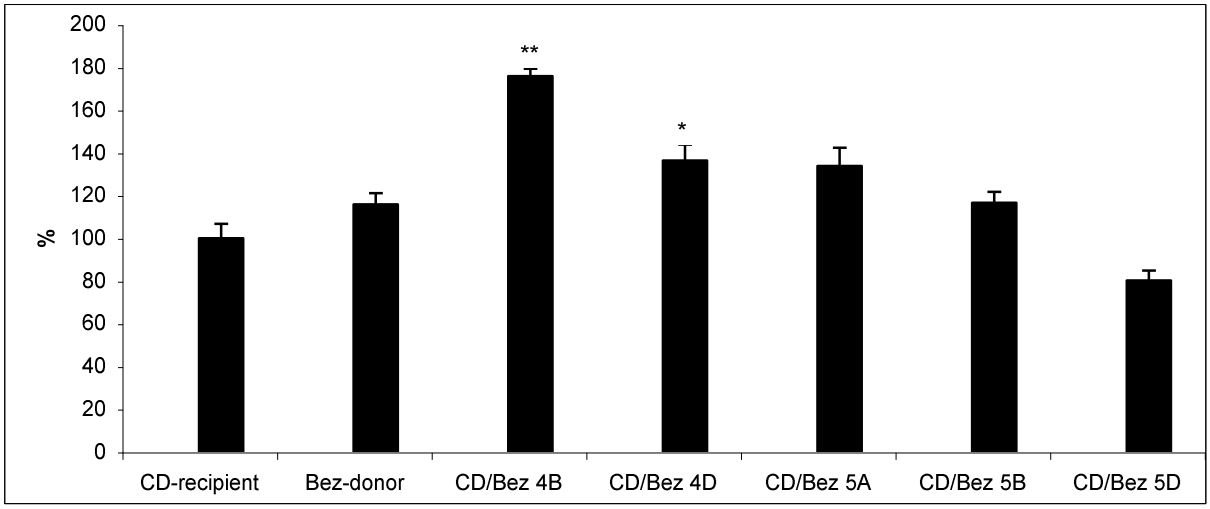

Fig. 3: Specific Lpx activity in the intervarietal substitution lines Cappelle-Desprez / Bezostaya (CD/Bez) on chromosomes of 4 and 5 homoeologous groups. *, ** - significantly different from CD; $P<0.05$, $P<0.01$, respectively.

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Relationship between specific lipoxygenase activity and technological characteristics of gluten in recombinant inbred lines of the ITMI mapping population

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Lipoxygenases (linoleat: oxygen oxidoreductase, Lpx, EC 1.13.11.12) are a group of enzymes that catalyze the dioxygenation of polyunsaturated fatty acids containing 1,4-pentadiene system to form a Z,E- conjugated hydroperoxide fatty acid (Grechkin1998). Lipoxygenases are widely distributed in plants and play an important role in responses to pest

attacks and wounding (Siedow 1991). Lpx of wheat mainly is studied in connection with its role in bleaching carotenoid pigments in durum wheat grain (Pastore et al. 2000).

Our interest to Lpx is conditioned by its participation in the formation of superoxide radicals that may *in vivo* oxidize SH-groups of wheat storage proteins with the formation of inter- and intramolecular disulfide bonds stabilizing gluten protein complexes in hexaploid wheat.

The ITMI population consists of recombinant inbred lines obtained from a cross between bread wheat 'Opata 85" and synthetic hexaploid wheat W7984. The parents have contrasting levels of Lpx activity, low and high, respectively. Every line inherited a different set of structural and regulatory genes determining the Lpx activity level. This allows studying the distribution of the character in the population and its correlation with such an important agronomy trait as technological properties of grain.

Materials and Methods

Seeds of 53 the recombinant inbred lines and the parental genotypes of the ITMI mapping population were studied.

Lipoxygenase activity was analyzed in Tris-soluble flour extracts according to Doderer (Doderer et al. 1992). Protein concentration was determined by the Lowry method (Lowry et al. 1951). The analysis of 17 technological traits in recombinant inbred lines corresponds to the "Methods of State Variety Testing of Crops" accepted in Russia. The lines were grouped according the value levels of the characters studied and the correlation coefficients were determined in the whole population and within groups. Correlation coefficients between the traits were calculated with the help of computer program Microsoft Excel 2000.

Results and Discussion

Calculation of correlation coefficients within the whole population showed that Lpx activity is significantly positively related to thousand grain weight (0.305*), vitreousnes (0.318*) and water absorbing capacity (0.289*).

The data of Table 1 shows that the correlation of Lpx activity with technological parameters depends on the values. At low values specific Lpx-activity shows negative significant correlation with Tris-soluble protein content. At high values Lpx-activity is positively related to thousand grain weight. Correlations of Lpx-activity with key quality parameters were negative at high Lpx values.

The relationship between technological parameters with various levels and Lpx activity is shown in Tables 2 and 3. Some of the parameters were found to be connected with Lpx activity only at high values. Only extensibility was significantly negatively connected with this trait at all values.

It was found that most of the gluten physical properties correlate with the content of disulfide bonds in the storage proteins, which are responsible for stabilizing space structure of the gluten complex (Làszity 1984). Wheat seed contains a specific system of enzymes, referring to as an oxidoreductase class and directly or indirectly regulating thiol-disulfide metabolism in proteins. Lpx is one such enzyme, which can oxidize SH-groups of proteins with the formation of SS-bonds (Shiiba et al. 1991). According to the data obtained in this investigation, it may be concluded that specific Lpx activity is related to the quality of wheat gluten. For example, this trait was found to be directly related to dough extensibility which is determined by the number of S-S bonds. These relationships also depend on the value levels of Lpx activity in different lines which, in turn, is determined by the genotype.

Table 1: Correlation relationships of specific Lpx-activity with technological parameters as dependence on Lpx values (in units of spectrophotometer, u.s.)

Traits	Low values n=26 80,3±14,0 u.s.	High Value n=29 144,6±29,0 u.s.
Milling properties		
Thousand grain weight (g)	-0.287	0.416*
Vitreousness (%)	0.333	-0.041
Gluten content (%)	0.317	0.103
Specific flour particles surface (cm^2/g)	-0.241	0.096
Particles size (μk)	0.303	-0.108
Alveograph		
Dough strength (units alveograph)	0.315	-0.402*
Tenacity (P, mm)	0.328	-0,324
Extensibility (L, mm)	-0.208	-0.059
P / L ratio	0.331	-0.041
Farinograph		
Water absorbing capacity (%)	0.359	0.003
Dough formation (min)	0.234	-0.348
Resistance to mixing (min)	0.074	-0.430*
Dough stability (min)	0.219	-0.389*
Dough liquefaction (units farinograph)	-0.308	0.301
Valorimeter number (units farinograph)	0.251	-0.381*
Tris-soluble protein contents (mg / ml)	-0.474*	0.061

* - $P < 0.05$

Table 2: Correlation relationships between technological and milling parameters with various values and specific Lpx-activity

Value levels of technological traits	Mean value	Standard deviation	Correlation with Lpx activity
Thousand grain weight (g)			
low value	54.8	2.6	0.126
high value	62.5	3.5	0.464*
Gluten content (%)			
low value	34.3	1.8	0.195
high value	39.7	2.3	0.433*
Dough liquefaction (units of farinograph)			
low value	54.1	15.1	-0.127
high value	102.2	30.2	0.443*
Valorimeter number (units of farinograph)			
low value	51.8	6.8	-0.382*
high value	66.5	3.2	0.094

* - $P < 0.05$

Table 3: Correlation relationships between alveograph parameters with various values and specific Lpx-activity

Value levels technological traits	Mean value	Standard deviation	Correlation with Lpx activity
Tenacity (P, mm)			
low value	64.9	10.6	-0.17
average value	92.7	7.9	0.30
high value	126.6	12	0.46*
Extensibility (L, mm)			
low value	67	16.6	-0.346
average value	92.9	6.7	-0.468*
high value	119.6	10.9	-0.538*
P/L ratio			
low value	0.57	0.12	-0.141
average value	1.02	0.13	-0.208
high value	1.69	0.45	0.505*

* - $P < 0.05$

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Microsatellite marker analysis of winter hardiness in wheat

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Introduction

Freezing temperatures often become the cause of losses in agricultural wheat productivity in Ukraine. Winter conditions expose young seedlings to many kinds of stress: direct frost effects, cold winds, snow cover, glaciation of the soil, the consequent deficiency of water and nutrients or frost lifting in spring. Frost resistance is one of the components of winter hardiness. Cereals, which are frost resistant generally survive the winter well (Sutka et al. 1986). But there is an insignificant number of varieties, growing up in conditions of the Southern Ukraine, that possesses sufficient frost and winter hardiness.

In the conditions when the constant snow cover is absent and long thawing weather changes often to frost (-15^{o}C), increasing of the frost tolerance is the main task for most breeding programs (Fayt 2004). Frost resistance is regulated through complex genotypic and environmental interactions inducing a large number of physical and biochemical changes in the plants. The cold acclimation process is associated with altered gene expression. A number of low temperature induced genes have been characterized.

Inheritance of frost hardiness in winter wheat is regarded as a quantitative trait controlled by many loci, at least on 15 of the 21 pairs of chromosomes in hexaploid wheat. The major genes affecting winter hardiness have been mapped on the long arms of chromosomes of homoeologous group 5: *Fr-A1* and *Fr-A2* (5A), *Fr-D1* (5D) and *Fr-B1* (5B) (Galiba et al. 1995; Snape et al. 1997; Toth et al. 2003; Vaguifalvi et al. 2003).

Material and Methods

Winter wheat varieties and six F_2 populations have been analyzed for frost resistance in this work (Tables 1 and 2).

Table 1: Test to identify the frost resistance of Ukrainian hexaploid wheat varieties in phytotron conditions (-11 ^{o}C)

Varieties	Percentage survival	Varieties	Percentage survival
Obriy	9	Erythrospermum 604	47
Odesskaya krasnokolosaya	17	Nikoniya	47
Zolotava	18	Leleka	47
Ol'viya	19	Pryboy	49
Odesskaya polukarlykovaya	21	Krasunya odesskaya	49
Yubileynaya 75	22	Prokof'evka	52
Burevestnyk odesskyj	23	Tira	52
Chervona	24	Lan	53
Odesskaya 66	25	Peresvit	53
Hostianum 237	25	Vympel odesskyj	53
Prometey	26	Ukrainka odesskaya	54
Selyanka	27	Luzanovka odesskaya	54
Zirka	28	Fedorovka	54
Bezostaya1	30	Fantaziya odesskaya	58
Kuyalnyk	30	Symvol odesskyj	58
Odesskaya ostystaya	30	Khvylya	58
Bryz	32	Stepova	58
Lada odesskaya	32	Pivdenna Zorya	59
Zlagoda	33	Progress	59
Brigantina	35	Krymka mestnaya	60
Odesskaya 132	36	Zastava odesskaya	61
Odesskaya 51	37	Ljubava odesskaya	61
Yakor odesskyj	37	Odesskaya 117	61
Syrena odesskaya	38	Mironovskaya 808	62
Odesskaya 266	38	Albatros odesskyj	63
Strumok	39	Povaga	64
Lelya	39	Odom	64
Viktoriya odesskaya	39	Albidum 114	65
Nagoroda	39	Chayka	69
Odesskaya 162	40	Prima odesskaya	75
Porada	40	Odesskaya 16	79
Odesskaya 130	41	Znakhidka odesskaya	87
Odesskaya 120	42		
Skorospelka	42	$\sum$	3207
Darunok	43	$\bar{x}$	44.54167
Odesskaya 267	44	σ	15.85226
Fregat odesskyj	45	N	72
Yunnat odesskyj	45	CV,%	35.58972
Odesskaya 26	46	Max	87
Panna	46	Min	9

The test to identify frost resistance was carried out using the procedure described by Musich (1987). Seeds were potted in boxes and grown in phytotron cambers at 20^0 C. After appearance of shoots, boxes were put in a chamber at 2^0 C and light intensity 2000-4000 lk during 12 days (first experiment) or 25 days (second and third experiment). Later the temperature in the chamber was reduced to -4^0 C for 1 day and to -6^0 C for 2 days. Then the temperature was gradually lowered to the freezing temperature, -12^0 C (-11^0 C), maintained there for 1 day. After freezing, the temperature was gradually increased to 16^0 C. Frost tolerance was estimated after 18 days. Microsatellite analysis of F_2 populations was performed as described by Röder et al. (1998) with some modifications.

Table 2: Analysis of F_2 populations in phytotronic conditions at (-12^oC), % of survived plants (number of survived plants/number of all plants)

F_2 populations	First experiment, %	Second experiment, %	Third experiment, %
Odom/Sirena odesskaya	22.3 (25/112)	17.7 (41/232)	67.6 (171/253)
Obriy/Progress	72.5 (166/229)	33.5 (73/218)	53.7 (264/492)
Odesskaya16/Bezostaya1	23.5 (52/221)	-	-
Erythrospermum2917/Odesskaya132	17.2 (28/163)	57.4 (81/148)	-
Albidum114/Odesskaya132	27.2 (44/162)	65.4 (100/153)	-
Luzanovka odesskaya/Odesskaya krasnokolosaya	37.3 (69/185)	40.3 (64/159)	49.0 (214/437)

Results and Discussion

Three groups of varieties have been determined essentially different in the level of frost resistance within the collection of Ukrainian varieties (PBGI) and some Russian wheat varieties as a result of three-year studies on conditions of Southern Ukrainian steppe (Table 1). The first group – the varieties with high and good frost resistance on conditions includes Albidum114 (53-88%), Odesskaya16 (72-100%), Odom (64-95%), Luzanovka odesskaya (54-86%), Progress (50-80%) and line Erythrospermum2917 (62-65%). The second group – the varieties with a medium frost resistance comprises Bezostaya1 (30-60%), Odesskaya132 (36-60%), Sirena odesskaya (38-44%). The third group includes the varieties with the low level of frost resistance: Odesskaya krasnokolosaya (17-44%) and Obriy (4-37%).

We have created F_2 populations: Odom/Sirena odesskaya, Obriy/Progress, Odesskaya 16/Bezostaya 1, Erythrospermum 2917/Odesskaya 132, Albidum 114/Odesskaya 132, Luzanovka odesskaya/Odesskaya krasnokolosaya. The large-scale introduction in breeding process in Southern Ukraine of the variety Bezostaya 1, the frost resistance of which is lower then average, promoted to growth of productivity but decreased the frost resistance of varieties. This was the first stage of decreasing frost resistance in South of Ukraine. Therefore, crossing combinations with participation of a variety with high level of frost resistance, Odesskaya 16, were created. The second stage of decrease of frost resistance in South of Ukraine is connected with the introduction of spring varieties having the intensive used *Rht* genes in their background. Semi-dwarf varieties Obriy and Odesskaya krasnokolosaya have such spring wheats as one of the parents in their pedigree and possess low level of frost resistance. Therefore, crosses Obriy/Progress and Luzanovka odesskaya/Odesskaya krasnokolosaya were made. The combinations of crosses will allow us to reveal genes promoting high frost resistance in the varieties Odesskaya 16, Luzanovka odesskaya, Albidum 114, selection line Erythrospermum 2917 and Progress with midle level of resistance in comparison with poorly cold-resistant varieties. The test to identify the frost resistance of varieties and F_2 populations is demonstrated (Table 2).

Parental forms were analyzed by using microsatellite markers (*Xgwm*, *Xbarc* and *Xwmc*). Polymorphism between parental varieties was observed for 24 of 41 microsatellite markers (59 %). The regions of homoeologous groups 5, where was mapped *Fr*-genes, have been studied by MS-analysis. 8 of 12 microsatellite markers, that localised on the long arm of chromosome 5A (regions *Fr-A1* and *Fr-A2*), detected polymorphism between parental varieties. Polymorphism between varieties for chromosome 5D (region *Fr-D1*) was observed for 4 of 7 MS-markers. Percentage of polymorphism between varieties of the combination of crossing Odom/Sirena odesskaya was 19,5%, Obriy/Progress – 24.4%, Odesskaya 16/Bezostaya 1 – 22.0%, Erythrospermum 2917/Odesskaya 132 - 22.0%, Albidum 114/Odesskaya 132 – 30.0%. Highest level of polymorphism was observed for the combination Luzanovka odesskaya/Odesskaya krasnokolosaya. Therefore RILs Luzanovka odesskaya/Odesskaya krasnokolosaya have been developed to be used in further studies.

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Technological properties of grain and flour in lines of common wheat with introgressions from *Aegilops speltoides*

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The wild relatives of common wheat are considered as a source of genes for improvement of bread-making quality (DePace et al. 2001). In Russia, after crossing of common wheat cultivar ‘Skala’ with *Triticum dicoccum* a new genotype was obtained, ‘Hybrid 21’. Its grain technological parameters are higher not only comparing to the parental cultivar but to cv. ‘Saratovskaya 29’ having an outstanding grain quality. In this work, technological characters of grain and flour were compared between the lines with introgressions from *Aegilops speltoides* and parental common wheat cultivar ‘Rodina’. The presence of alien introgression was confirmed through the molecular analysis using microsatellite markers and subtelomeric repeats (Table 1) (Adonina et al. 2004). The lines were obtained at the Agricultural Research Institute of Non-Chernozem Zone of Central Russia (Fig.1) (Lapochkina et al. 2003). The

grain for this investigation was obtained from lines grown in two replicates under field conditions near Novosibirsk. T-criterion of Student was used for statistical processing of data.

Table 1: Some characteristics of common wheat lines of cv. 'Rodina' with introgressions from *Ae. speltoides*

Lines	Growth habit	Gliadin composition	Substitution and molecular markers (2)
76/00i	Spring	Wheat only	7D/7S, microsatellites
81/00i	Spring	Secalins	7D/7S, microsatellites
84/00i	Spring	Secalins	*Subtelomeric repeat* Spelt52

The initial cultivar 'Rodina' has the cv. 'Kavkaz' in its pedigree. It may carry a mixture of grains containing secalins in their gliadin composition although we did not meet such a genotype in the laboratory population but only in the introgression lines. 'Rodina' may be attributed to the cultivars with medium milling characteristics (Table 2). Physical properties of dough in cv. 'Rodina' are rather low (Table 3). Two introgression lines, 84/00i and 81/00i have even a lower dough strength. Analysis of gliadin composition showed the presence of marker components for the 1B/1R substitution. It is known that this substitution negatively influences physical properties of dough decreasing its strength and extensibility (Bullrich et al. 1998). The same effect was detected in the two introgression lines.

Table 2: Milling properties of introgression lines of cv. 'Rodina', Novosibirsk, 2003

Parental cultivar and lines	Weight of 1000, g	Vitreousness, %	Wet gluten content in grain, %	PSH-4	
				Specific flour surface, cm2/g	Particle size, μk
Rodina	29,7±1,6	82±1,0	39,0±2,0	1884±24	21,38±0,0
76/00i	28,2±0,5	93±1,0**	40,0±1,0	1708±1	23,58±0,0
84/00i	29,8±1,5	83±0,9	43,0±1,0**	1986±10	20,28±0,1
81/00i	23,4±1,0***	89±1,9**	39,0±1,0	1997±26*	20,16±0,25*

*, **, *** - siginificant at 5%, 1% and 0,1%, correspondingly

Table 3: Physical properties of dough of introgression lines of cv.'Rodina', Novosibirsk, 2003

Parental cultivar and lines	Alveograph parameters			
	Dough strength, u.a.	Tenacity, mm (P)	Extensibility, mm (L)	P/L
Rodina	203±10	75±3	101±17	0,77±1,16
170/98i	305±33**	105±9**	101±7	1,05±0,14*
182/98i	178±4	81±1	75±16*	1,10±0,23**
178/98i	141±18*	74±6	73±1*	1,02±0,06*

*, **, - siginificant at 5% and 1%, correspondingly

The best alveograph parameters were found in the line 76/00i (Table 3). It has also high milling characteristics (Table 2), high dough strength and P/L ration near 1. Secalins were not detected in this line, so it does not carry the 1B/1R substitution. According to the molecular analysis (Table 1) the line 76/00i carries the 7S chromosome instead of 7D. It may be

supposed that this translocation renders a favorable influence on the physical properties of dough through an increase of tenacity and dough strength. This line exceeded the parental cultivar 'Rodina' in physical properties of dough and may be attributed to a "strong wheats" category.

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Discovering QTL controlling yield and yield components in wheat

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Introduction

Despite major increases in wheat yields over the last three decades in the UK, little is known of specific genes that control yield potential and yield components. This is because, to date, most of the selection for improved yield has come from the preference for high performing, observed phenotypes, rather than precise, desirable alleles known to improve yield traits (Foulkes 2003). With the development of genetic marker systems there are new opportunities for detailed genetic analysis of such economically important and biologically interesting traits (Snape 2002). Genetic maps can be fully annotated with the locations of genes for quality, disease resistance, adaptability, and yield, so that they can be manipulated in a directed manner by marker-assisted selection.

Methods

To discover quantitative trait loci (QTL) important in controlling yield in European germplasm, we developed and evaluated five recombinant doubled haploid populations from crosses between diverse European wheat varieties. The crosses were chosen for their genetic diversity and agronomic importance in order to maximise the genetic variation for marker polymorphisms and phenotypic traits.

Genetic maps were produced for each population using publicly available microsatellite markers and storage protein loci aiming for a marker density of every 10-20cM. The populations were evaluated in randomised, replicated field trials over three years, at five sites

across Europe, for a range of characteristics including yield and its components, to generate good agronomic data across a range of environments.

Table 1: Populations selected for DH production and QTL mapping

Spark x Rialto 'Average' yield, group 1 quality x High yield, group 2 quality. Also known to differ for physiological parameters that may determine yield under stress conditions (carbohydrate stem reserves). Allows the detection of genes controlling yield variation within a bread-making background.
Charger x Badger Cross between two diverse high yielding feed wheats. To evaluate the genes that determinine yield potential.
Trintella x Piko Parents of a commercial hybrid – Hybnos. For the evaluation of the genetic basis of heterosis.
Rialto x Savannah Cross between a high yielding bread-making variety and a high yielding feed wheat. Allows the evaluation of genes determining yield potential in the UK environment.
Savannah x Renesansa Cross between a high yield potential Northern European wheat and a high yield potential Southern European wheat. For the evaluation of genes determining yield potential in a European context.

Results and Discussion

QTL analysis revealed a large degree of variation for most of the traits analysed, in all environments, and identified many specific yield and yield related QTL. Major effects where consistently found for 1000 grain weight i.e. grain size, for all five populations. Those QTL replicated consistently across environments and years were identified as 'target' regions for further study.

For Spark x Rialto the majority of the yield related QTL were associated with the higher yielding parent (Rialto), however transgressive segregation was observed indicating some QTL dispersion. Consistent effects were observed on chromosomes 1B, 2A, 2B, 2D, 4D (*Rht-D1*), 5D, 6A and 7B.

In the Charger x Badger population the yield related QTL were evenly distributed between the two parents, which gave virtually identical performance. This resulted in large transgressive segregation and suggests that different genes for yield enhancement were selected in different breeding programmes. Major yield and grain weight effects were seen on 1B, 2B, 3A, 5A, 6A and 7A.

The Trintella x Piko cross did not produce any significant yield effects apart from those which appeared to be associated with hybrid necrosis, which caused a large detrimental effect on both yield and grain weight, especially effects associated with 7D.

The final two validation populations (Rialto x Savannah, Savannah x Renesansa) were only grown in the final two years of the study and at the two UK environments. However they did

produce some yield related QTL that appeared to support the findings of the other populations e.g. significant QTL on 2A, 5A, 6A, 7A and 7B.

The most consistent QTL across years, environments and populations were observed on 2A and 6A. Three of the populations, Spark x Rialto, Charger x Badger and Rialto x Savannah (all British germplasm) exhibited a QTL for both yield and 1000 grain weight reliably across environments and growing seasons on the long arm of 6A. This produced up to a 10% increase in plot yield and accounted for up to 50% of the variation in yield in one case.

For consistent QTL targeted in the Spark x Rialto and Charger x Badger populations, doubled haploid lines containing the positive alleles were selected from graphical genotypes and backcrossed to create a collection of near isogenic lines (NIL's). Microsatellite markers were used to select heterozygous plants after each of two backcrosses (BC2) to restore to 87.5% of the recipient background, before being selfed. Homozygotes for alternative parental alleles for the regions of interest were then selected using marker screens. The NIL's and controls will form the basis for further analysis of the targeted regions, enabling a predictive framework for directed plant breeding for yield in wheat.

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The relationship between vernalization requirement, freezing tolerance and cold regulated proteins in reciprocal substitution lines of two winter wheat cultivars

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Introduction

The ability of the wheat plant to develop and maintain frost tolerance (FT) decreases after the transition from the vegetative to the reproductive phase. Saturation of the vernalization requirement has been suggested as the main factor responsible for the seasonal decline in the frost tolerance of winter wheat (Roberts 1979). Vernalization is determined by *Vrn* genes that are primarily located on the group 5 chromosomes in wheat (Galiba et al. 1995). Molecular studies have shown a very close genetic linkage between the vernalization and frost tolerance (*Fr*) genes (Galiba et al. 1995; Toth et al. 2003).

In wheat, the expression of several genes during cold acclimation is related to the capacity of each genotype to develop FT. These genes belong to the Cor/Lea gene superfamily (Cold regulated/Late embryogenesis abundant) (Thomashow 1999).

The aim of our study was to compare the expression levels of the Cor/Lea gene superfamily (with emphasis on the *wcs120* gene family) with vernalization requirement and FT of wheat lines.

Material and Methods

Reciprocal substitution lines for chromosome 5D were produced from crossing aneuploid lines of two winter cultivars with different vernalization requirements (VR) Mironovskaya 808 (MIR) and Bezostaya 1 (BEZ).

After seed germination at 18 ºC for 4 d the seedlings were grown in soil at a constant temperature of 17 ºC under a 12 h photoperiod and an irradiance of 400 µmol m^{-2} s^{-1} provided by a combination of vapour lamps and high intensity discharge lamps (LU/400/T/40, Tungsram, Hungary) in a growth cabinet (Tyler, Hungary) (Prášil et al. 2004). After two weeks of growth at 17 ºC the plants at the three-leaf stage were exposed to a cold acclimation regime at 2 °C with the same photoperiod. The second fully expanded leaves were sampled for all analyses.

Total soluble proteins from wheat tissues were extracted in Tris buffer (100mM Tris-HCl, pH 8.0, 1mM PMSF). Proteins were separated on 2D SDS-PAGE (2-Dimensional Sodium Dodecyl Sulfate PolyAcrylamide Gel), and visualized by Coomassie Blue or silver staining (O'Farrell 1975).

Quantification of the chosen COR/LEA proteins was carried out by image analysis of 2D SDS-PAGE gels. Identification of COR proteins was made by mass-spectrometry (Shevchenko et al., 1996).

Results and Conclusions

Neither the cultivars nor substitution lines differed in the level of FT up to the sixth week of cold acclimation. Then the FT of B(M5D) and BEZ gradually decreased, and following a further six weeks the FT of MIR and M(B5D) also decreased. A similar order was observed in the saturation of VR where B(M5D) and BEZ showed much a shorter VR (about 6 weeks) than MIR and M(B5D) (9 weeks) (Figs. 1, 2).

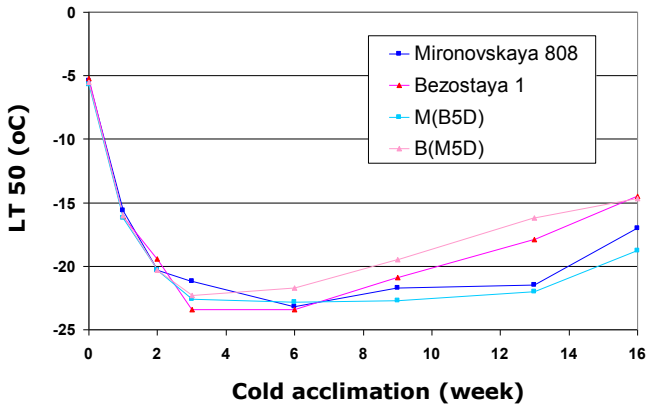

Figure 1: Frost tolerance (LT50) in wheat substitution lines and parental cultivars cold cultivars during cold acclimation

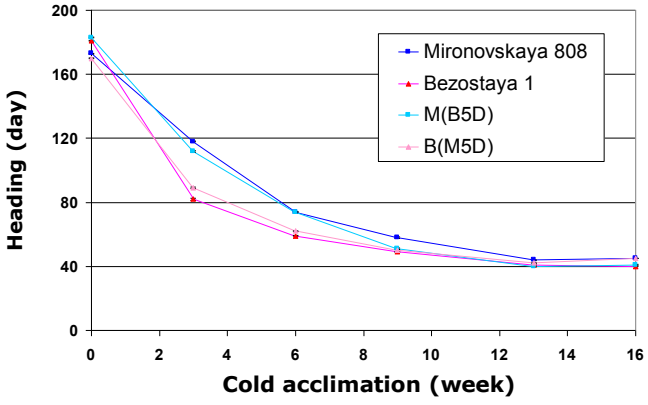

Figure 2: Frost tolerance (LT50) in wheat substitution lines and parental cultivars during cold acclimation

Neither the cultivars nor substitution lines differed in the level of FT up to the sixth week of cold acclimation. Then the FT of B(M5D) and BEZ gradually decreased, and following a further six weeks the FT of MIR and M(B5D) also decreased. A similar order was observed

in the saturation of VR where B(M5D) and BEZ showed much a shorter VR (about 6 weeks) than MIR and M(B5D) (9 weeks) (Fig. 1, 2).

The concentration of WCS120 proteins increased during cold-acclimation in both cultivars. The gradual decrease of FT after saturation of VR was accompanied by a decrease of WCS120 proteins. We detected a different pattern of soluble proteins in non-acclimated and cold-acclimated wheats by 2D SDS-PAGE and mass-spectrometry. After saturation of VR and reacclimation, the parental winter wheat showed similar accumulation of WCS120 proteins and FT as in a cold-acclimated spring wheat Leguan. Substitution lines for the 5D chromosome were similar to the parental cultivars in FT, VR and accumulation of COR proteins. However, protein patterns of proteins soluble upon boiling were reduced during 9 weeks of cold acclimation, but the level of WCS120 proteins did not distinctly change during this period.

The results support the hypothesis that genes for VR figure as a master switch regulating the low temperature induced FT.

Summary

Frost tolerance (FT) and the saturation of vernalization requirement (VR) are controlled by several major loci mapped to the homoeologous group 5 chromosomes in bread wheat (*Triticum aestivum* L.). New reciprocal substitution lines with substituted 5D chromosomes were produced using aneuploid techniques between two winter cultivars Mironovskaya 808 (MIR) and Bezostaya 1 (BEZ). The results support the hypothesis that genes for VR act as a master switch regulating the continuance of low temperature induced FT and that substitution of 5D influences FT as well as WCS120 proteins.

Acknowledgments

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Application of NBS profiling to durum wheat–*Thinopyrum ponticum* recombinant lines carrying the *Lr19* leaf-rust resistance gene

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The NBS profiling approach and the target materials

A number of specific plant disease resistance (R) genes have been cloned and characterised. The largest group of such genes, from both monocot and dicot species, encodes proteins with nucleotide-binding site (NBS)-leucine-rich repeat (LRR) domains (see e.g. Ayliffe and Lagudah, 2004). Structural conservation within the NBS-LRR class of R genes and R-gene analogs (RGAs) is particularly high in NBS regions, which share several motifs, including a P-loop and a kinase-2 motif. These conserved sequences, which have been widely used to clone RGAs from various plant species, have been recently exploited to generate a domain-anchored PCR assay targeted at R genes (Van der Linden et al., 2004). We have applied NBS profiling to durum wheat-*Th. ponticum* recombinant lines developed in the course of an introgression programme aimed at the transfer into tetraploid wheat of both *Lr19*, one of the few genes for resistance to leaf-rust which remain still largely effective, and the linked *Yp* gene, which is responsible for the determination of yellow endosperm pigmentation (Ceoloni et al., 1998, 2005).

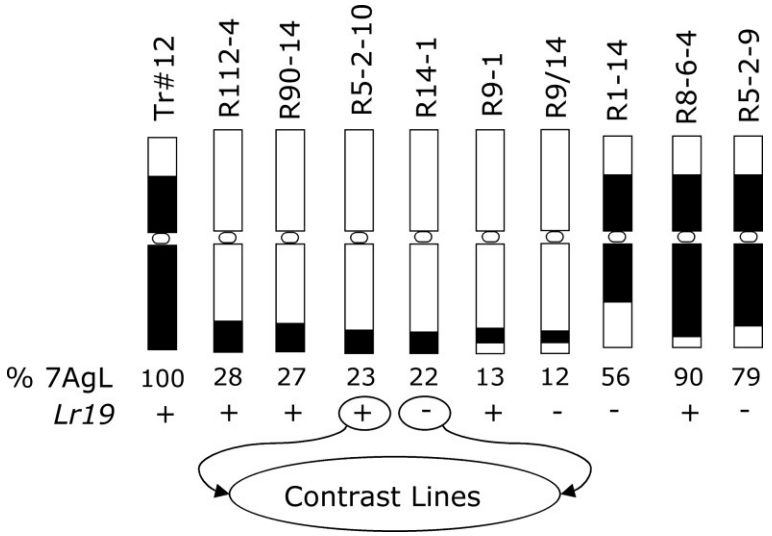

Fig. 1: Durum wheat-*Th. ponticum* 7A/7Ag recombinant chromosomes. *Th. ponticum* 7Ag chromosome segments (black), durum wheat 7A segments (white)

As a starting point for the introgression of *Lr19+Yp* from the alien chromosome arm 7AgL into durum wheat, we chose the line Transfer#12 (Sears, 1973) which carries a 7A/7Ag translocation in a hexaploid wheat background (Ceoloni et al., 1996). This line has retained too large a block of 7Ag chromatin to be easily tolerated in a tetraploid background, so we induced homoeologous recombination to reduce the size of the alien segment. Several secondary and tertiary recombinant lines were obtained by this route, and these lines have been shown to contain varying amounts of alien chromatin, some carrying and others lacking *Lr19* (Ceoloni et al., 1998, 2005).

To identify specific DNA fragments tightly linked to *Lr19* and to enrich the 7AL/7AgL region with new markers, we have focused on the two contrasting recombinant lines R5-2-10 and R14-1; the former carries *Lr19*, and has 23% of 7AgL substituting a distal 7AL portion, while the latter, with 22% of distal 7AgL, lacks *Lr19* (Fig. 1). To isolate R gene fragments located within the 1% 7AgL chromatin differentiating the two lines and containing *Lr19*, we compared the NBS profiles of R5-2-10 and R14-1 BC_{4-5} near-isogenic lines in a cv. Simeto (Italian durum wheat) background.

Results

NBS profiles using a HaeIII/NBS2 enzyme/primer combination identified AG15, a band specific to *Lr19* containing recombinant lines. AG15 was recovered from the gel, reamplified and inserted into a plasmid. Selected transformed plasmids were sequenced and checked for the presence of both adaptor and NBS2 primer sequences. The 223bp AG15 sequence was used to design amplicon-specific primers and to perform a BLAST analysis. A set of primers was tested to validate the polymorphism detected by NBS profiling. In all *Lr19*+ lines, a specific amplicon was generated, making these primer pairs functional for the tagging of *Lr19* (Fig. 2).

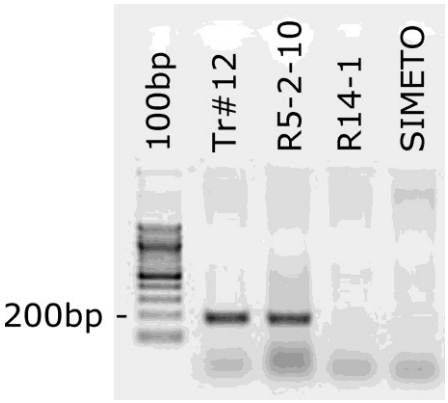

Fig. 2: AG15 primers, derived from a sequenced NBS polymorphic band, reveal high specificity for "*Lr19*+" lines

In a further step, the AG15 sequence was extended via a combination of inverse PCR (iPCR) and the use of digested/ligated DNA fragments as templates in a PCR reaction performed with the adaptor primer and one AG15-specific primer. This allowed the initial 223bp sequence to be extended to 811bp, which provided further possibilities for the design of specific primers. Based on these data, we were able to generate a multiplex PCR assay which provides a codominant assay for the presence of *Lr19*.

BLASTX analyses of AG15 and the extended sequence revealed 50% and 57% identity, respectively, with the sequence of the NBS-LRR wheat powdery mildew resistance gene *Pm3b* (Yahiaoui et al., 2004). A high level of homology was also detected to other known or putative R gene, which confirms that AG15 is a fragment of an NBS-LRR gene.

To study expression of the gene containing AG15, we used cDNA synthesised from uninfected *Lr19*+ seedlings as template in RT-PCR. This showed that the gene is constitutively expressed, as is usually the case for plant disease resistance genes (Hulbert et al., 2001; Feuillet et al., 2003). Further analysis with quantitative PCR is now underway to characterise expression of the AG15 sequence during the infection process.

We also used the cDNA of the two contrasting genotypes to search for NBS-like sequences encoded by the 1% of 7AgL containing *Lr19* and induced by leaf rust infection. To achieve this, it was necessary to modify the NBS profiling protocol. We have been able to identify some candidate NBS fragments, expressed at particular time points post infection, and specific to plants carrying *Lr19.* These sequences are currently being characterized.

The application of NBS-profiling to wheat-alien recombinant lines has been a fruitful strategy to describe, at both the DNA and RNA levels, the R-gene content of an introgressed segment. It may also represent a useful initial step towards the isolation of important R genes such as *Lr19*.

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Non specific adult plant disease resistance against stripe rust (*Puccinia striiformis*) – fine mapping and origin of *Yrns-B1*

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Introduction

Plant disease resistance against airborne pathogens of wheat may be caused by both specific and non specific resistance genes. Specific resistance genes determine a profound protection against the disease and are expressed usually at all stages of plant development (overall resistance). A completely different type of resistance is characterised by susceptibility against actual virulent races at the seedling stage, indicating that no specific resistance gene is present but a non race specific, broad range field resistance of adult plants. Börner et al. (2000, 2001) were able to map a major gene (*Yrns-B1*) determining non specific resistance against stripe rust (*Puccinia striiformis*), present in the line 'Lgst. 79-74'. Linkage to five Gatersleben wheat microsatellite (GWM) markers, mapped on chromosome arm 3BS, was discovered (Börner et al. 2000, 2001). In the present study we performed fine mapping of the gene *Yrns-*

B1 by adding new GWM markers. In order to find diagnostic markers and to study the origin of the gene *Yrns-B1*, both microsatellite analysis and resistance tests of a collection of old German and UK wheat varieties, including probable ancestors of 'Lgst.79-74' were carried out. In addition, a selection of Russian spring wheat varieties was investigated with the aim of estimating the capability of markers supposed to be usable as diagnostic ones.

Materials and Methods

The fine mapping of the gene *Yrns-B1* was performed by using an already existing mapping population (Börner et al. 2000, 2001), originating from the cross between 'Lgst. 79-74' (resistant) and 'Winzi' (susceptible). For analysis of the probable origin of *Yrns-B1* we used a collection of old German and UK wheat cultivars (Table 1). In addition a subset of 35 samples originating from a collection of Russian wheat cultivars described earlier (Khlestkina et al. 2004) was investigated. The disease tests were performed as described by Börner et al. (2000). The field test data for the Russian spring wheat varieties were kindly provided by Dr. Volker Lein, SAATEN UNION RECHERCHE, France. DNA extraction and marker analysis were performed according Röder et al. (1998). GWM were described in Röder et al. (1998). Seven GWM were kindly provided by the company 'Trait Genetics' (Gatersleben, Germany). Polymorphic markers were mapped applying the MAPMAKER 2.0 computer program (Lander et al. 1987) and the QGENE application (Nelson 1997) for the GWM markers and the disease resistance, respectively. GWM closely linked to *Yrns-B1* (*Xgwm533, Xgwm802, Xgwm1015, Xgwm1329, Xgwm3087*) were chosen for studying their capability to be used as diagnostic markers (Table 1).

Results and Discussion

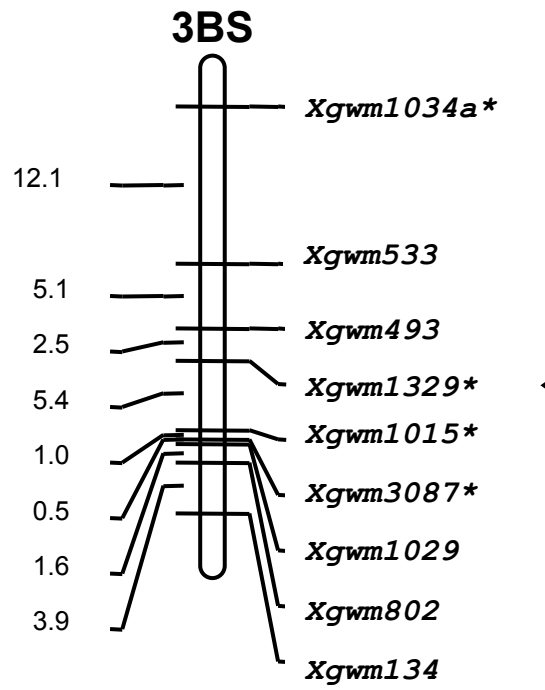
Molecular fine mapping of *Yrns-B1*

Four GWM (*Xgwm1015, Xgwm1034a, Xgwm1329, Xgwm3087*) were incorporated into the previous map. QTL-analysis revealed high LOD values for the resistance at all nine loci, whereas the largest LOD (20.76) was found for the newly mapped marker *Xgwm1329* (Fig.1). Comparing microsatellite mapping of *Yrns-B1* with molecular mapping data available for other resistance genes (or QTLs) on wheat chromosome 3BS, it was found to occur at a similar position as the adult plant disease resistance genes *Yr30* against stripe rust (Singh et al. 2000) and *Sr2* against stem rust (Bariana et al. 1998, Spielmeyer et al. 2003). Whether *Yrns-B1* and *Yr30* are identical can only be speculated. Furthermore, the map position is close to a cluster of other resistance genes including the leaf rust resistance gene *Lr27* (Nelson et al. 1997), a QTL for *Fusarium* head blight resistance (Zhou et al. 2003) and a QTL for leaf rust resistance (Börner et al. 2002). Other cases of an association of different resistance genes on wheat chromosomes are known (McIntosh et al. 2003).

Identification and utilisation of diagnostic markers for *Yrns-B1*

Considering the pedigree information available for 'Lgst. 79-74' the disease resistance most probably originated from the old German cultivar 'Rimpaus Früher Bastard' created by the famous German plant breeder Wilhelm Rimpau in 1882-1888 by a cross between 'Früher rother Amerikanischer Weizen' and 'Squarehead' (Rimpau 1891). Five (*Xgwm533, Xgwm802, Xgwm1015, Xgwm1329, Xgwm3087*) GWM closely linked to *Yrns-B1* were used to screen a collection of wheat varieties (Table 1) which include 'Rimpaus Früher Bastard' and other varieties, which may be related to it. Marker *Xgwm533*, known from previous studies (Röder unpubl.) to possess diagnostic characteristics, was found to amplify a 117 bp fragment, in 'Lgst. 79-74' and other cultivars, including 'Rimpaus Früher Bastard'. Disease resistance tests against stripe rust were performed for both German/UK and Russian wheat

collections. A high coincidence of non-specific adult plant disease resistance against stripe rust and the presence of *Xgwm533*-‘117 bp’ allele was observed among the German/UK cultivars tested (Table 1).



GWM	RSq	LOD
*Xgwm1034a**	0.2876	10.38
Xgwm533	0.3664	13.88
Xgwm493	0.3860	14.83
Xgwm1329*	0.5023	20.76
*Xgwm1015**	0.2327	8.28
*Xgwm3087**	0.2357	8.35
Xgwm1029	0.2924	10.29
Xgwm802	0.2619	9.36
Xgwm134	0.2567	9.15

Fig. 1: Molecular marker map constructed by using MAPMAKER 2.0. New integrated markers are indicated by asterisks. The target gene *Yrns-B1* was tagged as a QTL by using the QGENE computer program

The combination of historical information with the molecular and resistance data presented here, strongly indicates that the origin of the rust resistance is the squarehead genepool. Both ‘Rimpau’s Dickkopf’ (syn. ‘Original Rimpau’s Squarehead-Weizen’), the UK squarehead wheats and ‘Rimpaus Früher Bastard’ carry the 117 bp fragment of the microsatellite marker *Xgwm533*, characteristic for ‘Lgst. 79-74’. On the other hand, the diagnostic allele was also found in the cultivar ‘Amerikanischer Brauner’ which may be just another name for ‘Früher rother Amerikanischer Weizen’. Therefore, both parents of ‘Rimpaus Früher Bastard’ may have been contributors of the resistance (Table 1).

The 117 bp allele of *Xgwm533* was found in about 35% of the Russian cultivars analysed, however, none of them possessed the expected disease resistance. Thus, the utilisation of *Xgwm533* as diagnostic marker seems to be restricted to certain genepools.

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Table 1: Cultivar names, accession numbers, pedigree information, resistance type and microsatellite alleles of the German/UK wheats investigated (nsR = non specific resistance, sR = specific resistance)

Variety	**Accession number**	**Origin**	**Pedigree**	**Resistance**	**Allele of *Xgwm533* (bp)**	**Allele of *Xgwm802* (bp)**	**Allele of *Xgwm1015* (bp)**	**Allele of *Xgwm1329* (bp)**	**Allele of *Xgwm3087* (bp)**
Amerikanischer Brauner	TRI 4954	Germany		nsR	117	140	157	142	233
Basta [2)]	TRI 7727	Germany	See Rimpaus Bastard II	nsR	117	142	155	136	233
Rimpaus	TRI 4255	Germany		sR	null	134	155	158	233
Rimpaus 347/38	TRI 3823	Germany		sR	119	131, 140	157	154	231
Rimpaus 351/38	TRI 3821	Germany		sR	119	134	157	154	233
Rimpaus 365/38	TRI 3822	Germany		sR	null	134	157	154	231
Rimpaus 466/38	TRI 3819	Germany		sR	119	140	157	136, 156	231
Rimpaus 7730	TRI 4284	Germany		S	105,139	140	157	156	231
Rimpaus 9391	TRI 3818	Germany		nsR	105,139	136	157	154	231
Rimpaus Bastard I [1)]	TRI 4183	Germany	See Rimpaus Früher Bastard	nsR	117	142	155	136	233
Rimpaus Bastard II [2)]	TRI 3741	Germany	Salzmünder Standard / Rimpaus Früher Bastard (released 1939)	nsR	117	142	155	136	233
Rimpaus Bastardo, Temprano[1?)]	TRI 4555	Germany	See Rimpaus Früher Bastard	nsR	117	130	141	154	233
Rimpaus Bordeaux	TRI 826	Germany		nsR	117	136	143, 157	143	233
Rimpaus Braunweizen	TRI 2414	Germany	Rimpaus Früher Bastard / Modell (released 1939)	nsR	117	140	155	-	233
Rimpaus Dickkopf	TRI 5073	Germany	Selected from Shiriff's Squarehead (grown since 1891)	not tested	117	140	157	-	231
Rimpaus Früher Bastard [1)]	TRI 219	Germany	Früher rother Amerikanischer Weizen / Squarehead (widely grown since 1889)	nsR	117	142	155	136	233
Rimpaus Langensteiner	TRI 3260	Germany		sR	141	128	175	136	231
Salzmünder 14/44	TRI 3810	Germany		sR	null	140	161	148	231
Salzmünder Bartweizen	TRI 4529	Germany		sR	null	140	161	148	231
Salzmünder Ella	TRI 1345	Germany		nsR	117	136	157	156	231
Salzmünder Standard	TRI 5086	Germany		not tested	117	136	157	156	231
Squarehead Master	TRI 1177	UK		sR	117	140	157	154	231
Squareheads Master	TRI 5171	UK		nsR	117	140	157	154	231

[1), 2)] cultivar names are synonyms

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Genetic and molecular analysis of powdery mildew resistance in Polish common wheat 'Sigma'

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Wheat is one of the most widely grown crops in Poland. Foliar diseases like powdery mildew (*Blumeria graminis* (DC.) E. O. Speer f. sp. *tritici*), leaf rust (*Puccinia triticina* Eriks.) and stem rust (*Puccinia graminis* Pers. f. sp. *tritici*) cause severe yield losses. Breeding of common wheat cultivars with genetically determined resistance to this fungal disease is the most effective and envinronmentally safe approach to prevent these losses (Feuillet, Keller 1998; Hsam, Zeller 2002; Singrün et al. 2004b). To date more than 32 major gene loci conferring resistance to wheat powdery mildew have been detected, mapped to specific chromosomes (McIntosh et al. 2003; Hsam et al 2003; Singrün et al. 2004a). Up to now, Polish breeders use only a few powdery mildew resistance genes in common wheat breeding (Kowalczyk et al. 1998). Using host-pathogen tests and eleven differential isolates of *Blumeria graminis* f. sp. *tritici* to the major known resistance genes, we had trouble with identification of *Pm* genes in Polish common wheat cultivar Sigma. 'Sigma' was resistant to all isolates using in this test (Kowalczyk et al. 1998). After analysis of 'Sigma' pedigree it was very probable, that this cultivar has the *Pm2* gene, in a pyramid combination with other powdery mildew resistance genes. Therefore identification of powdery mildew resistance genes *Pm2* in 'Sigma' was conducted using allelic analysis and RFLP mapping.

Materials and Methods

The F_1 and F_2 hybridgs of Sigma×Ulka and Sigma×Zorza were used for powdery mildew tests with two isolates (No 5 and No10) specific to *Pm2* gene. Cultivars Ulka and Zorza carry powdery mildew resistance gene *Pm2*. Host-pathogen tests were conducted on primary leaf segments cultured on 6 g L^{-1} agar and 35 mg L^{-1} benzimidazole in plastic boxes. The methods of inoculation and condition of incubation were applied according to Hsam and Zeller (1997). Disease responses were assessed at 10 days after inoculation.

DNA from cultivars Sigma, Ulka and Zorza were extracted from leaves of 10 days old seedlings using the CTAB method (Saghai Maroof et al. 1984). DNA dissolved in TE buffer was digested with approximately 2 units of endonuclease EcoRV on 1 µg DNA in the presence of the appriopriate buffer. Digestions proceeded 16 h at 37^{o}C. Digested DNA was loaded onto gels, electrophoresed and transferred to Hybond N^{+} membranes. DNA on the membranes was hybridizated with two probes whs 350 and whs 295. The probes were earlier ^{32}P-labeled. After washing the membranes, they were exposed to X-ray films and screened after 10 days.

Results

The F_1 and F_2 were resistant to these isolates. The results from host-pathogen tests were obtained, but did not clearly reveal that cultivar Sigma carries *Pm2* gene, because 'Sigma' may carry another potent gene, avirulent for these isolates. After hybridization with the whs 350 probe, the absence of a 6.5 kb EcoRV fragment was indentified in Sigma, Ulka and Zorza. Absence of this fragment indicated the presence of the *Pm2* gene in cultivar Sigma. After hybridization with the whs 295 probe, presence of 3.2 kb EcoRV fragment was indentified in Sigma, Ulka and Zorza. Presence of this fragment indicated presence of *Pm2* gene in cultivar Sigma.

Conclusions

Lack of segregation in the F_1 and F_2 of Sigma×Ulka and Sigma×Zorza and RFLP analysis shown that Polish wheat cultivar Sigma carries the *Pm2* gene.

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Heterogeneity of powdery mildew resistance genes *Pm2* and *Pm6* in new Polish comomon wheat cultivars

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Powdery mildew caused by *Blumeria graminis* (DC.) E. O. Speer f. sp. *tritici* is one of the most destructive foliar of common wheat in Poland (Strzembicka, Łazarska 1996). Breeding of resistant cultivars provides an effective approach to eliminate the use of fungicides and minimize crop losses due to the disease (Hsam, Zeller 2002; Hsam et al. 2003). Cultivars with single resistance genes become susceptible shortly after their commercial exploitation due to frequent changes in the pathogen populations (Szunics et al. 1999). One strategy that has been applied to achieve more comprehensive protection is the pyramiding of multiple simply inherited resistance genes and to use combinations in a single genotype (Young 1996; Liu et al. 2000). In Poland a combination of *Pm2* and *Pm6* powdery mildew resistance genes were widely used in breeding programmes of common wheat (Kowalczyk et al. 1998).

Materials and Methods

The *Blumeria graminis* f. sp. *tritici* isolates used for identification of *Pm2+Pm6* genes in Polish common wheat cultivars were collected from different parts of Europe and selected from single spore progenies and classified under Wiehenstephan accesion numbers. The test of mildew resistance were conducted on primary leaf segments cultured on 6 g L^{-1} agar and 35 mg L^{-1} benzimidazole in plastic boxes. The methods of inoculation and condition of incubation were according to Hsam and Zeller (1997). Disease responses were assessed at 10 days after inoculation. Three major classes of host reaction were distinguished relative to susceptible control cultivar 'Kanzler': resistant, intermediate, susceptible.

Results and Discussion

Kowalczyk et al. (1998) tested 69 common wheat cultivars grown in Poland for resistance to powdery mildew using eleven differential isolates of *Blumeria graminis* f. sp. *tritici*. Among these 18 cultivars had the combination of *Pm2+Pm6* genes. There were: Alba, Almari, Arda, Astarte, Jubilatka, Juma, Kamila, Korweta, Lama, Maltanka, Oda, Olcha, Olma, Opera, Rada, Roma, Sakwa, Weneda and were homozygous for *Pm2+Pm6* genes. Three cultivars were registered before 1997. From new cultivars carrying *Pm2+Pm6* genes, Korweta and Soraja were homozygous. 'Korweta' was registered in 1997 year and Soraja was registered in 1998. At the end of the Ninties of the 20th century, the population of powdery mildew in Poland changed and became very virulent to the *Pm2+Pm6* combination. Polish new cultivars which were registered after 1999 were heterozygous for *Pm2+Pm6* genes. Probably lines carrying *Pm2+Pm6* did not exhibit differences in powdery mildew infection in field experiments in comparison to lines without Pm genes. Polish new cultivars as: Koksa, Narwa, Nutka, Tonacja were heterozygous for *Pm2+Pm6* genes combinations (Table 1).

Table 1: Reaction of Polish common wheat cultivars with *Pm2+Pm6* genes after inoculation of *Blumeria graminis* f. sp. *tritici* isolates

Cultivars	*Blumeria graminis* f. sp. *tritici* isolate No											Postulated *Pm2+Pm6* genes
	2	5	6	9	10	12	13	14	15	16	17	
Korweta	s	r	r	r	r	s	r	r	r	i	s	homogenous
Soraja	s	r	r	r	r	s	r	r	r	i	s	homogenous
Koksa	r	r,s	s/i	s,i,r	r,i	r,s	s	r,i	r,s	s	s	heterogenous
Nawra	s,r	r,s	s,i	r,s	s,r	r	s	r	r,i	s	s	heterogenous
Nutka	s	r	s	s/i	r	s	i/r	r	r,s	r,s	r,s	heterogenous
Tonacja	s	r	s	i/r	r,s	s	r,s	r,s	r	r/i	r,i	heterogenous

r- resistant; i-intermediate; s-susceptible

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Pyrenophora tritici-repentis - an important wheat leaf spot pathogen in the Czech Republic

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Introduction

Pyrenophora tritici-repentis (PTR), a homothallic ascomycete, is the causal agent of leaf spot disease of wheat known as "tan spot" or "yellow spot". This disease is found in all the major wheat growing areas of the world and it can cause yield losses from 3% to 50%. The development of resistant cultivars is thought to be the best way to control tan spot. *P. tritici-repentis* has been reported on many grass species from different parts of the World. Two qualitative types of symptoms, tan necrosis and extensive chlorosis, induced by *PTR* were identified on wheat (Lamari and Bernier 1989). Based on these two types of symptoms,

nowadays *PTR* isolates are separated into 8 races, which are grouped according to their virulence/avirulence combination to cultivars/lines used as a differential set (Strelkov and Lamari 2003). Variability of the *PTR* population was studied in different parts of the World and races 1 and 2 seem to be the most common. So far, four host-specific toxins have been characterized, one necrosis inducing toxin Ptr Tox A, two chlorosis inducing toxins Ptr Tox B and Ptr Tox C and one necrosis and chlorosis inducing toxin Ptr ToxD (Ballance et al. 1989, Brown and Hunger 1993, Orolaza et al. 1995, Ali et al. 2002).

In the Czech Republic *P. tritici-repentis* has been discovered on wheat more and more often since about 1998 (Šárová et al. 2003). The aim of this study was to test the reaction of winter wheat cultivars grown in the Czech Republic to *PTR* in greenhouse conditions and to study the race spectrum of *PTR* isolates from our country.

Material and Methods

Cultivars

The reaction of 45 winter wheat cultivars (predominantly Czech registered cultivars) and new breeding lines to artificial infection with *P. tritici-repentis* was tested in greenhouse conditions. In total, three different monosporic isolates of *PTR* originating from the Czech Republic were used for inoculation. The conidial suspensions (3000 spores/ml) of the isolates were applied on seedlings at the two-leaf stage. Reaction of the cultivars was rated about 7-10 days after inoculation, using the 1 to 5 rating scale (1 = resistant, 5 = susceptible) (Lamari and Bernier 1989).

Races

Four differential lines (Glenlea, 6B662, 6B365, Salamouni) were used for determination of the *PTR* races. The reaction of 95 monosporic *PTR* isolates from different parts of the Czech Republic to a differential set was analyzed. Inoculum was prepared using the procedure described (see above).

Results and Discussion

Cultivars

The study showed differences in the reaction of individual cultivars. The majority of the tested cultivars showed moderately resistant to moderately susceptible reaction. Cultivars/lines Clarus, Rheia, Cubus, SHMK WW 14-92, Šárka, Vlasta and SWS 799 were moderately resistant to *P. tritici-repentis* in our greenhouse experiments. Only cultivar PBIS 00/140 was susceptible to this pathogen. So only a few of the currently grown cultivars showed a high level of resistance, while a somewhat larger number possess a moderate level of resistance (De Wolf et al. 1998).

Races

In the Czech Republic race 1 seems to be the most frequent (67 %). Races 2, 3, 6 and avirulent race 4 were detected only sporadically (less than 4 %). The occurrence of race 3 in the Czech Republic is the first report of this race outside of Canada (Ali et al. 2004). Other races were not found yet. The reaction of 25 % of the isolates was impossible to assign to those of so far identified *PTR* races. It could confirm the existence of new races which are not able to be identified by the so far used differential set. The predominance of race 1 was described in the USA and Canada as well (Ali and Francl 1998, Lamari et al. 1998). The occurrence of race 2 has increased since nineties in North America and it would be expected to be similar to that of race 1.

But race 2 was detected very sporadically in our conditions. We do not have enough information about the race spectrum of *P. tritici-repentis* in Europe (Šárová et al. 2005).

Acknowledgements

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Clean gene technology to produce marker-free rice plants containing cystein proteinase inhibitor genes against nematodes

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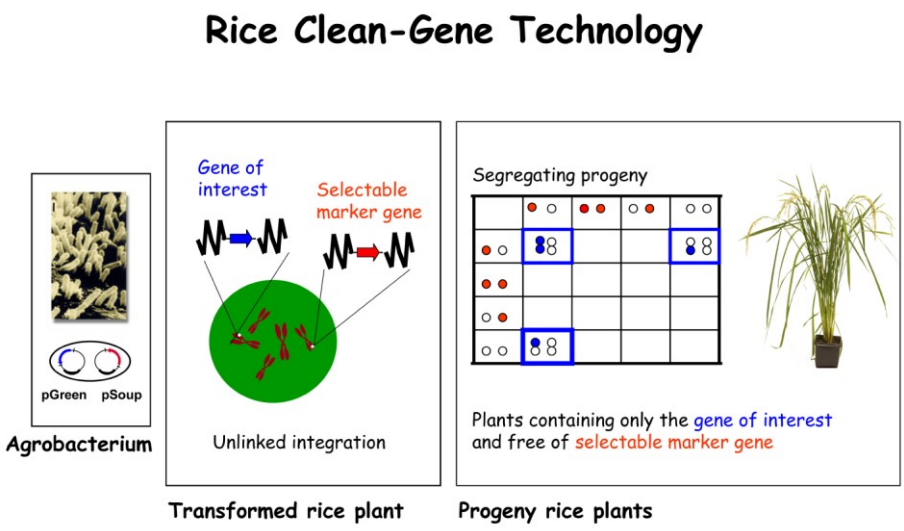
Half the world's population depends on rice as its major source of nutritional calories. Rice transgenic technologies hold great promise for increasing rice productivity especially in areas where rice farmers have little means to counter damage caused by pests and disease. In the tropics, average crop production is reduced by 11-25% as a result of damage by nematodes.

Plant transgenic approaches are still limited by uncontrolled factors affecting the integration and behavior of transgenes. Strategies to control transgene integration and to limit unwanted or multiple integrated DNA sequences are central to the development of advanced plant transformation technologies. Among these, the production of marker-free transgenic plants is particularly important. Selectable marker genes required for the transformation process are generally unwanted, especially in sequential transformation experiments or in transgenic crops.

A new "clean gene" technology has been developed to generate transgenic rice plants free of undesirable selectable marker genes (such as antibiotic or herbicide resistance genes) and containing simple transgenic locus. This technology involves the use of a natural vector for plant transformation (*Agrobacterium tumefaciens)* containing multiple binary plasmids (pGreen/pSoup system). pGreen is a small binary plasmid unable to replicate in *Agrobacterium* without the presence of another binary plasmid, pSoup, in the same strain. Both pGreen and pSoup can carry a T-DNA with different transgenes (Vain *et al.* 2003). When co-transferred into the rice genome, the transgenes carried by pGreen (in blue Figure 1) and pSoup (in red Figure 1) can integrate at unlinked loci allowing the recovery of rice plant progeny containing only the gene of interest (on pGreen T-DNA) but without any selectable marker gene (on pSoup T-DNA).

Transgenic locus composition and T-DNA linkage configuration were determined in a large population of transgenic rice plants transformed with the pGreen and pSoup plasmids in order to assess the performance of the "clean gene" system (Afolabi *et al.*, 2004). Around one third of the transgenic loci generated by this system contained only the pGreen T-DNA and 2% of the progeny plants recovered were free of selectable marker gene (plant containing only the gene of interest carried by the pGreen T-DNA).

Recently, this system has been used to produce rice plants containing only cystatin gene (*CC*) for nematode resistance. An *Agrobacterium* strain containing the pGreen-based plasmid pRT110 (*CC*) and the pSoup-based plasmid pRT47 (*APHIV* + *GFP*, Afolabi *et al.* 2004) were used to transform Nipponbare rice embryogenic calli derived from mature seeds. 98 independent T_0 plants expressing *APHIV* (HygroR) and *GFP* (GFP+) genes were produced. 57% were co-transformed with *CC*, *APHIV* and *GFP*. Progeny (T_1) plants were screened for *GFP* expression. GFP+ plants were eliminated. GFP- plants were analysed at the molecular level. *In fine*, 26% of the plant lines produced clean gene progeny plants and 1.8% (65/3472) of the T_1 progeny plants contained only the *CC* gene (clean gene plants)



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SSR mapping of two asynaptic mutations in rye (*Secale cereale* L.)

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Introductions

Meiosis plays a major role in sexual reproduction in eukaryotes, reducing the chromosome number and allowing gene recombination. The success of meiosis and stability of the genome depend to a large extent upon the exclusive interaction of homologous chromosomes. The recovery of a number of different meiotic mutants of rye indicates that this precise control is achieved by the involvement and interaction of a range of meiosis-specific genes. Assaying the phenotypic expression of the mutants thereby affords the possibility of dissecting the different components of the process. For this purpose a unique "Peterhof" collection of 21 meiotic mutants was established. Over the past 10 years the meiotic phenotype of 14 of lines has been precisely described (for review see, Sosnikhina et al. 2005). However, despite on the great progress in the molecular mapping of cereal genomes, meiotic genes have not been placed in genetic maps. In this study we have mapped the two asynaptic mutations in rye (*Secale cereale* L.).

Materials and Methods

Homozygotes for *sy1* and *sy9* non-allelic mutations form axial elements during leptotene of male meiosis, but fail to form synaptonemal complexes. Consequently, recombination is severely impaired, and high univalency is observed at metaphase I. The *sy1* and *sy9* mutants were originally isolated from individual plants of weedy rye and the rye variety Vyatka, respectively (Sosnikhina et al., 1992, 1998), and are maintained in inbreeding populations of diploid winter rye. The individual heterozygote plants *sy1*/+ and *sy9*/+ from both populations were crossed with the self-fertility inbred line N6 (originated from cv. Steel) to develop the F_2 mapping populations consisting of 129 and 122 plants, respectively.

DNA was extracted from fresh leaf material cut from the individual plants of both F_2 mapping populations. The procedures for DNA extraction, PCR and gel electrophoresis were performed as described by Khlestkina et al. (2004). A multipoint linkage map was calculated using MAPMAKER/EXP 3.0 computer program.

Results and Discussion

Thirty-four microsatellite loci previously mapped in the rye genome (Saal and Wricke, 1999; Hackauf and Wehling, 2001; Khlestkina at al., 2004) were checked for polymorphisms between the parental lines and F_2 populations. 12 of them were polymorphic and linked to the mutant loci. Two additional loci were detected by applying the izozyme markers.

The gene *sy1* was mapped on the long arm of chromosome 7R (Fig.1). It was clearly tagged in relation to the 6 rye SSR loci (*Xscm92*, *Xscm102*, *Xrems1188*, *Xrems1187*, *Xrems1135a* and *Xrems1135b*) and the isozyme locus *Got2*. The *sy1* gene was found to be flanked by the *Got2* locus and the two co-segregating loci *Xrems1188* and *Xrems1135a* located 0.4 cM proximal and 0.1 cM distal to the gene locus.

In turn, the gene *sy9* was mapped close (distal) to the centromere region on chromosome 2R (Fig.1) in relation to the 5 rye SSR loci (*Xscm31*, *Xscm32*, *Xscm35*, *Xscm43* and *Xrems1230*),

one wheat SSR locus *Xgwm132* and the izozyme marker *Sod2*. The target gene was found to co-segregate with two SSR markers *Xscm43* and *Xgwm132*. Both markers are known to map on the long arm of chromosome 2R close to the centromere.

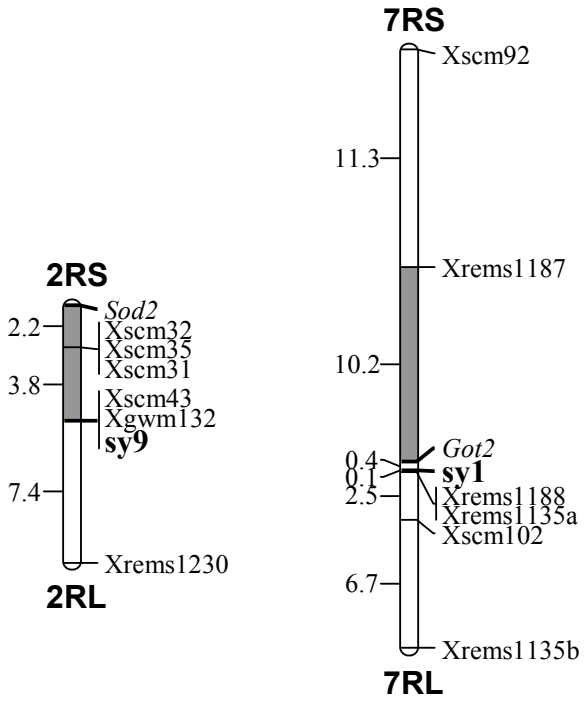

Fig.1: SSR maps of chromosomes 2R and 7R showing the map positions of the asynaptic mutant loci *sy1* and *sy9*. Genetic distances are given in centimorgans (Kosambi). Grey bars indicate the centromere regions. S = short arm, L = long arm

The high-resolution mapping of *sy1* and *sy9* in rye will provide, on one hand, the basis for testing candidate genes from other species and, on the other hand, simplifies maintaining sterile mutations in the heterozygous condition. Moreover, the availability of closely linked markers provides an option for the rapid, marker-assisted development of the double mutants in order to further study a specific interaction of these genes during meiosis.

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Ongoing and future co-operation within EWAC – business meeting

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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:

- Mandate of EWAC
- Maintenance of present and development of new stocks
- Ongoing and new co-operative projects
- The 14th EWAC Conference (where and when)

In order to broaden the activities of EWAC and to attract more people, Andreas Börner proposed the re-consideration of the mandate of EWAC and to re-name it. In fact, since several years the work of the co-operative was not restricted to wheat aneuploid research only. Stocks were developed and used for genetic studies including the localisation and mapping of genes/QTLs by employing molecular markers. Furthermore, after discovering the synteny within the Triticeae, other cereals like barley or rye became of interest for wheat researchers as well. In consequence it was proposed to extend the mandate of EWAC to co-ordinate genetic studies in cereals with the main focus on the development of new and the exploitation of existing genetic stocks. The participants decided to keep EWAC as a label but to use the following extended name in the future:

'EWAC – The European Cereals Genetics Co-operative'.

Opening the discussion about the development and maintenance of genetic stocks and their utilisation for co-operative projects Andreas Börner presented the stage of the development of single chromosome recombinant DH lines at IPK Gatersleben. In total 24 sets at different steps of development are available or in preparation (Table 1). In addition a set of 85 'Chinese Spring/Synthetics' D genome introgression lines have been developed at IPK Gatersleben. The material is available for evaluation within the EWAC community. Other mainly new developed material is offered by John Snape (DH lines of the reference mapping population 'Avalon x Cadensa', TILLING population), Antonio Blanco (DH lines and RILs of Durum mapping populations) or Tatyana Pshenichnikova (aneuploid sets of 'Saratovskaya 29' and 'Diamant 2').

There was no doubt among the participants about the necessity to apply for additional funding. An appropriate programme may be the 'COST action' supporting co-operation among scientists and researchers across Europe. Marta Molnar-Lang volunteered to find out more details and to check whether 'COST' may become a platform for EWAC. The new established web page (http://www.vurv.cz/ewac05) may become a good starting point. Katerina Pankova generous offered to maintain that web-page publishing the full papers of the present conference as well as forthcoming information about following EWAC activities.

Finally the place and the time for the next EWAC meeting were discussed. Tom Payne announced the willingness of CIMMYT to host the 14th EWAC Conference in Turkey. This

proposal was accepted by the participants. It provides the possibility to set up a linkage between EWAC and CIMMYT in the context of the global 'Generation Challenge Program' where some EWAC members arealready participating. As an appropriate time the year 2007 (spring) was selected being the 40th Anniversary of EWAC.

Table 1: Sets of single chromosome recombinant DH lines of IPK Gatersleben at different stages of development

Set	Markerdata available	Seed stocks available	DH plants developed	In preparation
Chinese Spring/Synthetics 6A	X	X		
Chinese Spring/Synthetics 1D	X	X		
Chinese Spring/Synthetics 6D	X	X		
Chinese Spring/Synthetics 3A				X
Chinese Spring/Synthetics 5A				X
Chinese Spring/Synthetics 3B				X
Chinese Spring/Synthetics 5B				X
Saratovskaya 29/Yanetzkis Probat 1A			X	
Saratovskaya 29/Yanetzkis Probat 2A		X		
Saratovskaya 29/Yanetzkis Probat 3A				X
Saratovskaya 29/Yanetzkis Probat 4A				X
Saratovskaya 29/Yanetzkis Probat 5A		X		
Saratovskaya 29/Yanetzkis Probat 3B		X		
Saratovskaya 29/Yanetzkis Probat 5B				X
Saratovskaya 29/Yanetzkis Probat 1D				X
Saratovskaya 29/Yanetzkis Probat 2D		X		
Saratovskaya 29/Yanetzkis Probat 4D		X		
Saratovskaya 29/Yanetzkis Probat 5D		X		
Saratovskaya 29/Yanetzkis Probat 7D			X	
Chinese Spring/Bezostaya 5A			X	
Chinese Spring/Bezostaya 5B			X	
Chinese Spring/Bezostaya 3D			X	
Chinese Spring/Bezostaya 5D			X	
Chinese Spring/*T. spelta* (7D)		X		