European Wheat Aneuploid Co-operative Newsletter

2003

Proceedings of the 12th International EWAC Workshop

1-6 July 2002

at the John Innes Centre, Norwich, UK

Cereals Rersearch Department, John Innes Centre, Norwich, UK

and

Institut für Pflanzengenetik und Kulturpflanzenforschung, Gatersleben, Germany

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In Memorial to Tony Worland

Edited by
A. Börner, J. W. Snape and C. N. Law

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

12th EWAC Workshop
JIC, Norwich 1 - 6th July 2002

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Preface

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The 12th meeting of the EWAC was held at the John Innes Centre, Norwich, UK from July 1st to 6th, 2002. It was organised especially to commemorate the life and work of Tony Worland, who passed away on April 24, 2001. Since the creation of EWAC in 1966, Tony was one of the most active members of EWAC and its secretary for many years. Colin Law stated in his presentation given in the historic section of this meeting: 'Without Tony's continuing efforts I am sure that EWAC would have foundered.'

Tony's involvement with EWAC was not limited to coordinating and organising workshops or preparing the EWAC Newsletters. Of even greater importance was his ability to motivate researchers to set up joint projects. He did this not only within Europe but also throughout the world. This very fruitful co-operation is apparent in the many contributions presented in this Newsletter. Genes for agronomic traits such as reduced plant height, flowering time, disease resistance or bread making quality were detected and their effects evaluated often in several environments. A prerequisite for success in this area of genetic analysis was often the utilisation of precise genetic stocks, many of which were developed by Tony and co-workers.

Participants from 15 countries came to Norwich to discuss the results of their recent wheat research and to express their willingness to continue and extend the work initiated by Tony. The results of this work will be presented at further EWAC meetings. It is a great pleasure to announce that Katharina Pankova, Research Institute of Crop Production, Prague, Czech Republic has offered to host the 13th EWAC meeting. We look forward to meeting each other in Prague, the 'Golden Town', in 2005.

Tony Worland – his life and work

C.N. Law

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Tony Worland came to work with me at the Plant Breeding Institute in Cambridge in 1962. He was my Assistant. At that time we had just started developing the Cappelle-Desprez monosomics and substitutions of that variety into Chinese Spring. I believe also I had made my first tentative forays into genetic analysis using Ernie Sears' Chinese Spring (Hope) substitution series. Tony came straight from the Cambridgeshire High School for Boys immediately after taking his Advanced level examinations. He obtained an external degree in Botany from the University of London in 1968. He climbed the academic ladder the hard way, holding down a job whilst working at nights and weekends studying to pass exams.

At the PBI it soon became apparent that Tony had exceptional qualities. He loved plants – caring for them, growing them, and propagating them. The work on aneuploids required someone with these qualities. Large numbers of cytologically characterised wheat plants needed to be grown in the glasshouse, selected, hybridised and their progenies evaluated, often in extensive field trials. Tony coupled a seemingly inexhaustible supply of energy with a genius for organizing all this, monitoring the throughput of an immense amount of plant material. He regularly would complete over 500 wheat hybridisations in a summer crossing programme – far more than anyone else could achieve. The same would apply to the numbers of chromosome counts he would make. Above all he enjoyed his work. I do recall even in those early days that I only had to mention that it might be a good idea to make a substitution set in a particular varietal combination for the varieties to be sown and the initial crosses made. As Liz Sayers and I have found to our cost over the last few months, as we plod through the thousands of seed packets residing in the JIC seed store, each with AJW written in the corner, the remnants of some of these endeavours can still be seen today. As you can appreciate, it is difficult to recall why such and such a cross was made more than thirty years ago even if you were the initiator in the first place.

Tony was present at the first EWAC conference in 1966/67 and his help in running the meeting was acknowledged in the EWAC Newsletter published shortly afterwards. Thereafter his contribution to future conferences steadily increased. Tony took over the Secretaryship of EWAC in 1981 and edited the next two Newsletters, reporting the 1981 meeting in Wageningen and that in Versailles in 1984. In the following meetings in Cordoba (1991), Gatersleben-Wernigerode (1994), Viterbo (1997) and Novisibirsk (2000), Tony jointly edited the Newsletters and had a major role in initiating and coordinating each of the meetings. Without Tony's continuing efforts, I am sure that EWAC would have foundered. It is very appropriate therefore that we meet here today to acknowledge this contribution. Tony did more than anyone to sustain this interest in the development and usage of wheat aneuploids within Europe and in fact in many countries outside Europe.

Indeed, whilst on the subject of EWAC, I might mention that at one time there was a copycat organisation being developed in the US which was referred to as the Non-European Wheat Aneuploid Cooperative or NEWAC. There was also a phantom and rather secretive organisation in Eastern Europe whose aim seemed to be to shadow what was going on in the West. At times and in its early days, EWAC became jokingly confused with AWAC – the Airborne Warning and Control System. Indeed, John Hermsen, reporting on the founding of EWAC, made the remark that "the world would be a much better place if there were more EWACs around rather than AWACs"!

Tony's first independent research was a study of the genetical control of grass-clump dwarfs. I think we got into this research simply because grass-clump dwarfs began to appear in some of the lines being developed as part of our aneuploid programmes. Tony showed conclusively that four genes were responsible for producing this extreme phenotype. This had been suspected before but had not been unequivocally established. The results of his work were published in 1980 in Zeitschrift für Pflanzenzüchtung and this was his first paper where he was the senior author. However, I do recall this work in particular because a rather intriguing result occurred when Timstein, a *D1* and *D3* carrier, was crossed to each of the Cappelle-Desprez monosomics. All the monosomic hybrids apart from that involving 2D were grass-clump. This indicated that Cappelle-Desprez carried the gene *D4* on chromosome 2D. However, the interesting aspect of this study was the behaviour of the other hybrids. All the grass clump hybrids produced by crossing to the group 3 monosomics were profusely tillering and vigorous and survived for up to a year whereas those from groups 4 and 7 were much enfeebled and scarcely produced any tillers before dying within a few weeks. This was reported in the PBI Annual Report of 1976 and the only comment made was that it showed a marked agreement within homoeologous groups. It totally ignored the cryptic variation that had been uncovered controlling the growth and longevity of the wheat plant not to mention its significance in the behaviour of grass-clumps. I mention this example here because it is a good example of how aneuploids can be used to reveal physiological control mechanisms. Physiologists in particular should take note but I have being saying this to them to no avail for years. However, perhaps even wheat cytogeneticists should also take note because many are just sitting on the aneuploid stocks they have. Somewhere amongst the EWAC Newsletters is the comment that 70 monosomic series are in being. In 1988, Tony listed 75 monosomic series, their developers and maintainers in the proceedings of the 7th International Wheat Genetic Symposium in Cambridge. What has happened to them? Where are they? If found, who has the drive and imagination to use them?

I think the next major break that Tony had in his research was the discovery of a further source of dwarfism in the Japanese variety Saitama 27. This resulted from the screening of European wheats for GA insensitivity. This showed there were several wheats, mainly of Italian origin, which were insensitive to GA but had no connection in their parentage with the usual Norin 10 sources. Tony was able to show that these insensitive varieties were traceable back to an old Japanese wheat, Saitama 27, and that this variety carried a less potent allele of *Rht1* than the allele in Norin 10. It has been reasoned that the popularity of the Saitama allele in southern European varieties was due to its being less prone to temperature stress than the Norin 10 allele. Collaborative experiments, that no doubt will be mentioned by Steven Petrovic later today, have shown that the Saitama allele in varieties grown at Novi Sad in Serbia reduced height by about 11 per cent, increased grain number in the ear but had reduced grain size. This resulted in no net increase in yield. Experiments with isogenic lines carrying the *Rht* genes from Norin 10, on the other hand, and grown at Szeged in southern Hungary, showed that yields were down for the *Rht* lines compared with their taller counterparts.

This work with Saitama 27 is a good example of the type of work at which Tony excelled - tracing genes through varietal lineages and writing the history as it were of wheat breeding. It was the forerunner of other and more extensive investigations into gene origins and their impact on breeding programmes.

One and perhaps the best example of this is the study of the GA sensitive dwarfing gene *Rht8* on chromosome 2D. This emerged from the genetic analysis of chromosome 2D of Mara substituted into Cappelle-Desprez. As an aside, I never seem to tire from citing this particular analytical work as a direct outcome of our involvement with EWAC. Without the agreements made some 35 years ago to collaborate, to select key varieties and to develop monosomic and substitution lines involving them, the substituted series of Mara into Cappelle-Desprez would never have arisen. The identification of *Rht8* would probably never have occurred. Nor would

we have investigated Italian and former Yugoslavian wheats to the extent that has been the case.

Anyway, to revert to the story of the analysis of chromosome 2D. This was carried out using a set of single chromosome recombinant lines derived from the cross of Cappelle-Desprez onto Cappelle-Desprez (Mara 2D). These lines were studied for a range of agronomic characters of which final plant height and flowering time were but just two investigated in detail. The study of height was carried out based on a segregational analysis, an approach seemingly ignored today, in which the 2D recombinant lines could clearly be classified into two equal sized groups. This was the effect of *Rht8*. A similar result giving distinct groups of equal numbers was also obtained for the character flowering time. This was due to *Ppd1*, the photoperiodic gene, linked closely to *Rht8*.

The identification of *Rht8* and *Ppd1* led directly to questions as to their origins which in turn led to the breeder Strampelli and his use of Japanese germplasm and in particular the variety Akagomugi. At this point, microsatellites began to make an impact with the arrival of Victor Korzun and colleagues at Gatersleben to help with the project. This illustrates another good quality that Tony had and that was his ability to collaborate. In common parlance - he was good at networking. I shall come back to this later. Again using the 2D recombinant lines, Victor was able to show that *Rht8* was closely linked to a microsatellite marker WMS 261. Furthermore, different alleles of WMS 261 could be distinguished in Mara and Cappelle-Desprez and a third variety Ciano 67 by different fragment sizes of 192bp, 174bp and 165bp, respectively. Moreover, these variants at the WMS 261 locus were correlated with allelic variants at the *Rht8* locus, the taller allele being found with the 165bp, the shorter allele with the 192bp and the intermediate height allele with the 174bp fragment. A survey of southern and central European wheats showed that the 192bp fragment marking the allele of *Rht8* for reduced height was widespread and could be related directly to the introduction of the variety Akagomugi by Strampelli in the 1930s. The Cappelle-Desprez allele on the other hand was concentrated in Northern European wheats presumably as a consequence of its linkage to the sensitive allele of *Ppd1* which would have been favoured in more Northern climes. The tall producing allele marked by the 192bp fragment was found in CIMMYT wheats. Interestingly, the three alleles of *Rht8* were all introduced in the initial three way cross made by Strampelli. Akagomugi carried the 192bp fragment, Wilhelmina the 174bp and Rieti the 165bp. Tony was able to show in a paper published in 1998 how these three alleles had descended through many different breeding programmes starting in Italy and reaching Mexico via Brazil and Argentina and also into Russian wheats such as Bezostaya 1 via the Italian wheat Ardito.

This is the best example of gene tracing in wheat that I know. Indeed, it might qualify as the best for any crop plant. The tools are available to study other gene descents in wheat and I would hope that some of you will be encouraged to follow in Tony's footsteps because such information will be of immense value to wheat breeding in the future.

Another important contribution that Tony made was the development and characterisation of isogenic lines. This development usually followed the identification of a particular gene and the need to study its agronomic performance. The first genes to be treated in this way were the Norin 10 genes, *Rht1* and *Rht2*, followed by other dwarfing genes such as *Rht3*, *Rht1S* and *Rht10*. These were introduced into a number of backgrounds, the most recent being into the variety Mercia. These lines have been distributed widely and have been used to explore the effects of these genes in different environments. In particular, large-scale collaborative experiments have been carried out particularly with Andreas Börner in Germany and Steven Petrovic in Serbia and these have formed the basis of a number of papers. These confirmed and extended the understanding of how these genes influence final plant yields and how their interactions with the environment can affect yields adversely. *Rht3* isogenics have also been sown on a farm scale in the UK and apparently, these gave acceptable yields.

Isogenic lines carrying the day-length insensitive alleles, *Ppd1* and *Ppd2*, were also produced by Tony, again in a number of backgrounds. These formed the basis of a study of the adaptive role that these genes have in wheat. Collaboration was also a feature of the use of these isogenic lines and projects to explore their behaviour in different environments were set up with Gatersleben and Novi Sad. Again these spawned a number of papers pointing to the crucial role that such genes have on varietal performance. In Germany, for instance, where *Ppd1* had not been used, the introduction of this gene gave higher yields compared to lines carrying the sensitive allele. They even suggested that there could be a positive role, leading to higher yields, for the insensitive *Ppd1* allele in UK breeding. In either case, breeders would hardly have considered this a worthwhile approach before these assessments had been made.

A striking feature of much of Tony's research work is the extent to which he collaborated with others. As I mentioned earlier, he was a good networker. He formed links with researchers in many countries throughout the world. Many of these were under the umbrella of EWAC. This is borne out in the papers being presented at this meeting, many of which bear Tony's name. He made useful and productive links outside this framework. Many of these arose from visitors to the Institute either here in Norwich or in Cambridge. He was good at welcoming and befriending visitors, helping them to settle in and seeing to their problems. In this, he was supported greatly by his wife Barbara and family. I am sure that out of these developing friendships came opportunities to set up collaborative projects, many of which started during these visits. Apart from the names associated with EWAC, I recall the names of Li Weiming from China, Kenji Kato and H Miura from Japan, all of whom linked successfully with Tony but I am sure there are many others. Li Weiming in particular became very close to Tony and a number of important and original papers emerged from their collaboration.

Also, and because of these links, opportunities emerged for Tony to travel, and latterly he made important visits to Mexico, Argentina, Chile, Brazil and China, accompanied in some instances by Barbara. During these visits, he seems to have scattered advice and genetic stocks as he went along and I daresay *Rht8* and the *Ppd* genes are as well known now in these parts of the world as they are in Europe! I still can't get over visiting with farmers in the former Yugoslavia where they were full of comments such as "Tony says this variety has got *Rht8* and this hasn't"! He had managed to get to them as well as the farmers in East Anglia. These visits abroad however were not just about carrying the message, they also resulted in many new and interesting lines of wheat finding their way to the JIC.

Tony was a friend to all, so much so that at times it could lead him into difficulties. I remember on one occasion, Tony being cornered by a research worker at a conference. It was evident that this person was being a nuisance and was totally monopolising Tony who was as usual trying to be helpful but was having great difficulty extricating himself. The only way out of the predicament was for someone to barge in and rescue Tony by using the trumped up excuse that the bus was about to depart and drag him away. But this was symptomatic of Tony, he was always willing to put himself out for others.

He certainly looked after me down at the old PBI when I was required to put on and describe displays of plants to visiting dignitaries. I could always rely on him to provide all the plants that I required and often more than I originally asked for because he had additional material available to make an extra point or two! And as always the plants were in immaculate condition. The same comments also apply to the annual field demonstrations at the PBI. They were always superb. Also, who could forget the marvellous field demonstration at the 7th International Wheat Genetics Symposium in Cambridge in 1988? Those of you who were fortunate in attending will surely never forget. It was by far the best display of aneuploid wheat stocks ever presented at such Symposia - again all down to Tony's efforts.

However, the chief legacy that Tony leaves to us all must be the genetic stocks that he was so much involved in making. These meant a lot to him. I know that just before we moved from Cambridge to Norwich, he was approached to move to another organisation. He talked this over with me at the time and, although I didn't want to lose him, I had to say that I couldn't say which was the best course of action for him. However, the stocks decided it. He had invested such a lot in them that he could not bear to be cut off from them so he stayed.

The extent of the genetic stocks in wheat assembled over the years predominantly by Tony but also by numerous colleagues including myself has become evident from the review of aneuploid seed stocks that Liz Sayers and I have been doing over the last few months. At the present time we are only as far as cataloguing Hobbit sib and its derivatives, having completed the initial reviews of the Chinese Spring, Bersee and Cappelle-Desprez materials. We have still to touch the more recently developed seed stocks, plus all the isogenic lines, which number quite a few, as well as the considerable number of stocks derived from other aneuploid programmes. We have identified 14 different sets of single chromosome recombinant lines in Cappelle-Desprez alone, covering chromosomes 1A, 1B, 1D, 2A, 2B, 2D (two different sets), 3A, 3B, 4B, 5A, 6B, 6D, 5BS-7BS. Most but not all of these sets involve Bezostaya 1 chromosomes substituted into Cappelle-Desprez. We expect to find several more recombinant sets in Hobbit sib backgrounds, not to mention those that have been derived in Chinese Spring. This is without doubt some of the best material available in any crop plant for identifying genes of agronomic importance. I have just completed the analysis of the 3A recombinant set, one of the last experiments Tony completed, and I have identified and mapped two genes, one affecting loaf volume directly and the other Hagberg Falling Number. The allelic differences are not large for either gene and would have been difficult to recognise without the availability of a recombinant substitution set for this chromosome. This material in my view is priceless and surely must be taken up by future researchers. I do not accept the criticism that the material is no longer relevant to current varieties of wheat. I suspect the variation present in today's wheats is fully represented in the aneuploid stocks available. The reason for believing this is the case is that we deliberately set out to sample the germplasm widely at the start of the aneuploid development programme. After all, wasn't this the main reason for creating EWAC in the first place?

Tony Worland gave a great deal to wheat genetics. He also did much for wheat breeding and the farming communities. This latter aspect was very evident at the annual Cereal exhibitions where he could always be seen, at the centre of a knot of interested farmers, describing what this gene or that gene was doing to performance of the varieties they were using. I am sure that if he had lived he would have done much more. His commitment to developing and characterising precisely defined genetic stocks would surely have ensured that this would have been the case. However, in these stocks he has left a legacy to those still active in the field of wheat genetics. Hopefully this legacy will be an enduring one. It will be a fitting memorial to Tony if these genetic stocks were to be made freely available and, above all, were to be fully used in the future.

I commend them to your attention and USE.

Tony Worland and his "foot print" on wheat genetics in the Southern Cone of America

S. Lewis and E. Y. Suárez.

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Scientific aspects

Tony's influence on South America Wheat Genetics started in 1980 when he advised us about the development first of aneuploids and then substitution series within South American germplasm. Since then, his influence has been continuous and the extent of his collaboration has grown to cover many of the countries of the Southern Cone of America.

His trips and initiatives

His first visit to Argentina in 1989 provided us with many new ideas about research work in wheat. During his stay, he visited different Experimental Stations of INTA, private breeders and Universities. Since then, Tony has returned to visit different places in Argentina as well as other countries from the Southern Cone, Uruguay, Brazil, Chile, Paraguay and Bolivia. After these visits, several collaborative projects were established between Tony and different researchers. A proposed research project on "Improving selection efficiency for genetic resistance to complex pathogens of wheat by utilizing molecular tagging techniques" was also put together and an application was made to the EU. Although this major effort by Tony, involving eight partners from South America and four from Europe, was unsuccessful, several collaborative programmes nevertheless emerged from this initiative.

Results from Tony's activities

As consequence of personal advice, material interchanges and joint projects between different centres in South America and Europe, Tony created a net work between researchers in both continents. In this way, many centres were linked because of his participation (Table 1).

Main wheat genetic topics studied, based on Tony's advice or material obtained

Many wheat genetic topics were studied, several of which gave important results and were the first chromosome analyses carried out in South American wheat varieties. They included new rust resistance genes on chromosome 5A, 1A and 4D, pest tolerance, *Septoria* resistance on Synthetic 7D, etc. (Díaz *et al.,* 1993; Brammer *et al*., 1998; Ramos *et al*., 1999; Castro *et al.,* 2000; Tranquilli *et al*., 2000; Simon *et al.,* 2000). However, the most original results were obtained by using aneuploids to study host pathogen interactions. This showed that a change from resistance to susceptibility could result from altering just the dosage of host alleles confronted by the same pathogen alleles (Suárez and Favret, 1984; Tranquilli et al., 1997; Sacco et al., 2001). Table 2 shows a summary of the main topics analysed.

Publications and theses

Several publications and theses emerged from the help that Tony Worland was able to provide. These are summarised in Table 3.

Training carried out with the help of Tony

Tony Worland helped greatly with the training of many visiting workers either at Cambridge (PBI) or Norwich (JIC) (Table 4).

Table 1. Centres linked through Tony's participation

Country	Center
ARGENTINA	-Institute of Genetics- INTA -Institute of Biological Resources- INTA -University of La Plata (UNLP) -University of Buenos Aires (UBA) -Buck S.A. (Breeding Company) -Cargill S.A. (Breeding Company)
BOLIVIA	-Santa Cruz de la Sierra- IBTA -Cochabamba- IBTA -La Paz- IBTA
BRAZIL	-Passo Fundo- EMBRAPA -Rio de Janeiro (Technological Centre- EMBRAPA).
CHILE	-La Platina- INIA -Temuco- INIA
PARAGUAY	-Capitán Miranda- DIA
URUGUAY	-La Estanzuela- INIA
GERMANY	-Gatersleben- IPK
HOLLAND	-Wageningen Institute
U. KINGDOM	-Norwich- JIC and UEA -Cambridge- PBI

Table 2. Topics studied using materials supplied by Tony (PBI-JIC) (1980-2002).

Specific topic		Involved Researchers
Chromosome effects		Favret, E.A.; Suárez, E.Y.; Pérez Camargo, B.; Appendino, M.L.; Nencini,A.; Torroglosa, J.; Covas, G.; Cetour, I.; Formica, B.; Bullrich, L.; Zelener, N.; Lorences, M.; Arteaga, M.; Suárez, A.; González, L.; Lewis, S.
Preferential Pairing and Recombination		Gorgoschidse, L.; Sacco, F.; Saione, H.; Suárez, E.Y.; Tranquilli, G.; Dubcovsky, J.; Khan, I.A.; Pfluger, L.; Rousset, M.; Dvorak, J.; Tanos, B.
Polyploidy (Unreduced gametes)		Naranjo, C.A.; López, A.; Suárez, E.Y.
Host-pathogen interaction	Variation	Sacco, F.; Naranjo, T.; Suárez, E.Y.; Antonelli, E.; Schlatter, A.; Bullrich, L.; Toom, G.O.; Lewis, S.; Simón, M.R.; Barcellos, A.
	Dosage effect	Tranquilli, G.; Saione, H.; Sacco, F.; Tozzini, A.; Suárez, E.Y.; Favret, E. A.
Baker quality		Dubcovsky, J.; Bullrich, L.; Echaide, M.; Schlatter, A.R.; Manifesto, M.; Tranquilli, G.; Pfluger, L.; Feingold, S.; Barneix, A.; Hopp, E.; Suárez, E.Y.; Fatta, N.; Kade, M.
Cultivar identification		Manifesto, M.; Schlatter, A.; Hopp, E.; Suárez, E.Y.; Dubcovsky, J.
Pests		Castro, A.M.; Ramos, S.; Vasicek, A.; Giménez Clúa, A.; Suárez, E.Y.; Boland, W.; Tacality, M.;
Earlines per se		Appendino, M.L.; Suárez, E.Y.
Day Length response		Bullrich, L.; Appendino, M.L.; Suárez, E.Y.
Vernalization requirement		Whitechurch, E; Slafer, G.
Dwarfness		Miralles, D.
Breeding behaviour		Buck, H.; Gonzalez, L.; Hewstone, C.

Table 3. Publications and theses resulting from Tony's influence

Derived publications	Number	South American participants
Meetings , Symposiums , Newsletters .	31	35
Refereed articles	29	49
-Graduate thesis -Postgraduate Thesis	10	14

Human aspects

The most important "footprint" left by Tony in South America was his spirit of collaboration and close friendship. Tony will of course be recognized worldwide for his scientific work. However, more importantly, he will be remembered for his kindness and human values. More than as a colleague, Tony will stay in the minds of South American people as a friend and as a person impossible to forget.

Table 4. Training with the help of Tony

Person trained	Period	Main results
-Suárez, Enrique (INTA - Argentina)	1980/82 1993 1995	-Development of new aneuploids. -Genetic analysis. -RFLPs analysis. -Agreements.
-Lewis, Silvina (INTA - Argentina)	1994/95 1999	-MSc. In Plant Breeding for Agricultural Development (Master Project). -Development and utilization of DH in wheat.
-Tranquilli, Gabriela. (INTA - Argentina)	1996 1997	-Biochemical markers in genetic analysis. -Molecular markers in genetic analysis.
-Bullrich, Laura (INTA - Argentina)	1997 1999	-RFLP and microsatellites analysis. -Development and utilization of DH in wheat.
-Ana M. Castro (UNLP - Argentina)	2000	-Chromosome identification showing tolerance to aphids.
-Eileen Whitechurch (UBA - Argentina)	1999/2000	-Vernalization responses

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Ramos, S., Vasicek, A., Worland, A. J, Giménez-Clúa, A. A., Suárez, E. Y. 1999. Plant Breeding 118: 131-137.

Sacco, F., Suárez, E. Y., Saione, H. A., Tanos, B. E. 2001. J. Phytopathology 149: 538-588.

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Suárez, E. Y. and Favret, E. A. 1984. Z. Pflanzenzuchtung 92: 289-294.

Tranquilli, G., Suárez, E. Y., Saione, H. A., Sacco, F., Tozzini, A. 1997. Plant Breeding 116: 98-100.

Tranquilli, G., Antonelli, E., Schlatter, A.R., Bullrich, L., Toom, G. O., Suárez, E. 2000. Eleventh EWAC, Siberian Branch of the Russian Academy of Science, Novosibirsk, Russia.

A. J. Worland – The leader of EWAC cooperative projects designed for studying the effects of important characters in contrasting environments

S. Petrovic

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Cooperative projects were an extremely important part of EWAC. Almost all such studies were between PBI Cambridge or JIC Norwich on the one side and Institutes from other European countries on the other. The leader and driving force behind many of these common studies was Tony. Between UK Institutes – PBI Cambridge & JIC Norwich (A.J.Worland) and Novi Sad Institute for Field and Vegetable Crops (S.Petrovic), this cooperation was carried out for over twenty years and included many different subjects.

The results obtained in these studies were published in over 30 papers (many others remain to be published) and they were used as subjects in many conferences and lectures. Cooperative studies were supported by the British Council and the International Research Department of Yugoslavia and were of benefit to both sides. In the present paper the results of these cooperative projects are discussed and summarized.

Riley and Law (1967) suggested proposals for EWAC and the first meeting was organized in Cambridge in 1967 at which the first cooperative programmes were agreed.

The cooperation between PBI/JIC and Novi Sad started at that time and included many different subjects. These are listed below along with a brief description and the relevant references:

- Development and characterisation of monosomic series including the varieties Sava and Novosadska Rana 1 (Petrovic & Worland, 1993,1994)
- Comparing monosomic series to identify chromosomes controlling important quantitative characters (Worland *et al.* 1988).
- The two Novi Sad varieties were compared with the Bersee monosomics for all chromosomes and for the group 2 chromosomes of the varieties Mara and Hobbit. The use of reciprocal monosomic analysis to study variation between certain chromosomes of the wheat varieties Bersee and Sava (Petrovic & Worland 1988, 1998, 1999), showed that among the 11 studied chromosomes, 2D was the most, if not the only, important one.
- The genetic differences between recombinant lines, planted in contrasting environments, and their influence on agronomically important characters (Worland et al. 1988, 1991, 1996, 1998, Law et al. 1993). These included the Cappelle Desprez-Mara 2D recombinants and the study of the effects of the genes *Rht8*, *Ppd1* and *Yr16*. Several other recombinant lines were also analyzed but the results have yet to be published.
- The importance of chromosome 2D in Yugoslavian wheat varieties (Worland & Petrovic 1988, Worland et al. 1990, 1991, 1992) including Bersee/Sava 2D reciprocal monosomics and Cappelle Desprez/Mara 2D recombinants, but only for *Rht8* and *Ppd1* genes.
- Determination of height reducing genes in Yugoslavian wheat varieties and the pleiotropic effect of GA insensitive dwarfing genes on a range of agronomic characters (Worland & Petrovic 1988, Petrovic & Worland 1992, 1993) was the subject of numerous studies for many years. Over 100 varieties and lines, mainly those from Novi Sad, were tested. All NS winter wheat varieties were GA sensitive and spring varieties

GA insensitive. At present, several newly developed NS winter wheat varieties are GA insensitive, but are not grown to any extent. Varieties from other former Yugoslavian institutes, or institutes from neighboring countries are usually GA insensitive.

- Study of *Rht* isogenic lines developed in several varieties. The agronomic performance of these lines was evaluated under field conditions and over many years (Worland et al. 1992, 1998, Petrovic & Worland in preparation). The following experiments in Novi Sad are finished, but not published:
  (1) varieties : April Bearded, Bersee, Maris Huntsman, Maris Widgen,
  genes: *rht*, *Rht1*, *Rht2*, *Rht1+2*, *Rht3*, *Rht2+3*;
  (2) varieties: Mercia and Bersee,
  genes: *rht*, *Rht1*, *Rht2*, *Rht3*, *Rht(Bz.dw.)*, *Rht12*.

- Interaction of alien cytoplasms and hexaploid wheat nucleus affecting a number of quantitative characters (Petrovic et al., 1990, 1996a, 1996b, 1996c, 1998, 1999) were studied at the Institute of Field and Vegetable Crops, Novi Sad. The material was produced at PBI/ JIC. Material included the wheat varieties Bersee, Cappelle-Desprez and Dwarf A in normal *Triticum aestivum* cytoplasm and alien cytoplasms derived from *Aegilops mutica, Ae. variabilis, Ae. squarossa*. The results were based upon observations made over five years. In *Ae. mutica* cytoplasm, the expression of agronomic characters was much reduced compared to the controls in *aestivum* cytoplasm. The data from the other cytoplasmic lines, on the other hand, were similar to the controls.

- Introduction of alien cytoplasms into NS varieties (Petrovic *et al*. in press) is a project to study the same effects in adapted varieties. The recipient varieties are Sava and Novosadska Rana 1. The alien cytoplasms, mainly in Dwarf A background (donor), originated from *Triticum araraticum, Ae. bicornis, Ae. speltoides, Ae. squarrossa, Ae. uniaristata* and *Ae. variabilis.* The introduction of alien cytoplasm includes seven more alien cytoplasms, but the number of backcrosses is at the moment too few for evaluation.

- Crossability of Yugoslavian wheat varieties with *Hordeum bulbosum* (Worland & Petrovic 1986) was tested on 16 winter and 4 spring wheat varieties by pollinating 100 florets of each variety with *H. bulbosum*.

- The production of haploids and double haploids in NS wheat varieties (Petrovic & Worland 1986) was successful for the winter wheat variety, Jugoslavija, and the spring varieties, Jarka and Radusa (one in each). The numbers obtained however were low suggesting that the method has little to offer the wheat breeding programme in NS.

Cooperative studies and projects have proved to be very important in the past and will be even more important in future research work. New methods and techniques, new projects, should be coordinated internationally and EWAC is a valuable vehicle for achieving this.

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Development and use of precise genetic stocks for study of technological parameters of grain in common wheat

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During the last 30 years impressive progress has been achieved in studying the genetic basis of variation in the technological aspects of grain quality in common wheat. Precise genetic stocks, both aneuploids and substitution lines, have played a key role. With the aid of these stocks, the chromosome assignment of genes for the storage proteins that go to make up wheat gluten were established (Wrigley and Shepherd, 1973; Orth and Bushuk, 1974; Payne et al. 1982a). The considerable positive or negative effects of chromosome substitutions on separate technological

parameters were also observed (Payne et al. 1982b; Mansur et al. 1990). These achievements stimulated the successful investigation of the structure and function of storage protein genes (Harberd et al. 1987; Halford et al. 1989). The use of modern mapping techniques contributed greatly to our knowledge of the genetic control of wheat gluten quality. In particular, the results of QTL analysis have enlarged the number of chromosomes and loci involved in the genetic control of this character (Perretant et al. 2000; Rousset et al. 2001).

The aim of this paper is to describe and discuss the main investigations undertaken to understand the genetic control of quality using the wheat cytogenetic collection developed at the Institute of Cytology and Genetics SB RAS, Novosibirsk.

Investigation of technological parameters in aneuploids

Several aneuploid stocks were developed in Novosibirsk by Olga Maystrenko and co-workers with the initial aim of studying the genetics of quality. Two key cultivars were chosen for this purpose - Saratovskaya 29 (S29) and Diamant (Dm). The former was found to have a low gluten content (26-28%) but outstanding technological properties, the latter, on the other hand, had a high gluten content (38-42%) but poor quality. To carry out this study, a special technological laboratory was established in our Institute. This enabled the analysis of milling properties, the physical properties of dough and baking tests (18 parameters in all) to be carried out. The scheme of analyses corresponds to the Method of State Variety Testing of Crops accepted in Russia. It should be emphasised that each of these parameters is a necessary and important part of fully characterising the genetic basis of grain quality in wheat.

Initially the influence of aneuploidy on different technological parameters and the identification of "critical" chromosomes were the main objectives. The Chinese Spring ditelosomics of Prof . E.R.Sears and the two monosomic sets of S29 and Dm were investigated (Maystrenko et al. 1973; Arbuzova et al. 2001). Major changes in quality parameters were found in lines lacking a chromosome arm or one homologous chromosome. Both plus and minus effects on various parameters were observed. Common for the three sets of aneuploids was the deterioration of quality in lines involving chromosome 1D. Other chromosmes showed diverse effects but genotype specificity was also important. It is necessary to mention that separate parameters of one technological method, for example, alveograph, often demonstrated independence from others, in particular, increased flour strength was not always correlated with an increased P/L ratio. This might be explained if the traits were under independent genetic control. Overall, aneuploidy led to genetic unbalance in wheat genotypes and revealed hidden variability. However, the variability in the high-quality cultivar S29 was found to be much higher than in the low quality Dm.

Substitution lines and bread-making quality

In the 1980s when the efforts of investigators were concentrated on studying the effects of separate proteins of gluten on bread-making quality (Branlard and Dardevet, 1985; Payne et al. 1987), we developed intervarietal substitution lines for chromosomes of the homoeologous groups 1 and 6 carrying the genes for storage proteins. Dm served as a recipient and the high-quality Siberean cultivar, Novosibirskaya 67 (N67), as a donor. After the first field testing the line having the 1A substitution showed the greatest effect in improving the quality of Dm. This may be attributed to the well-known influence of the active *a* allele of the *Glu-A1* locus of the donor N67 substituted for the null *c* allele of recipient. Nevertheless, this line, whilst still preserving the high gluten content inherited from Dm, did not reach the quality level of N67. The line Dm/N67 6D also improved loaf volume and, consequently, the double substitution line Dm/N67 1A, 6D was developed. This line inherited the higher flour strength of chromosome 1A of N67 and had the better mixing properties, probably connected with the 6D substituion. But

both Dm/N67 1A and the double substitution lines had unfavorable P/L ratios resulting in a low loaf volume.

Another model used in our investigations of wheat quality is represented by a full set of substitution lines of Saratovskaya 29/Janetskis Probat (S29/JP) where the recipient has very high technological properties and the donor has moderate ones. In this case, no significant effects of chromosomes 1A and 1D were found. The largest positive effect on flour strength was shown by chromosome 5A of the donor. Reduced strengths were found for the lines S29/JP 1B, S29/JP 2A and S29/JP 7D. In lines with substitutions for chromosomes 3B, 5D, 6A and 6D the mixing properties were improved significantly and in the 3B line it coincided with a decrease of flour strength. This may be due to a separate control of alveograph and farinograph characters. A wide diversity of P/L ratios occurred among the lines. This means that the intervarietal substitution breaks the rational balance between stiffness and extensibility in S29 gluten. This was also characteristic of the aneuploid studies.

From these investigations, using precise genetic stocks, it is evident that chromosomes of different homoeologous groups participate in the control of this composite character and that the composition of the storage proteins although important in this control is not the only factor. This agrees with recent results obtained by mapping technological parameters using QTL markers (Perrentant et al. 2000; Rousset et al. 2001). Thus while the genetic control of storage proteins and their role in quality determination has been intensively studied, the factors situated on other chromosomes have yet to be characterised.

Enzymes of thiol-disulfide exchange – a candidate system participating in determination of wheat quality

From the biochemical point of view, gluten is a complex three-dimensional protein structure which is maintained by different types of chemical bond. Among them, the inter- and intra-molecular disulfide bonds are considered most important. It is known that their number correlates with many technological parameters of dough (Trufanov, 1994). Folding of such protein complexes are catalyzed by the system of SH/SS metabolic enzymes. Wheat seed contains a specific system of enzymes, referred to as oxidoreductases, which directly or indirectly regulate thiol-disulfide metabolism in cells. The activity of several of them, for example lypoxygenase, has been shown to correlate with quality parameters (Frazier et al. 1977).

The activities of thiol-oxidase and SS-reductase, having reducing and oxidation functions of SS-bonds, has been observed (Kichatinova et al. 1993; Trufanov, 1994) in wheat endosperm. Enzymes activity is localized on membranes of endoplasmic reticulum where the proteins are synthesized. Physiological investigations have shown that the maximum SH-reductase activity coincides with the formation of molecular complexes of functional glutenin during the ripening of seed (Trufanov, 1994). Genetic variation affecting the activity of these enzymes has been discovered as well as a close correlation between the ratio of specific activities of SH-oxidase/S-S reductase and wheat quality (Trufanov, 1999).

Although chromosomal location of genes for these two enzymes is still unknown the activity of one of them, S-S-reductase was investigated using the substitution lines of Dm/N67. This enzyme decomposes S-S bonds and therefore, high activity in grains is unfavorable. It was found that in the Dm/N67 1A substitution line, having improved bread-making quality, the activity of the enzyme is 16% lower than in the recipient Dm. The same was found in the double substitution line Dm/N67 1A, 6D, confirming the link with chromosome 1A of the donor. The line having the 6B substitution has the highest S-S-reductase activity but the lowest quality of the whole substitution series. On the base of 3-years experiments with this set, the correlation coefficients were estimated between the activity of this enzyme and several technological

parameters. A highly significant positive correlation (0.888*) was found between specific S-S reductase activity and P/L ratio, the latter determining elasticity of dough and loaf volume.

To date the genetic information about the enzymes of thiol-disulfide metabolism in common wheat is very poor. For only two of them – lypoxygenase and disulfide isomerase – is the chromosomal location known but the regulation of their activity may be under separate genetic control. Taking into account their important physiological role in endosperm, further genetic studies look to be extremely important.

Further possibilities of using precise genetic stocks to study the technological properties of common wheat should reveal new genetic factors participating in gluten formation. For this purpose, more diverse genetic material should be developed and involved in the work as should cooperation with plant physiologists and biochemists. The detailed technological analysis of different parameters involved in wheat flour quality seems to be a very productive area of research. It will allow more successfully to separate quality into different genetic components and to use molecular techniques to map them for breeding purposes.

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Telosomic mapping in durum wheat

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Monosomics in tetraploid wheat were practically useless to locate genes on specific chromosomes because they were low in vigour and null-gametes were transmitted with much lower frequencies compared to hexaploid wheat (Sears, 1969; Mochizuki, 1968; Longwell and Sears, 1963). The use of monopentaploid lines (2n=34) between "Chinese Spring" monosomics and *Triticum durum* was an alternative method to locate genes on the chromosome present in hemizygous condition (Bozzini and Giorgi, 1971). The use of durum wheat primary trisomics was useful to locate genes (Simeone et al., 1983). The *Gai1* gene, conferring insensitivity to gibberellic acid, which is a marker for the reduced height *Rht1* gene, was located on chromosome 4B by means of F_2 trisomic analysis (Blanco & Simeone, 1982).

Double-ditelosomics and di-monotelosomics in durum wheat

Joppa (1988) and Nishikawa (personal communication) developed the double-ditelosomics of durum wheat by crossing each of the 14 CS A- and B-genome double ditelosomics (2n=40 +4t) with the durum wheat parent. The F_1 plants were backcrossed to recurrent durum parents. In succeeding generations, the plants which have a heteromorphic trivalent (t1t'''), were selected and repeatedly backcrossed to recurrent durum wheat parent. After 9-10 backcrosses had been completed, these selected progenies were selfed, and double-ditelosomics (dDT; 2n = 26 + 4t) were selected among the progeny. They are useful to map the genes once the chromosome arm location is known and to identify chromosomes in other aneuploids. Because of normal transmission of both male and female, these aneuploids are superior to ditelosomics for mapping studies.

The loss of one arm of one chromosome is usually less severe than the loss of an entire chromosome in durum wheat. Therefore, the ditelosomics of CS were crossed with durum wheat to produce telosomics. Using backcrossing methods, many of the aneuploids were produced in durum wheat (Nishikwa, 1971; Joppa, 1988). Considerable cytological observation was required to identify and maintain them, because transmission of the telosomics was low.

The ditelo-monotelo-disomics (dimonoteloes, dMT; 2n=26+3t), which have a pair of telosomes for one arm and a single telosome for the opposite arm, can be produced by crossing a telosomic to its respective double ditelosomic. When selfed, they segregate dimonoteloes (2n = 26 + 3t) and double–diteloes (2n = 26 + 4t) in 65:35 ratio on the average, but there was considerable variation from one chromosome arm and to another. Nishikawa (personal comunication) and Joppa *et al.* (1987) have produced nearly complete stets of theses aneuploids. These aneuploids also can be used for determining arm location and gene to centromere distances in durum wheat.

Telosomic stocks in the background of two durum wheat varieties, Langdon and LD222, are available for the research. Klindworth *et al.* (1997) first mapped four genetic markers in durum wheat using telosome in tetraploid wheat. We describe the procedure of telosomic mapping in tetraploid wheat.

Chromosome arm location of genes for brittle rachis

To determine the chromosome arm location of genes for brittle rachis, dimonotelosomic (dMT) 3AL (13" + tL" + tS') and dMT 3AS (13" + tS"+ tL') plants were crossed as female to LDN(DIC 3A). F_2 progenies of monotelodisomic (13" + t1") F_1 plants were grown and classified for brittle rachis. The critical cross should have an excess of dominant phenotype because the F_1 plant receives only the chromosomes with dominant allele. For example, if *Br₂* were on 3AS, the cross between the dMT 3AL and LDN(DIC 3A) would produce a dimonotelosomic (13" + t1") F_1 plant with a 3A chromosome from LDN(DIC 3A) and a chromosome arm 3AL from the dMT 3AL. The dimonotelosomic F_1 plant produces gametes (either male or female) carrying either one monosomic chromosome or one telosomic chromosome. The telosome will not be transmitted through the male gamete, but not the female gametes. Hence, all euploid (2n=28) plants will have brittle rachis phenotype. In the F_2 of cross of dMT3AL/LDN(DIC 3A), all euploid (2n=28) plants had brittle rachis, therefore the *Br₂* designated by Watanabe & Ikebata (2000) located on 3AS (Table 1). Segregation of F_2 progenies of dimonotelosomic F_1 plants had a significant excess of plants with brittle rachis. The cross of dMT 3AS/LDN(DIC 3A) segregated into the ratio of 3:1 (Table 1). These results indicate that *Br₂* is located on 3AS.

Table 1. Segregation for chromosome number and brittle rachis in the F_2 progenies of monotelodisomic (13" + t1") F_1 plants.

		Number of plants			
Chromosome Arm	Chromosom e Number	Brittle	Tough	N	χ^2 (3:1) for brittle rachis
3AL	28	123	0		
	27+t	41	1		
Total		164	1	165	52.3657*
3AS	28	91	31		
	27+t	38	12		
Total		129	43	172	0.0000^{NS}

[1)] Values for significance at P=0.05; 3.84 (df=1).
*: Significant at P=0.05.
NS: Non-significant.

Map distance of the genes for brittle rachis from centromere

To assess the map distance of the gene for brittle rachis and centromere, a double-ditelosomic (dDT) 3A (13" + tL" + tS") was crossed as female to LDN(DIC 3A). The F_1 plants (13"+ t1t"') as male were crossed to Langdon. Testcross progenies for each cross were grown and classified for chromosome number and brittle rachis. In the test cross progenies, chromosome number of plants are either 28 or 27+2t and segregation was expected 1:1 ratio. Segregation for brittle rachis was expected for 1:1 ratio. As shown in Table 2, calculated distance between the gene for britle rachis and the centromere was 20.5 cM (Table 2).

Taxonomy-related characters of tetraploid wheat and their mapping

Long glume of *Triticum polonicum* and *T. ispahanicum*, purple pericarp of *T. aethiopicum*, tetraristatus (simultaneous presence of awns on glume and palea) of *T. carthlicuim* and branched spike of *T. turgidum* have been used for botanical classification in tetraploid wheat. Biffen (1905) first mentioned long glume phenotype of *T. polonicum*, however the gene has not been mapped until Watanabe (2001). Klindworth *et al.* (1997) mapped the gene for

branched spike of *T. turgidum*. The gene for tetraristatus of *T. carthlicuim* can be mapped in the future. We summarized the genes to centromere distances estimated by use of teosomic stocks in durum wheat (Table 3). The major genes affecting polyphenol oxidase (PPO) activity were mapped on the long arm of homoeologous group 2 chromosomes. The genes on the short arm of homoeologous group 3 chromosomes controlled the brittle rachis of tetraploid wheat. The genes on the long arm of group 7 chromosomes determined chlorina and long glume phenotypes. Watanabe *et al.* (1996), Watanabe (1999) and Watanabe *et al.* (2002) confirmed the linkage relationship between chlorina and long glume phenotypes.

Table 2. Joint segregation for chromosome number and brittle rachis in Langdon/(dDT 3A/LDN(DIC 3A)), associated χ^2 values and map distances.

	Rachis			χ^2 analysis	
Chromosome No.	Brittle	Tough	N	Ratio	Value[1)]
				Dihybrid (1:1:1:1)	66.2265*
28	76	19		Chromosome No. (1:1)	0.4476 [NS]
			181	Brittle rachis (1:1)	0.1381 [NS]
27 + 2t	17	69		Linkage χ^2	65.6409*

Linkage (centromere - *Br_2*): 20.5±0.8 cM.
[1)] Values for significance at P=0.05; 3.84 (df=1), 7.81 (df=3), respectively.
*: Significant at P=0.05.
[NS]: Non-significant

Table 3. Distance of gene to centromere estimated by use of telosomic stocks in durum wheat.

Chromosome arm	Character	Locus	Distance (cM)	References
2AS	Branched head	*bh*	8.5	Klindworth *et al*.(1997)
2AL	PPO activity	*Tc1*	48.2	(unpublished data)
2BL	PPO activity	*Tc2*	43.2	(unpublished data)
3AS	Brittle rachis	*Br2*	20.5	(unpublished data)
3BS	Brittle rachis	*Br3*	20.1	(unpublished data)
7AL	Chlorina	*cn-A1*	46.6	Klindworth *et al* (1997)
	Long glume	*P1*	14.7	Watanabe *et al*.(2002)
7BS	Chocolate black chaff	*cc*	33.5	Klindworth *et al*.(1997)
7BL	Chlorina	*cn-B1*	42.6	Klindworth *et al*.(1997)
7BL	Long glume	*P2*	11.7	Watanabe *et al*.(2002)

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The study of the authenticity of three sets of inter-varietal chromosome substitution lines of wheat (*Triticum aestivum* L.).

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Chromosome substitutions between wheat (*Triticum aestivum* L.) provide an effective mean of analyzing the genetics of quantitative characters (Law and Worland 1973). The development of such lines is a time-consuming procedure involving repeated backcrossing to recurrent monosomic or monotelocentric parents that must be selected cytologically as must be the monosomic segregants of each backcross. A number of problems may arise during backcrossing including human errors of incorrect plant labeling and incorrect cytological analysis of chromosome number and errors from natural causes principally 'univalent shift' and (or) 'univalent switch'. Up to date about 45 monosomic series and 24 single chromosome inter-varietal substitution series have been developed. The European Wheat Aneuploid Co-operative (EWAC) had chosen three inter-varietal substitution series for tests of their chromosomal constitutions. One set of substitution lines using Saratovskaya 29 and Yanetzkis Probat (S29/YP) as recipient and donor, respectively, was produced by Olga Maystrenko in Novosibirsk (Russia). Other two sets 'Cappelle-Desprez/Bezostaya'(Cap/Bez) and 'Chinese/Synthetic'(CS/Syn) were developed by Tony Worland in Cambridge/Norwich (England).

The test of target chromosome authenticity in inter-varietal chromosome substitution lines

Ninety-five, eighty-eight and sixty-eight polymorphic wheat microsatellite markers (WMS) were used to verify the authenticity of the substitution sets S29/YP, CS/Synt and Cap/Bez, respectively. Polymorphic markers covered all of the 21 wheat chromosomes, and their number per chromosome varied from 2 to 8. The size of the amplifying products was in the range of 70 – 320 bp, and the differences between the parental varieties varied from 2 bp to 46 bp. The results of microsatellite test of target chromosomes in the three inter-varietal substitution series are shown in Table 1.

The results demonstrated that most of the lines were correct. Out of 21 lines of each set tested 15-18 showed the expected microsatellite pattern of the donor variety. The incorrect substitution lines were different in the inter-varietal substitution sets. Only the entire chromosome 7A and chromosome arm 3AL were substituted incorrectly in two sets out of three. A translocation of the 3A chromosome occurred during the development S29/YP and Cap/Bez using repeated backcrossing to a recurrent monotelocentric and monosomic line, respectively. It is unlikely that the reason for such a translocation is connected with the way the substitution lines were developed. The 3A and 6D substitution lines of S29/YP were incorrect even though monotelocentric lines were used as the recurrent parent. (Pestsova *et al*. 2000). Thus, the utilization of a cytologically identifiable marker chromosome cannot exclude completely errors in the development of a substitution line.

It was demonstrated that the Cap/Bez lines (5BL-7BL, 6A and 7D), which contain the translocated chromosome according to cytological analysis, had wrong substitution of target chromosomes (Table 1). In case of the chromosome substitutions for 3A and 4B of Cap/Bez where the most advanced backcross line of the substitution line was found to be incorrect, plants from earlier backcross generation were checked. It was shown that the mistake occurred for chromosome 3A before the 3rd backcross and for 4B between the 1st and 4th backcross (Korzun et al. 1997).

The test of background of several Cap/Bez substitution lines

It is expected that repeated backcrossing allows the removal of the undesirable donor parent genome and to make the background very nearly identical to the chromosomes of the recipient variety. However, despite many backcrosses, background variation may still exist and can lead to errors in assigning genetic effects to particular chromosomes (Law and Worland, 1973).

Background analysis of 12 lines of Cap/Bez has been done (Table 2). In half of the studied lines, background variation could not be detected. However, in the remaining six lines, the presence of donor genes was observed in the background. This did not correlate with the number of backcrosses. It was shown that in two cases of incorrect substitutions, one was due to ‘univalent shift’ (1D/5D) and the other to ‘univalent switch’ (7D).

Table 1. Characterisation of target chromosomes in the substitution lines analyzed

A genome

Chromosome / Lines	1A	2A	3A	4A	5A	6A	7A
S29/YP	mt *	*	mt	*	mt *	*	
CS/Synt.	*		*		*	*	
Capp/Bez	*	*		*	*		*

B genome

Chromosome / Lines	1B	2B	3B	4B	5B	6B	7B
S29/YP	*	*	mt *	*	*	mt *	*
CS/Synt.	*		*	*	*		*
Capp/Bez	*	*	*		5BL 7BL	*	5BS * 7BS

D genome

Chromosome / Lines	1D	2D	3D	4D	5D	6D	7D
S29/YP	*	*	mt *	*	*	mt	mt *
CS/Synt.	*	*	*	*	*	*	*
Capp/Bez		*	*	*	*	*	

* - correct substitution; | - wrong substitution; | - translocation ;

mt – backcrossing to monotelocentric parents

Table 2. The background variation in inter-varietal chromosome substitution lines

	Target chromosome	Background 'Bez traces'	Conclusion
Line '1D a' *[10]	1D - Capp	entire 5D + 1/4 1AL + 1/2 6AL	' shift'
Line '2B ab' *[10]	2B - Bez	not found	Substitution line -OK
Line '2D b' *[9]	2D - Bez	1/3 3BL	Background with 'Bez'
Line '3A b' *[7]	3A - Bez	not found	Substitution line -OK
Lines '5BS/7BS' a *[11] '5BS/7BS' b *[9]	5BS;7BS – Bez *	not found 1/8 7DL	Substitution line –OK Background with 'Bez'
Lines '6D aa' *[9] '6D ba' *[11] '6D ac'	6D – Bez * *	not found not found 1/3 2DS	Substitution line –OK Substitution line –OK Background with 'Bez'
Lines '7D a' *[10] '7D acb' '7D bcb'	7D – Capp * *	1/8 1AS 1/4 1AS not found	'switch' 'switch' 'switch'

*[7-11]- the number of backcrosses

Conclusions

These investigations confirm:

- The problems of 'univalent shift' and (or) 'univalent switch' can lead to errors during anyone of the backcross stages of substitution line development.
- There are background variations present in some of the inter-varietal chromosome substitution lines. The amount of donor genome in the background does not correlate with the number of backcrosses.
- The use of microsatellite markers is a valuable tool in testing the authenticity of the genetic stocks.

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Application of microsatellite markers in the development of defined *Triticum aestivum - Aegilops tauschii* introgression lines

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Since 1957, intervarietal chromosome substitution lines of bread wheat have been widely used for studying inheritance of quantitative traits. In recent years the development of many molecular markers for wheat has allowed the production of more precise genetic stocks like single chromosome recombinant lines that can be used to locate agronomically important genes accurately.

Based on a set of *Triticum aestivum* cv. 'Chinese Spring' / 'Synthetic' chromosome substitution lines we created single chromosome recombinant lines for seven D-genome wheat chromosomes by backcrossing with 'Chinese Spring'. Microsatellite marker assisted selection for single and small pieces of 'Synthetic' genetic material resulted in the development of a set of introgression lines containing different segments of individual chromosomes of *Aegilops tauschii* in the 'Chinese Spring' background (Pestsova et al., 2001).

Aegilops tauschii is known to represent a valuable source of genes for disease and insect resistance (Knott, 1994; Lutz et al., 1995). Recent data showed that despite its inferior phenotype *Ae. tauschii* contains genes that can improve quantitative traits (Börner et al., 2002; Huang et al., 2002). The introgression lines produced in our study have a high potential to reveal and study resistance genes as well as quantitative trait loci (QTLs) of the wild species.

Material and methods

Plant material

Substitution lines of 'Chinese Spring' in which single chromosomes of the D genome had been replaced by homologous chromosomes of a synthetic wheat were developed and kindly provided by A.J. Worland (John Innes Centre, Norwich, UK). Synthetic wheat used for the production of the substitution lines had been obtained earlier from a cross of tetraploid emmer and wild grass *Aegilops tauschii* (McFadden and Sears 1947). The seven wheat D-genome chromosome substitution lines carrying different chromosomes of *Ae. tauschii* were crossed to *T. aestivum* cv. 'Chinese Spring'. Genotypic and phenotypic analyses were done for the BC1, BC2 and BC1F2 generations.

Genotypic analysis

DNA was isolated from the leaf material at the seedling stage according to a modified procedure of Plaschke et al. (1995). Genotypic analysis was carried out using microsatellite markers previously mapped on the chromosomes of the D genome of wheat (Röder et al. 1998, Pestsova et al. 2000a). PCRs and gel electrophoresis were performed as described by Röder et al. (1998). The number and chromosomal location of markers polymorphic between the synthetic wheat and cv. 'Chinese Spring' are shown in Table 1.

Phenotypic analysis

Unvernalised BC1 and BC2 progeny plants and vernalised BC1F2 plants (4 weeks at $+4^0$C) were grown in the greenhouse under long day photoperiod (14 h light) at temperatures of 20^0C and 16^0C during light and dark intervals, respectively. Nine phenotypic traits such as

flowering time, plant height, spikelet number, spike length, tiller number, grain weight per ear, grain weight per plant, fertility and thousand kernel weight were evaluated. Fertility was calculated as the number of grains divided by the total number of spikelets per spike. The association between phenotype and marker genotype data was investigated using the QGENE software application (Nelson 1997). The proportion of observed phenotypic variance attributable to a particular QTL was estimated by the coefficient of determination (R^2) from single marker analysis (SMA).

Table 1. Number of polymorphic microsatellite markers and developed introgression lines per each chromosome of the D genome

Chromosome	Number of microsatellite markers	Number of developed introgression lines
1D	9	4
2D	15	9
3D	9	9
4D	6	1
5D	13	7
6D	8	8
7D	11	3
Total	71	36

Results and discussion

Microsatellites assisted development of introgression lines

Seventy one microsatellite markers (Röder et al., 1998, Pestsova et al., 2000, unpublished data) located on the chromosomes of the D genome of bread wheat were found to be polymorphic between varieties ‘Chinese Spring’ and ‘Synthetic’. These microsatellites were used to study the genetic composition of plants that originated from backcrossing of the seven D genome substitution lines 'Chinese Spring' / 'Synthetic' with the recipient variety ‘Chinese Spring’. Totally 259 BC1-progeny plants and 450 BC2-progeny plants were genotyped. Fifty BC1 plants and 60 BC2 plants carrying different segments of the donor chromosomes were chosen to get homozygous introgression lines. Further microsatellite analysis of 500 BC1F2-progeny plants resulted in the detection of 36 homozygous individuals (Table 1). Respective screening of BC2F2 is now in progress. The selected introgression lines from BC1F2 together with the candidate lines from BC2F2 represent a good coverage of the chromosomes of the D genome.

QTL-analysis for different phenotypic traits

Individual plants from BC1, BC2 and BC1F2 were evaluated for several phenotypic traits. The results of the search for putative QTLs are presented in the Table 2. Totally 45 QTLs were detected. Only 1 QTL was common for three generations and 3 QTLs were common for two generations. The low number of common QTLs could be a result of variations in environmental condition (differing sowing date and vernalization treatment) as well as unequal representation of *Ae. tauschii* alleles in three generations.

Since no vernalization treatment was done on the BC1 plants a strong negative effect of the 'Synthetic' chromosome 5D on flowering time was detected. Surprisingly no effect of chromosome 5D on flowering time was detected in BC2 but an effect of chromosomes 1D, 3D and 7D was found. The occurrence of *Ae. tauschii* alleles on chromosomes 1D and 3D delayed flowering while the presence of alien alleles on chromosome 7D accelerated the target trait.

As a result of the strong pleiotropic effect of the vernalization gene, 15 out of 18 QTLs, detected in BC1, were located on chromosome 5D. The vernalization sensitivity of the 5D substitution line in BC1 was coupled with an increased tillering. Most of the other traits, especially fertility and grain weight per ear, were negatively influenced. The QTLs found in two other populations were distributed more uniform over the chromosomes.

The lowest number of QTLs was detected on chromosome 4D (0) and 2D (1). The low number of markers on chromosome 4D (6) and their non-random distribution was probably the reason for the failure to detect QTLs on this chromosome. However, as many as 15 markers were used for genotyping of plants that segregated for chromosome 2D. Since many agronomically important genes are known to be located on chromosome 2D the low number of QTLs detected on this chromosome could be a specific feature of the analysed genetic material.

A positive effect of *Ae. tauschii* alleles on different phenotypic traits was found. For example, wild alleles located on chromosomes 1D determined a higher spikelet number, alleles on chromosomes 2D and 6D increased ear length. Chromosomes 3D and 7D were interesting since they carried QTLs for yield components. Chromosome 3D possessed a QTL for thousand grain weight (TGW) detected in BC1 and BC2, a QTL for plant grain weight (PGW) and a QTL for grain weight per ear (GWE). Wild alleles decreased TGW but increased PGW and GWE. On chromosome 7D two QTLs were detected for GWE, two QTLs for PGW and one QTL for TGW. For all the QTLs, *Ae. tauschii* alleles had a positive effect.

The correlation coefficients between traits (r) were calculated. Flowering time correlated negatively with all other traits ($-0.04<r<0.60$). Strong correlation was found for ear length and spikelet number ($r=0.80$, $p<0.001$). Fertility is highly correlated with GWE ($r=0.88$, $p<0.001$) and PGW ($r=0.73$). There was weak correlation of TGW with all other traits except low positive correlation with GWE ($r=0.37$, $p<0.001$).

This paper presents data on a QTL analysis which were obtained from heterozygous and partly homozygous populations during the development of introgression lines. Further extended studies using homozygous introgression lines will be performed to confirm the current results, to detect new QTLs and to analyse genotype by environment interactions.

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Table 2. Putative QTLs for different phenotypic traits. QTLs designated in bold are common for different generations.

Trait	QTL	Marker with LOD max	Population	Increased effect from	LOD (SMA)	Var-SMA (%)
Flowering time	*QFlt.ipk-1D.1*	*Xgwm337*	BC2	Synthetic	4.45	4.61
	QFlt.ipk-1D.2	*Xgwm126*	BC2	Synthetic	3.75	3.91
	QFlt.ipk-3D	*Xgdm8*	BC2	Synthetic	2.02	2.13
	QFlt.ipk-7D	*Xgwm1187*	BC2	CS	2.29	2.40
	QFlt.ipk-5D.1	*Xgwm205*	BC1	Synthetic	3.98	7.10
	QFlt.ipk-5D.2	*Xgwm174*	BC1	Synthetic	3.84	6.85
	QFlt.ipk-5D.3	*Xgwm982*	BC1	Synthetic	3.17	5.69
Plant height	*QPH.ipk-3D*	*Xgwm161*	BC1F2	Synthetic	2.32	5.17
	QPH.ipk-5D.1	*Xgdm99*	BC1F2	CS	2.11	4.72
	QPH.ipk-5D.2	*Xgwm1252*	BC1F2	CS	2.02	4.52
	QPH.ipk-5D.3	*Xgwm272*	BC1	CS	2.99	5.36
Ear number	*QEN.ipk-5D*	*Xgwm1246*	BC1	Synthetic	5.44	9.64
	QEN.ipk-6D	*Xgwm774*	BC1F2	CS	2.32	5.15
Ear length	*QEL.ipk-2D*	*Xgwm1099*	BC1F2	Synthetic	2.10	4.73
	QEL.ipk-5D.1	*Xgdm99*	BC1F2	CS	2.48	5.56
	QEL.ipk-6D	*Xgwm732*	BC1F2	Synthetic	2.54	2.92
	QEL.ipk-5D.2	*Xgdm116*	BC1	CS	4.35	7.73
Spikelet number	*QSpN.ipk-1D*	*Xgwm106*	BC1F2	Synthetic	4.00	8.80
	QSpN.ipk-5D.3	*Xgwm174*	BC1F2	CS	2.34	5.30
	QSpN.ipk-5D.1	*Xgdm116*	BC1	CS	5.65	9.92
	QSpN.ipk-5D.2	*Xgwm205*	BC1	CS	4.13	7.32
	QSpN.ipk-5D.3	*Xgwm174*	BC1	CS	3.95	7.02
Fertility	***QFr.ipk-5D.1***	*Xgwm272*	BC1F2	CS	5.25	11.39
	QFr.ipk-5D.1	*Xgdm116*	BC2	CS	4.56	5.18
	QFr.ipk-7D.1	*Xgwm1187*	BC2	Synthetic	3.15	3.60
	QFr.ipk-7D.2	*Xgdm130*	BC2	Synthetic	2.93	3.36
	QFr.ipk-5D.1	*Xgdm116*	BC1	CS	13.72	22.41
	QFr.ipk-5D.2	*Xgdm3*	BC1	CS	3.94	7.00
Grain weight per ear	*QGWE.ipk-3D*	*Xgdm8*	BC1F2	Synthetic	3.00	6.98
	QGWE.ipk-5D	*Xgdm99*	BC1F2	CS	2.16	5.08
	QGWE.ipk-7D.1	*Xgwm1187*	BC2	Synthetic	2.76	3.22
	QGWE.ipk-7D.2	*Xgwm437*	BC2	Synthetic	2.14	2.51
	QGWE.ipk-5D	*Xgwm982*	BC1	CS	15.18	24.39
	QGWE.ipk-5D	*Xgdm3*	BC1	CS	5.37	9.43
	QGWE.ipk-7D.1	*Xgdm130*	BC1	Synthetic	3.21	5.74
Grain weight per plant	*QPGW.ipk-3D*	*Xgwm1243*	BC1F2	Synthetic	2.72	6.32
	QPGW.ipk-7D.1	*Xgwm437*	BC1F2	Synthetic	3.21	7.41
	QPGW.ipk-7D.2	*Xgwm1187*	BC2	Synthetic	2.49	2.77
	QPGW.ipk-5D.1	*Xgwm982*	BC1	CS	9.14	15.50
	QPGW.ipk-5D.2	*Xgdm3*	BC1	CS	4.19	7.42
Thousand grain weight	*QTGW.ipk-5D.1*	*Xgdm116*	BC1F2	Synthetic	4.17	9.62
	QTGW.ipk-5D.2	*Xgdm99*	BC1F2	CS	3.24	7.51
	QTGW.ipk-3D	*Xgwm664*	BC2	CS	2.09	2.46
	QTGW.ipk-7D	*Xgdm130*	BC1	Synthetic	10.08	16.94
	QTGW.ipk-3D	*Xgwm383*	BC1	CS	3.36	6.00

Approaches for the introgression of *Hordeum chilense* into wheat

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Naturally occurring genetic variation of the wild barley *Hordeum chilense* is available for durum and bread wheat breeding using the tritordeum amphiploids as backcross source. There are a number of agronomic traits already identified in the wild species that are expressed in wheat background. These include resistances to biotic and abiotic stresses and various quality traits. Several strategies have been tested to achieve bread and durum wheat introgressions. The potential of molecular markers to assist *H. chilense*-wheat introgressions has been assessed by the marker-assisted generation of novel addition lines.

For the bread wheat set, addition forms of *H. chilense* line H7 in the Spanish bread wheat T20, were obtained by selfing the backcross of the hybrid of genomic constitution **AABBDDH^{ch}** (2n = 7x = 49). For the durum wheat set, addition forms of *H. chilense* line H16 in the durum wheat cv. 'Yavaros', were obtained by backcrossing and selfing the hybrid of genomic constitution **AABBH^{ch}** (2n = 5x = 35). Marker-assisted selection during three generations of selfing was carried out using *H. chilense* chromosome-specific RAPD, SCAR, STS and SSR markers. Capillary electrophoresis of SSR markers and real-time PCR of single product STSs and SCARs was the most efficient approach to detecting introgression. As a result, addition forms containing six of the *H. chilense* chromosomes in bread wheat, and all chromosomes for durum wheat have been identified. Nevertheless, it has not been possible to obtain all the disomic addition lines in durum wheat, probably due to a lower buffering effect of the tetraploid genome in comparison with the hexaploid level.

Hordeum chilense genetic resources

Hordeum chilense Roem. & Schult. (Bothmer *et al*., 1995) is a native South American diploid wild barley. Its agronomically interesting characteristics and high crossability with other members of the Triticeae tribe makes it a potential genetic resource for cereal breeding (Martín *et al*., 1998). The availability of fully fertile hexaploid and octoploid tritordeums (2n=6x=42, **AABBH^{ch}H^{ch}** and 2n=8x=56, **AABBDDH^{ch}H^{ch}** respectively, Martín and Sánchez-Monge, 1980b, Martín and Chapman, 1977), and the generation of translocations involving wheat and *H. chilense* chromosomes (Ballesteros *et al.*, in press) makes this wild barley a new source of genetic variation for durum and bread wheat breeding.

As a result of a 20-year tritordeum breeding programme with the aim of developing a new crop, advanced lines have been obtained with similar yields to those of hexaploid wheat under Mediterranean conditions. The starting genetic material was a set of 251 primary tritordeums obtained using more than 100 different *H. chilense* lines as an alien genetic source (Martín, 1988). Previous studies have shown resistance to diseases and quality traits as the best candidates for wheat introgression.

Both *H. chilense* and tritordeum are resistant to *Septoria tritici*. Studies with *H. chilense* chromosomal addition and substitution lines in bread wheat indicated that resistance is mainly controlled by factors on chromosomes 4**H^{ch}** and to a minor extent by chromosome 5**H^{ch}** (Rubiales et al, 1996). Although there is a slight dilution of the resistance at higher ploidy

level (for bread wheat) resistance to *Septoria* seems to be an amenable trait to chromosome manipulation.

Tritordeum as a crop is usually susceptible to *Fusarium culmorum*, but some *H. chilense* genotypes enhance resistance to *F. culmorum* in its tritordeum offspring (Rubiales et al, 1996).

H. chilense is resistant to common bunt (*Tilletia caries*). Resistance is conferred mainly by chromosome 7$\mathbf{H}^{ch}$ (tn a minor extent by chromosome 6$\mathbf{H}^{ch}$), and is expressed on a wheat background (Rubiales and Martín, 1999).

H. chilense is resistant to brown rust, but tritordeum follows in all instances the phenotype of the wheat parent (Rubiales et al, 1991, 1993) therefore making useless the transfer of the *H. chilense* gene/s responsible for increasing wheat resistance because most probably they will not be expressed on a wheat background. A quantitative contribution of *H. chilense* to the tritordeum's resistance to wheat powdery mildew has been described (Rubiales et al, 1993a). The complex control of resistance makes the transfer of resistance to wheat difficult and unreliable, particularly so if the agronomic performance of wheat has to be maintained unchanged.

H. chilense exhibits resistance also to the smuts *Ustilago nuda* and *U. tritici* (Nielsen, 1987), to karnal bunt (*Tilletia indica*) (Chauhan and Singh, 1997), to take-all (*Gaueumannomyces graminis*) (Jorgensen and Jensen, 1976), to *Pyrenophora tritici-repentis* and *P. teres*, and to *Rhynchosporium secalis*. *H. chilense* is resistant to the root-knot nematodes *Meloidogyne naasi* (Person-Dedryver et al, 1990), and to the Columbia root-knot nematode (*Meloidogyne chitwoodi*) (Jensen and Griffin, 1994).

The resistance to the root-knot nematode is located on the short arm of the chromosome 1$\mathbf{H}^{ch}$ and is expressed in a wheat background (Person-Dedryver et al, 1990). Varying levels of resistance to aphids such as Schizaphis *graminum* (Castro et al, 1994), *Diuraphis noxia* (Clement and Lester, 1990) and *Rhopalosiphum padi* (Weibull, 1987) have been described in *H. chilense*, but, although it has been located chromosomally (Castro et al, 1996), no simple and therefore transferable genetic control has been found.

Addition lines of *H. chilense* in bread wheat were used to locate genes for tolerance to salt on chromosomes 1$\mathbf{H}^{ch}$, 4$\mathbf{H}^{ch}$ and 5$\mathbf{H}^{ch}$ (Foster et al, 1990). The complexity of the genetic control of this trait makes it difficult to think of *H. chilense* as a source of salt tolerance in wheat breeding.

Hordeum chilense is also a source of variability for carotene pigment content, as well as endosperm storage proteins. Both are expressed in tritordeum and wheat addition lines (Alvarez et al. 1998, 2001) and could be useful for durum wheat breeding.

Introgression of *Hordeum chilense* into wheat

The availability of addition lines in *T. aestivum* allowed us to develop specific markers for *H. chilense* chromosomes (Hernández *et al.*, 1996, 1999a, 1999b). Those markers that were conserved across distant accessions were used to assist the development of new sets of addition lines in bread and durum wheat (unpublished results). In the same way, these markers are valuable tools to track the chromatin of *H. chilense* in a wheat background. RAPDs and SCARs were the first molecular markers developed for *H. chilense*, due to the lack of sequence information available for this wild species. SCARs have proven very valuable for the introgression programme, and allow high numbers of individuals to be characterized each generation. Specific and single amplification products of SCAR markers can be directly detected on the amplification plate, either by ethidium bromide staining under UV illumination (Hernández et al., 2002) or by detection on real-time PCR equipment

(unpublished results). Nevertheless, their development requires a high sequencing investment, and no comparative relationships are directly obtained.

Wild species like *H. chilense* can benefit from marker resources developed in related Triticeae crop species such as barley and wheat, which in turn could provide additional comparative mapping information. In this way, subsets of SSR and STS primers developed for wheat and barley have an additional application for marker-assisted *H. chilense* introgression into wheat (Hernández *et al.* 2001, 2002).

The most effective markers for wheat introgression breeding are those permitting higher throughput at a reasonable labour and consumable costs. Single-product SCARs or STSs, permitting direct detection on the PCR plate, thus avoiding electrophoresis, and capillary electrophoresis of fluorescently-labelled SSR markers, allowing multiplexing and simultaneous detection of the wheat alleles, are the detection systems of choice.

The hybrid bread wheat × hexaploid tritordeum (**AABBH^{ch}D**) is in some varietal combinations self-fertile and seed set can always be obtained after backcrossing with either of the parents (wheat or tritordeum). In this way, tritordeum can be used as a bridge for transferring desirable traits from *H. chilense* into bread wheat avoiding the development of the addition lines first (Martín et al. 1998).

The hexaploid tritordeum collection synthesized with 103 different *H. chilense* accessions is the basic material for the introgression of characters of interest into durum wheat. With this purpose, *Triticum monococcum* (2n=2x=14, **A^{m}A^{m})** has been crossed with hexaploid tritordeum (2n=6x=42, **AABBH^{ch}H^{ch}**). The hybrid **A^{m}AB H^{ch}** (chromosome number 28) is male sterile but after backcrossing to durum wheat (2n=4x= 28, **AABB**) set some seeds. The chromosome number of the backcrossing progeny is always close to 35 and occasionally close to 42. Most probably these backcrosses have the genome constitution **AABBH^{ch}** (2n=35) and **A^{m}AABBH^{ch}** (2n=42), respectively. Clearly functional female gametes produced for the hybrid **A^{m}ABH^{ch}** are unreduced gametes, **ABH^{ch}** (in which **A** is a mixture of **A^{m}** and **A**) or **A^{m}ABH^{ch}**. Therefore, the chance of obtaining introgressions of *H. chilense* into durum wheat is quite low, given the need of backcrossing to restore fertility.

After this failure, alternative crosses have been made between chromosome addition lines 4**H^{ch}** and 7**H^{ch}**, which are the lines of interest for introgressing Septoria resistance and carotenoids content, with *T. urartu* (2n=2x=14, **AA**). The hybrid **AAB** plus one **H^{ch}** chromosome is easily obtained and backcrosses, again indicated the production of unreduced gametes.

A new approach we are working on uses as male parent the addition lines of *H. chilense* to durum wheat crossed to the **D**-genome substitution of durum wheat (Joppa and Williams, 1977). In this way, we expect to avoid the problem of unreduced gametes that occurred in the previous crosses. The objective of this cross is to produce, for groups 4 and 7, a hybrid with only one chromosome of the three genomes **B**, **D** and **H^{ch}** and, thus, to improve the chance of recovering translocations involving the **H^{ch}** chromosome.

We have synthesized amphiploids between *H. chilense* and durum and bread wheat carrying *Ph1* (pairing homoeologous) gene mutants. Until now they have not been used for promoting recombination between barley and wheat chromosomes, given that the pairing between *H. chilense* and wheat genomes is nil even in the absence of 5**B** chromosome in hybrids of *H. chilense* with N5B T5D bread wheat (Martín and Sánchez-Monge, 1980). Nevertheless, spontaneous translocations, terminal, interstitial or centromeric, are frequently observed on the progeny of the hybrid bread wheat x hexaploid tritordeum, **AABBDH^{ch}**. This material could be an alternative source for *H. chilense* introgressions in wheat.

Conclusions

Hordeum chilense could be a source of beneficial genes for resistance to biotic and abiotic stresses and quality for wheat breeding. Tritordeum, the amphiploid barley-wheat hybrid, is the basic genetic material for the transfer of *H. chilense* genetic variability. Resistance to Septoria, located on chromosome 4$\mathbf{H^{ch}}$ and high carotene pigment content and resistance to common bunt, both located on chromosome 7$\mathbf{H^{ch}}$ are among the traits of immediate utility to be transferred to durum wheat. Both traits as well as endosperm storage proteins could be also of interest in bread wheat breeding.

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Using AS-PCR and HPLC in characterising glutenin genes of a collection of Spanish common wheats

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Proteins are the most important components governing bread-making quality. The wheat technological properties are correlated with glutenin polymers that consist mainly of high and low molecular weight subunits. Both the functional properties and molecular structure of glutenin subunits have been studied intensely. Common wheats contain three *Glu-1* loci, coding the HMW glutenins located on the long arms of homoeologous group-1 chromosomes. The genes Glu-3 located on the short arms of the same chromosomes code for the LMW glutenins. Considerable differences in the SDS-PAGE patterns can be seen for the HMW glutenin subunits of different wheat varieties. Molecular studies have shown that each *Glu-1* locus contains two tightly linked genes (x and y) coding for protein subunits of different molecular weights (Harberd et al. 1986). Different cultivars of common wheat have up to six HMW subunit genes, with two genes (x+y) present on each of *Glu-1* loci. The silencing of specific genes leads to variation in the number of subunits. Moreover, different alleles for the complex *Glu-1* genes have been reported. The properties of wheat flour have been related to the allelic combination of these genes (Payne 1987, Shewry et al. 1992). Payne (1987) demonstrated that good breadmaking quality is firmly associated with the presence of HMW subunits *GluA1x1* and *GluD1 x5+y10.* According to Shewry et al (2001), the HMW subunits account for between about 45 and 70% of the variation in breadmaking performance within European wheats, despite representing only 12% of the total flour proteins. De Bustos et al (2001) developed and applied a series of new PCR markers that amplify the complete coding sequence of each specific allele (AS-PCR) of the main *Glu-1* genes.

Gliadins are the major storage protein fraction in wheat endosperm. They are monomeric and do not have disulfide bonds in their subunit structure. Complex gene families control gliadin proteins. *Gli-1* loci code for ω- and γ- gliadins and are located on the short arms of group 1 chromosomes, while *Gli-2* loci code for β- and α- gliadins and are located on the short arms of group 6 chromosomes (Payne, 1987). The present work reports the characterisation of a collection of 28 common wheats cultivated in Spain. The allelic composition of the main gene loci was determined combining SDS-PAGE electrophoresis and AS-PCR molecular markers previously developed by the authors (De Bustos et al. 2001). The breadmaking quality

GluB1x was analysed using the alveograph to test dough elasticity and strength of flour taken from wheat cultivated at different locations. Gluten proteins of these wheats were quantified using a RP-HPLC procedure, to correlate the technological properties with the quantities and ratios of different types of gluten proteins present in each cultivar.
Genomic DNA from wheat was extracted from leaves of single plants. A set of primers was useful in distinguishing the main alleles of the subunits x and y of *GluA1* and *GluD1* loci, as described by De Bustos et al. (2001). For the quantification of the type of proteins, it was followed by sequential extraction of the samples of flour after the crushing of the embryo-less grain. Aliquots of the extract were analyzed by SDS-PAGE, as well as by RP-HPLC, using a column Vydac C_{18}. The quantification was realized by means of a detector of ultraviolet to a wavelength of 210 nm. A linear elution gradient (0 min 24% ACN + 0.07% TFA, 30min 60% ACN + 0.07% TFA) was applied to separate gliadin and glutenin subunits. The coefficient of average separation for the total procedure was between ±1.5 and 4.1 % (three determinations). Separation and quantification of HMW subunits was performed using the method of Melas *et al.* (1994). To obtain the fraction of the HMW, the same RP-HPLC system was used modifying the temperature of the column, increasing the percentage of the TFA in the solution of 0.1 % and reducing the flow to 0.8ml/min. Statistical evaluations were performed using the program Statgraphics Plus 5.

Table 1. Common wheats analysed for dough properties, HMW allelic constitution, and quantities of the main gluten proteins.

Wheat	Code	W	Extensibility P/L				HMW Alleles			Total prot Glu + Gli.	mAU Glu				mAU Gli
			P/L	G-1 <0.5	G-2 0.5-1	G-3 >1	A1 x	B1 x+y	D1 x+y		HMW	x	x/y	LMW	
Astral	AST	100	0.4	+			Null	7+8	2+12	651	56	14	1,23	181	414
Fiel	FIEL	90	0.4	+			Null	7+8	5+10	912	100	37	3,08	293	519
Marius	MAR	80	0.3	+			Null	7+9	4+12	1124	114	31	2,58	243	767
Bolero	BOL	160	0.4	+			2*	7+9	2+12	811	101	30	3	280	430
Agrosa 10	S10	179	0.3	+			Null	7+8	5+10	2953	249	16	1,6	601	2103
Agrosa 44	S44	150	0.4	+			1	7+8	2+12	1503	198	6	1,5	303	1002
Agrosa26	S26	230	0.6	+			1	17+18	5+10	1278	236	15	2,5	298	744
Agrosa 11	S11	181	0.3	+			2*	7+8	2+12	1493	98	67	6,7	412	983
Bavaro	BAV	200	0.3	+			1	7+9	5+10	1791	156	56	3	444	1191
Gazul	GAZ	330	0.9		+		2*	7+8	5+10	1320	216	56	4,3	363	741
Anza	ANZ	100	0.8		+		Null	7+8	2+12	942	94	16	2,28	217	631
Pompeyo	POM	160	0.8		+		1	7+8	5+10	1728	322	134	7,44	445	961
Pata Negra	PN	280	0.6		+		1	17+18	5+10	1124	142	60	2,5	286	696
Agro.5801	S5801	350	0.9		+		1	7+8	5+10	1811	397	239	13,27	432	982
Agrosa 28	S28	268	0.6		+		1	7+9	5+10	1805	252	53	4,42	358	1195
Rinconada	RIN	300	0.7		+		1	7+8	5+10	2804	509	128	4,6	782	1513
Agrosa 12	S12	283	0.8		+		2*	7+9	5+10	2081	262	52	7,4	456	1363
Soissons	SOI	220	0.8		+		2*	7+8	5+10	1296	127	31	1,7	489	680
Agrosa 47	S47	240	0.8		+		2*	17+18	2+12	1654	324	45	7,5	640	690
Yecora	YEC	280	0.9		+		1	17+18	5+10	1471	266	34	2,61	479	726
Agrosa 9	S9	253	0.3		+		1	17+18	5+10	941	149	24	6	342	450
Zentos	ZEN	277	0.6		+		Null	7+9	5+10	995	141	52	3	464	390
Horzal	HOR	350	0.8		+		1	7+8	5+10	1321	288	61	8,7	519	514
Livio	LIV	260	0.6		+		1	7+8	5+10	1268	195	122	4,2	419	654
Agrosa 30	S30	269	1.3			+	Null	7+9	2+12	1397	282	143	7	365	750
Agrosa 8	S8	324	1.6			+	1	17+18	5+10	1517	240	157	4	372	905
Pané 247	PANE	110	1.3			+	Null	7+8	2+12	1773	223	30	3,75	487	1063
Agrosa 34	S34	140	1			+	1	7+9	5+10	1316	182	107	2,1	251	883

Results on dough quality

The results of the analysis with the Chopin alveograph allowed the wheats to be classified into three groups depending on the P/L alveograph values. This parameter indicates the balance between dough elasticity and plasticity (P/L), P being a measure of dough resistance to deformation and L, a measure of extensibility, the interval between the point where the dough bubble starts to inflate and the point where the bubble bursts and the pressure drops suddenly. The baking strength W values are also shown in Table 1. This table also includes the composition of the types of HMW alleles of the cultivars studied both by the SDS-PAGE and AS-PCR procedures. It is evident that the combination x2+y12, and x5+y10, usually correlated with low and high baking quality, respectively, is not always the case. Some cultivars for instance show very different extensibilities (P/L) and strength (W) values but have the same allelic composition.

The quantification and correlations of protein types

The last column of Table 1 summarizes the amounts of glutenins and gliadins expressed as mAU (HPLC absorbance units) and the ratio x/y. These demonstrate a large range of flour specific differences, which could be due to genotype as well as differences in growing conditions. The correlation coefficients (r) between protein quantity and the ratio x/y and the parameters measured by alveograph are summarized in Table 2.

Table 2. Correlation coefficients (r) for the relationships between quantities of protein type and wheat properties

			Glutenins subunits					Ratios						
	Gluten Protein	Gliadins Total	Total	HMW	LMW	x	y	GLI/ GLU	GLI/ HMW	GLI/ LMW	GLI/ x	GLI/ y	LMW/ HMW	x/y
[c]Ext	0,11	-0,01	0,27	0,40*	0,15	0,50**	0,44*	-0,26	-0,45*	-0,11	-0,32	-0,25	-0,42*	0,25
W	0.26	0,06	0,54**	0,57**	0,45*	0,49**	0,17	-0,51**	-0,51**	-0,39	-0,31	-0,15	-0,28	0,53**

[b] Level of significance: r=0,35 0,45, p=0,05 (*) ; r= 0,49 0,57, p= 0,01 (**).

[c] Ext= Extensibility; W= Strength

Strength showed moderate correlation with total glutenins, and their components HMW, x-type, LMW, and the ratio x/y. The dough strength was negatively correlated with the ratios GLI/GLU and GLI/HMW. In agreement with the studies of Wieser and Kieffer (2001), strength was not correlated with the total amount of gliadin or any of their different types. The correlation between strength with x-type and the ratio x/y glutenins denotes the importance of the active alleles of *Glu-A1* (x1 or x*2**) with respect to the Null allele in increasing the amount of glutenin. The decrease of dough strength and extensibility caused by the increase in the ratio GLI/GLU, and GLI/HMW, respectively, could be explained on the basis of their functional behaviour. It has been shown that glutenins form heterogeneous aggregates of multiple chain polymers in which the polypeptide subunits are linked by disulphide bonds (Payne and Lawrence 1983). However, gliadins are maintained as monomers and could act as a solvent for glutenins. Wieser and Kieffer (2001) previously demonstrated the decrease in dough strength caused by the addition of gliadins.

Dough extensibility is mainly influenced by HMW (both subunit types). This is indicated by the correlation between extensibility and the quantities of these proteins. The correlation coefficients of extensibility with the ratios GLI/HMW and LMW/HMW were negative and consistent with the positive correlation of extensibility with HMW, probably caused by the solvent effect of both gliadins and LMW for glutenins. Uthayakumaran *et al*. (1999) found that increasing the glutenin-to-gliadin ratio decreased extensibility in dough properties.

Finally, the analysis of RP-HPLC chromatograms demonstrates a possible effect of the *GluB1x* subunits on extensibility, mainly determined by *GluD1x* glutenins. It showed a progressive increase in the *GluB1x* peak from samples having high value of extensibility and high *GluD1x* content (AST, MAR, BOL) to wheats with the lowest values of extensibility and *GluD1x* (PAN, S26, GAZ). This could be explained by the interaction of particular HMW subunits in stabilizing polymers or other effects on polymer's conformation affecting the visco-elastic properties of gluten.

Conclusions

1. Dough strength is mainly dependent on the total amount of glutenins (HMW and LMW), particularly the HMW x-type subunits,
2. The correlation of the ratio GLI/HMW and LMW/HMW to extensibility of wheat flour suggests that monomeric gliadins and LMW act as a solvent for glutenins. A high ratio of GLI/HMW leads to a more viscous material.
3. Dough extensibility is mainly influenced by HMW. The allele *GluB1x7* seems to be a determinant for dough extensibility,
4. Novel effects resulting from the presence or absence of certain alleles or small differences in their molecular structures may explain rheological differences. It is necessary to know the optimal proportion of individual subunits to model the gluten structure.

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Pleiotropic effects of *Ppd-D1* on yield and its components in recombinant lines of common wheat

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Introduction

Day length is one of the most important factors affecting the rate of wheat development. Under tropical conditions, the length of the vegetative period for spring wheat is about 10 weeks, but for photoperiodically sensitive winter wheats in the cool European climate, this can be extended to almost a year (Worland *et al.*, 1993).

Many genes present on different chromosomes determine genetic control of day length insensitivity. Genes *Ppd-D1* (formerly *Ppd1*), *Ppd-B1* (formerly *Ppd2*) and *Ppd-A1* (formerly *Ppd3*) are localized on chromosomes of the homoeologous group 2 (2D, 2B and 2A, respectively) and have the major influence on this trait (Welsh *et al.*, 1973; Law *et al.*, 1978; Scarth and Law, 1984, Snape *et al.*, 2001). Moreover, genes localized on other chromosomes from the homeologous groups 1 and 6 (Law, 1998) and on 3D (Miura and Worland, 1994) or 4B (Halloran and Boydell, 1967) also affect the date of heading.

In order to evaluate precisely the effects of *Ppd-D1*, a series of homozygous recombinant lines from the cross of Cappelle-Desprez (Mara 2D) on to Cappelle-Desprez was created at the John Innes Centre, England (Worland *et al.*, 1988). These lines were tested in several countries: England (Worland *et al.*, 1988), Yugoslavia (Worland *et al.*, 1988; 1990), Germany (Worland *et al.*, 1991; Börner *et al.*, 1993). From these studies the effects of the *Ppd-D1* gene were evaluated on heading and flowering date, on yield components as well as its interaction with different environmental conditions (Börner *et al.*, 1993; Worland *et al.*, 1993).

In order to find out whether the effect of *Ppd*-D1 was dependent on genetic background, a series of substitution lines were developed at the John Innes Centre. These involved the varieties Avalon, Brigand, Brimstone, Mercia, Norman and Randezvous into which chromosome 2D, carrying *Ppd-D1,* was substituted from the varieties Mara and Ciano 67.

Materials and methods

Experiments were carried out over seasons 1991-93 for the Cappelle-Desprez(Mara 2D) recombinant lines and 1998-2000 for the substitution lines, Avalon(Mara 2D), Avalon(Ciano 2D), Brigand(Mara 2D), Brimstone(Mara 2D), Brimstone(Ciano 2D), Mercia(Mara 2D), Norman(Mara 2D), Randezvous(Mara 2D) near Lublin in Czeslawice, (Eastern Poland). Every year about 550 germinating kernels per m^2 were machine-sown in 5 m^2 plots in four replicates. 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 and used to calculate the number of spikelets/spike, number of grains per ear, weight of grains in ear, 1000 grain weight and spikelet fertility. The results obtained were statistically analyzed by ANOVA, each year being treated separately.

Results and discussion

It was found that gene *Ppd-D1* accelerated heading date. There were significant differences between the recombinant lines and the controls in the number of days to heading from May 1st. Lines carrying *Ppd-D1* headed earlier than lines with *ppd*-D1 by more than 3 days in 1991 and almost 5 days in 1993 (Tab. 1). Comparing lines Avalon (Mara 2D), Avalon (Ciano 2D), Brimstone (Mara 2D) and Brimstone (Ciano 2D) showed that *Ppd-D1* introduced from Mara 2D accelerated heading slightly more than Ciano 2D throughout the three-year study. However, the differences were small (Tab. 2). This contrasts with the work of Worland *et al.* (1998) who analyzed recombinant lines of Cappelle-Desprez in which alleles of *Ppd-D1* were substituted from Mara and Ciano. They established that the *Ppd-D1* allele originating from Ciano accelerated heading date to a greater extent than the allele from Mara, although again the differences were small. Many authors, on the basis of experiments performed upon recombinant, substitution and monosomic lines, as well as varieties carrying *Ppd-D1,* have demonstrated accelerated ear emergence compared to forms having alleles *ppd-D1* (sensitive to day length) (Worland and Law, 1986; Petrović and Worland, 1988; Worland *et al.*, 1988; Worland *et al.*, 1990; Börner *et al.*, 1993).

Recombinant lines derived from Cappelle-Desprez (Mara 2D) and containing *Ppd-D1* were significantly shorter compared to lines with *ppd-D1* (Tab. 1). Height reduction varied from 5 cm in 1991 to 4.5 cm in 1992. No significant differences of plant height were found in 1998-2000 between the recombinant lines and the controls (Tab. 2). The values of the observed traits for *Ppd-D1* and *ppd*-D1 lines were similar throughout the three-year experiment. Börner *et al.* (1993) who studied lines of Cappelle-Desprez (Mara 2D) in Germany, found that *Ppd-D1* reduced plant height. The level of reduction depended on the year and varied from 1.86 cm in 1990 to 6.18 cm in 1991. From studies performed upon recombinant lines of different varieties it follows that plant height of lines carryings *Ppd-D1* greatly depend upon the experimental year (Tab. 2).

When analyzing number of spikelets/ main spike of the lines compared to the control, significant differences were found in four combinations. Lines of Cappelle-Desprez containing *Ppd-D1* gave significantly less spikelets/ spike than the control in 1991 and 1993 (Tab. 1). In later experiment, Brigand (Mara 2D) formed significantly less spikelets/ main spike than the control in the first year and significantly more in the second (Tab. 2). In a Brimstone background, Brimstone (Mara 2D) in the first and third year of study, and Brimstone (Ciano 2D) in 1998 formed significantly less spikelets/ main spike. Börner *et al.* (1993) and Worland *et al.* (1998) found similar differences in the recombinant lines of Cappelle-Desprez.

Avalon (Mara 2D) in 1999 as well as Brigand (Mara 2D), Brimstone (Mara 2D) and Brimstone (Ciano 2D) in the first year set significantly more kernels/ spike compared to the control. However, no significant differences were found between the lines and their controls in the third year of study. Values obtained for the Cappelle-Desprez (Mara 2D) lines were similar (Tab. 1). Worland *et al.* (1998) tested Cappelle-Desprez lines with *Ppd-D1* substituted from Mara and Ciano in England and Germany. They found that Cappelle-Desprez (Mara 2D) in both countries set more kernels/ spike. However, in recombinant lines of Cappelle-Desprez (Ciano 2D), they found higher (in England) and lower (in Germany) value of the trait than in the control.

Recombinant lines with *Ppd-D1* were characterized by higher weight of kernels/ spike compared to the control, except for the Brigand and Brimstone series in the second year of study as well as Mercia and Randezvous in 2000. Significantly higher values of the trait were observed in lines Brimstone (Ciano 2D) in 1998 as well as Avalon (Mara 2D) and Norman (Mara 2D) in 1999. In the third year of study, no significant differences between the lines and their controls were found. Values of traits in lines derived from Cappelle-Desprez (Mara 2D)

were similar (Tab. 1) in experiments performed in 1991-1993. Worland *et al.* (1998) found that in Germany, the trait value was higher, but only in recombinant lines of Cappelle-Desprez (Mara 2D).

Recombinant lines of Cappelle-Desprez (Mara 2D) had similar spikelet fertilities in all years of study. In the second experiment in 1998, all the recombinant lines with *Ppd-D1* had higher spikelet fertility compared to those with *ppd-D1* (except for Randezvous (Mara 2D). In 1999, only Brigand (Mara 2D), Brimstone (Mara 2D) and Brimstone (Ciano 2D), were values of the trait lower than in the controls, but the differences were insignificant. Significant higher spikelet fertility was recorded in lines Avalon (Ciano 2D), Brigand (Mara 2D), Brimstone (Mara 2D) and Brimstone (Ciano 2D) in the first year of study, and Avalon (Mara 2D) in the second year (Table 2). In the third year of study, values of this trait for all the lines were similar. Worland *et al.* (1998) analyzed the pleiotropic effects of genes *Ppd-D1* in recombinant lines of Cappelle-Desprez (Mara 2D) and Cappelle-Desprez (Ciano 2D). They showed that lines carrying *Ppd-D1* in England had higher spikelet fertility. In Germany, however, lines of Cappelle-Desprez (Mara 2D) had higher, and Cappelle-Desprez (Ciano 2D) slightly lower fertilities than the control forms.

In experiments carried out in 1991-1993, 1000-kernel weights in the Cappelle-Desprez (Mara 2D) lines were similar (Tab. 1). In the experiments performed in 1998-2000, involving substitution lines from different varieties, it was shown that 1000-kernel weight depended mainly on experimental year and to a lesser extent on the line. Higher values of the trait were found in 2000. Significantly higher 1000-kernel weight was observed in lines Norman (Mara 2D) and significantly lower in Mercia (Mara 2D) compared to the controls in the second year of study. In the third year, significantly higher values of the trait were recorded in lines of Avalon (Mara 2D), Brimstone (Mara 2D) and Norman (Mara 2D) in comparison to the controls. Worland *et al.* (1998) demonstrated that unfavourable pleiotropic effects of *Ppd-D1* originating from Mara and Ciano on grain weight in England. In Germany, Cappelle-Desprez (Mara 2D) lines carrying *Ppd-D1* had lower, and Cappelle-Desprez (Ciano 2D) higher weights than the controls.

Grain yield/ plot was variable depending on the year and the line. In recombinant lines of Cappelle-Desprez (Mara 2D), no significant differences in grain yield were found (Tab. 1). The highest grain yields per plot were recorded in 2000. Significantly lower grain yield/ plot was found for line Brigand (Mara 2D) in 1998 and Rendezvous (Mara 2D) in 1999. Significantly higher value of this trait was observed in 1998 and 1999 for lines Avalon (Mara 2D), Avalon (Ciano 2D) and Brimstone (Mara 2D). In the third year of study, significantly higher grain yield per plot was found only in lines Mercia (Mara 2D). Börner *et al.* (1993) found variable values of this trait depending on the year.

Conclusions

- *Ppd-D1* affected the acceleration of heading date among recombinant lines.
- Significant reduction of plant height compared to the control was found in the recombinant lines of Cappelle-Desprez (Mara 2D) containing *Ppd-D1*.
- On the basis of a six-year study, it was found that number of spikelets per spike, number and weight of kernels per spike as well as spikelet fertility in analyzed forms depended mainly on the experimental year and to a lesser extent on the line.

Table 1. Pleiotropic effects of *Ppd-D1* genes in recombinant lines on yield and its components in Eastern Poland (Czeslawice 1998-2000)

Recombinant lines	Ear emergence (days from 1V)			Plant height (cm)			Number of spikelets in ear			Number of grains per ear			Weight of grains in ear (g)			Fertility of spikelets			Weight of 1000 grains (g)			Plot yield (t/ha)		
Year	1991	1992	1993	1991	1992	1993	1991	1992	1993	1991	1992	1993	1991	1992	1993	1991	1992	1993	1991	1992	1993	1991	1992	1993
Cappelle-Desprez (Mara 2D) /Ppd1/	37.8*	35.2*	28.1*	99.1*	98.3*	84.8*	17.9*	15.1	16.4*	43.7	32.5	38.3	1.98	1.85	2.03	2.43	2.21	2.32	45.6	53.0	53.2	5.10	6.06	6.75
Cappelle-Desprez (Mara 2D) /ppd1/	41.1	39.6	33.0	104.1	102.8	89.5	18.6	15.8	17.2	42.2	34.3	40.1	1.94	1.87	2.18	2.26	2.15	2.33	45.9	54.7	54.5	5.50	6.50	6.57

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

Table 2. Pleiotropic effects of *Ppd-D1* genes in recombinant lines on yield and its components in Eastern Poland (Czeslawice 1998-2000)

Recombinant lines	Ear emergence (days from 1V)			Plant height (cm)			Number of spikelets in ear			Number of grains per ear			Weight of grains in ear (g)			Fertility of spikelets			Weight of 1000 grains (g)			Plot yield (t/ha)		
Year	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000	1998	1999	2000
Avalon(Mara 2D)	29.0*	32.7*	22.2*	72.3	84.0	96.9	20.6	14.9	14.8*	52.7	35.9*	39.5	2.02	1.63*	2.21	2.56	2.39*	2.66	37.9	45.6	56.1*	6.43*	5.94*	7.76
Avalon (Ciano 2D)	30.3*	33.0*	23.2*	68.8	83.1	96.8	20.1	17.3	17.0	55.4	29.0	41.3	1.90	1.16	1.84	2.76*	1.66	2.41	34.6	41.5	44.6	8.38*	5.36*	8.26
Avalon	35.3	37.5	25.0	68.8	79.3	95.3	22.1	16.0	16.8	48.3	26.1	47.6	1.72	1.06	2.33	2.20	1.64	2.83	35.1	41.3	48.8	5.45	4.11	7.48
Brigand (Mara 2D)	32.5*	33.5*	22.5*	66.2	79.2	96.8	17.9*	17.5*	16.0	54.6*	30.4	42.5	1.53	1.27	2.20	3.02*	1.72	2.66	26.5	42.5	51.9	4.03*	5.30	7.60
Brigand	37.0	38.2	25.2	65.4	80.7	98.4	20.2	15.0	14.4	45.1	31.2	38.5	1.41	1.45	2.19	2.37	2.09	2.66	35.8	46.3	56.8	7.80	5.74	7.38
Brimstone (Mara 2D)	28.3*	32.5*	22.2*	71.6	83.6	99.4	19.5*	16.0	14.5*	53.4*	23.9	37.9	1.63	1.11	2.19	2.74*	1.51	2.61	30.1	45.3	58.0*	7.83*	5.54*	7.21
Brimstone (Ciano 2D)	29.0*	33.0*	23.5*	71.7	81.1	94.5	18.2*	16.5	17.9	51.3*	27.1	43.7	2.15*	1.25	2.25	2.80*	1.68	2.43	42.4	46.3	51.4	6.68	5.86*	7.20
Brimstone	31.8	36.5	25.5	68.9	78.1	94.4	22.0	16.8	17.9	34.0	30.4	42.7	1.25	1.24	2.04	1.54	1.81	2.37	37.0	41.7	47.9	6.25	4.51	7.65
Mercia (Mara 2D)	29.3*	33.7*	22.5*	76.5	77.4	93.6	20.2	17.0	18.3	47.7	30.1	43.0	1.56	1.13	2.16	2.38	1.76	2.34	32.7	37.5*	50.4	7.30	5.98*	8.72*
Mercia	37.3	38.2	25.5	79.7	79.6	96.3	20.9	17.7	17.7	45.2	24.5	45.2	1.50	1.13	2.08	2.17	1.38	2.54	33.7	46.8	46.1	6.86	4.34	7.54
Norman (Mara 2D)	27.8*	33.5*	23.0*	74.1	81.9	91.1	18.4	16.6	16.9	41.7	28.3	46.8	1.83	1.29*	2.31	2.29	1.71	2.77	43.8	46.1*	49.5*	7.33*	5.85	8.51
Norman	36.0	37.0	25.2	72.9	78.8	87.2	19.8	17.1	16.3	42.8	21.9	45.2	1.48	0.87	1.83	2.14	1.28	2.76	35.8	40.0	40.5	6.24	6.17	7.62
Randezvous (Mara 2D)	32.5*	33.0*	22.5*	66.2	87.8	93.7	20.2	16.0	16.4	46.7	28.8	44.9	1.54	1.18	2.38	2.33	1.81	2.73	33.0	41.6	53.0	3.72*	5.61*	8.76
Randezvous	36.5	36.2	25.5	59.9	84.3	90.4	20.0	15.1	17.6	48.5	24.6	49.3	1.32	1.12	2.57	2.43	1.66	2.80	26.7	45.6	52.1	2.72	6.73	8.43

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

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The study of wheat substitution lines for homoeologous group 5 chromosomes carrying dominant loci *Vrn*

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The presence or absence of *Vrn* genes determines the spring or winter habit of common wheat (*Triticum aestivum L.*). Winter wheats are vernalization sensitive, needing a period of cold temperature before the plants are able to go to flowering. Winter growth habit is usually diagnostic for a recessive constitution of all *Vrn* alleles (*vrn*). These prevent the premature development of the plants through to the generative stage of flowering following autumn sowing (Pugsley 1972). Spring growth habit, on the other hand, occurs when a dominant allele of *Vrn* is present, inhibiting the vernalization requirement and allowing the plants to flower after a spring sowing.

Besides the major vernalization requiring genes, *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, located on the chromosomes 5A, 5B and 5D, respectively (Law et al. 1976, Maystrenko 1980), other loci are known to regulate flowering time, especially *Vrn-B4* (formerly *Vrn5)* on chromosome 7B (Law 1966), *Vrn-A2* on the translocated segment of chromosome 4A to chromosome 5A, the *Vrn-3* series on the homoeologous group 1 chromosomes (Law and Worland 1997), and a series of vernalization sensitive genes on chromosomes of homoeologous group 6 (Islam-Faridi et al. 1996). The vernalization requirement of a variety is determined by a combination of alleles at all these loci, many of which occur as multiple alleles (Pugsley 1971; Snape et al. 1976, Pánková and Košner 1998).

The distribution of the major *Vrn* loci in spring wheat cultivars was studied in 482 cultivars sampled world-wide by Stelmakh and Avsenin (1985)**:** *Vrn-A1 vrn-B1 vrn-D1* = 125; *vrn-A1 Vrn-B1 vrn-D1* = 35; *vrn-A1 vrn-B1 Vrn-D1* = 18; *Vrn-A1 Vrn-B1 vrn-D1* = 251; *Vrn-A1 vrn-B1 Vrn-D1* = 29; *vrn-A1 Vrn-B1 Vrn-D1* = 22; *Vrn-A1 Vrn-B1 Vrn-D1* = 2. These numbers give frequencies of dominant alleles at these major loci: of *Vrn-A1*= 84.4 %, *Vrn-B1*= 64.3 % and *Vrn-D1*= 14.7 %.

A study of growth habit of 642 wheat genotypes (Stelmakh 1987) revealed the presence of the loci *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in the genotypes of spring wheat (*Triticum aestivum*). Only the substitution line Chinese Spring (Hope) was found to carry the dominant allele *Vrn5*, detected on chromosome 7B, the source of which could be *Triticum dicoccum,* cv. Vernal.

The causes of the different allelic frequencies of *Vrn* globally may be due to their different breeding values, resulting in their differential selection either by natural or artificial means (Stelmakh, 1990). The best example of this is the expansion of *Vrn-D1* (formerly *Vrn3*), introduced by N. Strampelli from the Japanese landrace Akakomugi into Italian cultivars and through the variety Mentana to Mexican cultivars at the beginning of 20th century. Later this became the bases of modern wheat cultivars, spread mainly in the countries, contiguous to the equator. Stelmakh (1993) found a correlation between the influence of *Vrn* genotypes in spring wheat and grain mass per plant and suggested a stronger effect of the *Vrn* genotypes in comparison to that of the genetic background and the environment.

The effect of different dominant *Vrn* genes on time to heading in the spring background of Zlatka

Epistatic effect of the dominant *Vrn-A1* allele over the alleles of *Vrn-B1* and *Vrn-D1* and the influence of the present alleles on time to heading were studied in the spring wheat cultivar ´Zlatka´ and its substitution lines Zlatka (ČP5B), Zlatka (CS5D), obtained by substitutions of chromosomes 5B and 5D of ´Česká Přesívka´ and ´Chinese Spring´, respectively, into ´Zlatka´.

The field experiment was carried out in the spring of 2000 with 6 different sowing dates. Time to heading and dates of heading of the plants were evaluated. The shortest mean time to heading, 81.22 days, was found for Zlatka, an almost identical time to heading, 81.94 days, was shown by Zlatka (CS5D), whereas Zlatka (ČP5B) headed four days later at 85.36 days. This corresponds with dates of heading: Zlatka 10.6., Zlatka (CS5D) 11.6., and Zlatka (ČP5B) 12.6. No significant differences were observed between Zlatka (*Vrn-A1*) and Zlatka (CS5D) (*Vrn-A1, Vrn-D1*) or between the genotypes Zlatka (CS5D) (*Vrn-A1, Vrn-D1*) and Zlatka (ČP5B) (*Vrn-A1, Vrn-B1*); a significant difference was found between Zlatka and Zlatka (ČP5B): Time to heading was slightly delayed by substitution of chromosome 5B carrying *Vrn-B1* into the genotype carrying *Vrn-A1*. The delayed sowing date led to reduction of vegetation period by 0.74 days per one day of delay in sowing.

The effect of substitutions of homoeologous group 5 chromosomes carrying dominant *Vrn* genes into winter backgrounds Košutka, Vala and Zdar on time to heading and photoperiod response

Spring growth habit was studied in wheat substitution lines, obtained by the substitution of homoeologous group 5 chromosomes, carrying dominant *Vrn* genes. Three genotypes of winter wheat, Košutka, Vala and Zdar, differing in their photoperiod responses were chosen to ake genetically defined lines with changed growth habit. The substituted chromosomes came from genotypes with known *Vrn* genes (Law et al, 1976; Košner 1984; Košner, 1987; Košner and Bromová 1993). The lines obtained by a series of backcrosses with monosomic lines for the given chromosomes of recipient cultivars are: Zdar (Zlatka 5A), Zdar (Česká Přesívka 5B),

Zdar (Chinese Spring 5D), Vala (Zlatka 5A), Vala (Česká Přesívka 5B), Vala (Chinese Spring 5D), Košutka (Zlatka 5A), Košutka (Česká Přesívka 5B), Košutka (Chinese Spring 5D). The experiment aimed at verifying the sources of the substituted chromosomes, checking the substitutions in individual sub-lines, and concurrently detecting differences between the effects of the loci *Vrn-A*, *Vrn-B1* and *Vrn-D1* present in both identical and different genetic backgrounds on time to heading.

Grains of the substitution lines were germinated at weekly intervals and vernalized (1 - 3 ^{0}C) so that variants with 4, 3, 2, 1 and 0 weeks of vernalization were prepared for planting on the 20th April when the day-length exceeded 14 hours (long-day photoperiod), and there was a low probability of additional vernalization. The grains of all of the lines and covering all the variant vernalization treatments were sown as plots in the field. The grains of all the lines, vernalized for 4 weeks, were also sown under conditions of a 10 hours´ light regime to detect the influence of day length and eliminate different effects of *Vrn* loci. The time from planting to heading was evaluated. The data obtained on approximately 1400 plants were statistically assessed.

The experiment confirmed the correctness of the chosen sources of dominant *Vrn* genes. All of the the substitution lines gave the anticipated spring growth habit. Thus, the genotypes of cultivars Zlatka, Česká Přesívka, and Chinese Spring are reliable sources of *Vrn-A1*, *Vrn-B1* and *Vrn-D1*, respectively. The experiment also showed cases where the transferred chromosome or gene had been lost during the development of the substitution lines - such sub-lines did not go to heading as they evidently did not carry the transferred dominant *Vrn* genes. It reaffirms the necessity of often and precisely checking the correctness of the transferred chromosomes during the process of obtaining chromosome substitutions.

The statistical analysis showed significant influence of all the studied factors: *Vrn* genes, genetic background and duration of vernalization on time to heading:

The estimates of genetic differences between lines carrying homoeologous group 5 chromosomes were significant in all of the observed cases; *Vrn-A1* versus *Vrn-B1* = -3.532 days, *Vrn-A1* versus *Vrn-D1* = 3.407 days, and *Vrn-B1* versus *Vrn-D1* = 10.852 days. The effect on earliness was detected in the following order: *Vrn-D1* > *Vrn-A1* > *Vrn-B1*. We can conclude that the *Vrn* genes influence earliness significantly. Stelmakh, Avsenin, (1985) established additive effects of *Vrn* genes on earliness: *Vrn-A1* (-12.8 days), *Vrn-D1* (-12.2 days), *Vrn -B1* (-10.2 days). The least effect was found for *Vrn-B1*; effects of *Vrn-A1* and *Vrn-D1* were similar to each other.

The estimates of differences between genetic backgrounds were also significant in all of the cases: Košutka versus Vala = -15.950 days, Košutka versus Zdar = -9.839 days a Vala versus Zdar = 12.273 days. The ranking of effects of the backgrounds on earliness was similar to the different photoperiod sensitivities of the varieties, i.e., Košutka > Zdar > Vala.

In conclusion, the experiment showed the major effect of vernalization on time to heading. Regression analysis also demonstrated that the duration of vernalization was dependant upon the background into which the *Vrn* genes had been introduced. Lines with *Vrn-B1* gave the weakest response to vernalization, lower than either of the lines with *Vrn-A1* or with *Vrn-D1,* which responded similarly. This was surprising since the lowest sensitivity to vernalization had been expected to be *Vrn-A1*, which is known to be epistatic over the other *Vrn* genes. The differences between the coefficients that characterize time to heading following incomplete vernalization were probably caused by interactions between the genetic background (mainly influenced by differing responses to photoperiod sensitivity) and the respective *Vrn* loci.

Freezing tolerance of wheat lines carrying homoeologous group 5 chromosomes with dominant *Vrn* genes substituted into winter backgrounds Košutka, Vala and Zdar

Besides the three main loci governing vernalization, genes controlling tolerance to freezing have also been reported to be carried by the homoeologous group 5 chromosomes (Prášil et al. 2002). The effect of these chromosomes on the induction, maintenance and loss of freezing tolerance of hexaploid wheat was studied using the set of intervarietal chromosome substitution lines between spring (Chinese Spring, Zlatka), alternative (Česká Přesívka) and winter (Vala, Košutka, Zdar) wheat cultivars carrying *Vrn-A1*, *Vrn-B1* and *Vrn-D1* in the winter wheat backgrounds of Košutka, Vala and Zdar. The evaluation of freezing tolerance of the substitution lines was carried out by the pot method under natural conditions (provocation test). The study revealed that substitutions of homoeologous group 5 chromosomes carrying *Vrn* genes in the three different winter wheat backgrounds (Košutka, Vala and Zdar) led not only to changes from winter to spring growth habit, but also to a marked decline in tolerance to winter frosts of the substitution lines that was far lower compared to the parental winter lines. The winter survival of the substitution lines carrying 5A and 5D chromosomes from Zlatka or Chinese Spring was low and similar to winter survival of each of the two spring cultivars. The survival of the substitution lines carrying 5B chromosome from the alternative cultivar Česká Přesívka was lower than in the parental winter cultivars but higher than in the substitution lines carrying 5A or 5D chromosomes from the spring cultivars. The lesser effect of the 5B chromosome with *Vrn-B1* on decreasing of freezing tolerance is being studied further because Ĉeská Přesívka (donor of 5B chromosome) gives a high degree of tolerance.

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Quantitative trait loci mapping in wheat

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Introduction

Since molecular markers became available a range of genes determining qualitatively inherited characters have been tagged in cereals. Examples for wheat are mapping of genes determining reduced plant height (Börner et al. 1997, Korzun et al. 1997, 2000), vernalisation response (Galiba et al. 1995, Korzun et al. 1997) or photoperiod response (Worland et al. 1998). Another, even higher impact from applying molecular markers is expected by analysing quantitative inherited traits. In wheat, quantitative trait loci (QTLs) for several characters including disease resistance, winter hardiness, plant height or tissue culture ability have been described by Nelson et al. (1995a, b, c), Galiba et al. (1995), Ben Amer et al. (1997), Cadalen et al. (1998) or Keller et al. (1999a, b).

In the early Nineties the 'International Triticeae Mapping Initiative' (ITMI) was established as a world-wide platform for joint mapping of RFLP (Nelson et al. 1995a, b, c, Van Deynze et al. 1995, Marino et al. 1996) or microsatellite markers (Röder et al. 1998). In the present study a set of 114 molecular well characterised recombinant inbred lines (RILs) of the ITMI mapping population was evaluated for morphological, agronomical and disease resistance traits under several environments. Mapping data for QTLs determining the traits plant height, ear emergence time and flowering time are presented.

Materials and methods

The ITMI mapping population was created by crossing the spring wheat variety 'Opata 85' with a synthetic hexaploid wheat generated via a cross of the *Triticum tauschii* accession 'CIGM86.940' with the tetraploid wheat 'Altar 84' (Nelson et al. 1995b). In total 114 RILs, obtained by Dr. Philippe Leroy, INRA, Clermont-Ferrand, France were chosen for the experiments. After multiplication the seeds were divided and grown in plots during four seasons at three sites. Days to ear emergence and flowering time were recorded when >50% of the ears of each RIL had left the flag leaf sheath or flowered, respectively. Plant heights were recorded just before harvest. The phenotypic data obtained were integrated into the existing framework map. QTL analysis was performed with MAPMAKER/QTL 1.1 (Paterson et al. 1988). Loci with a LOD score between 2 and 3 will be designated minor QTLs, the ones with LOD scores >3 major QTLs.

Results and discussion

Scoring the two related traits ear emergence time and flowering time major and/or minor QTLs were detected. For ear emergence time (Figure 1) major loci were found to map on chromosome arm 2DS and on chromosome arm 5DL. In two experiments minor QTLs were detected in the distal region of chromosome arm 7DS.

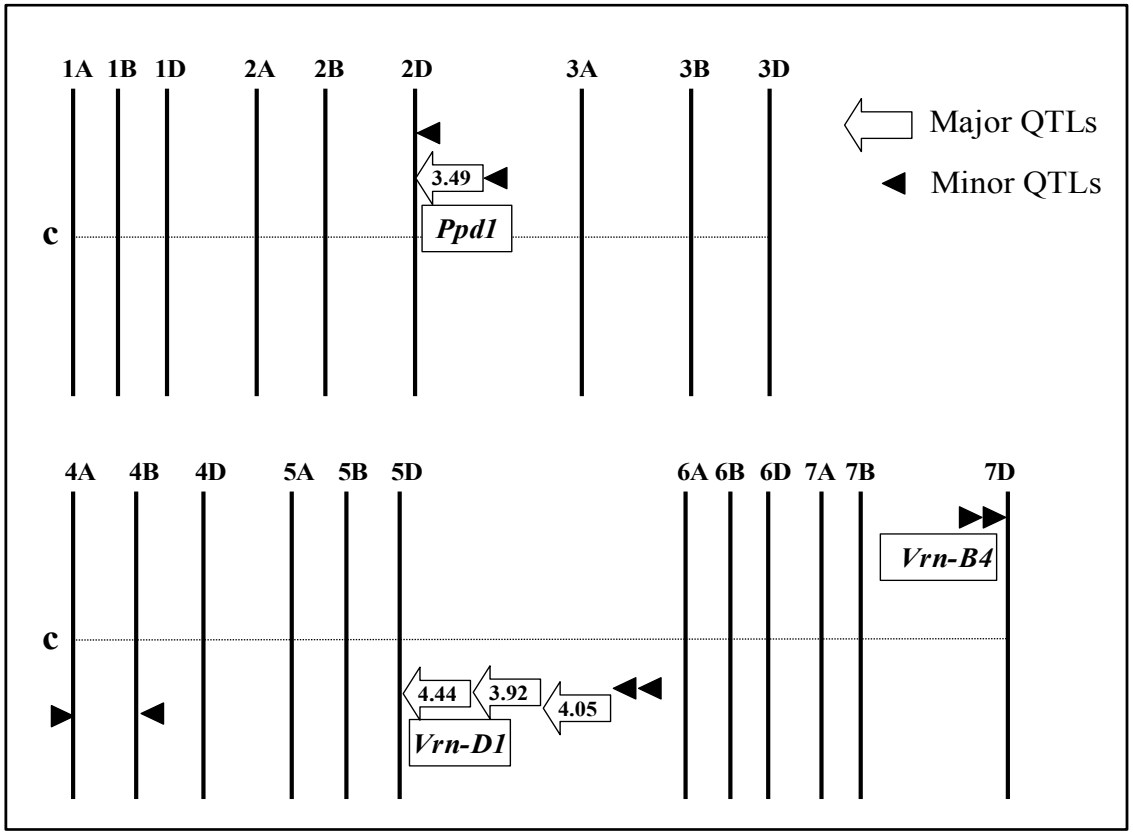

Fig. 1: Map positions of QTLs determining the trait ear emergence time. The effects contributed by the synthetic wheat and 'Opata 85' are given on the left and right hand side of each chromosome, respectively. Corresponding major genes known from literature are boxed.

Loci determining flowering time (Figure 2) were again mapped on chromosome arm 2DS. Minor loci were also found in the homoeologous region on chromosome arm 2BS. Further QTLs were found on chromosome arm 5DS and on chromosome arm 3AL.

The map positions of the detected QTLs are highly comparable to those of major genes for photoperiod and vernalisation response in wheat (Mc Intosh et al. 1998) and other Triticeae (Börner 1999). On chromosomes 2BS and 2DS the major genes *Ppd2* and *Ppd1*, respectively, are located whereas the detected QTLs on 5DL correspond with *Vrn-D1*. For the QTLs detected on chromosome arm 7DS homoeology to a vernalisation response gene in the distal region of chromosome 7BS described by Law (1966) and Law and Wolfe (1966) and designated *Vrn-B4* (Mc Intosh et al. 1998) may be suggested. The flowering time locus on chromosome arm 3AL may correspond with an earliness *per se* gene (*Eps-A1*) affecting the plant development independent of response to vernalisation and photoperiod (Miura et al. 1999, McIntosh et al. 2000).

Four major QTLs but 14 additional minor QTLs were found for final plant height (Figure 3). This was not surprising, because the trait is known to be determined by many genes (Börner et al. 1996). One locus was found to be in a position of a known major dwarfing gene (*Rht8*) on the short arm of chromosome 2D (Korzun et al. 1998), although the effect on height may be also caused by pleiotropy of the detected *Ppd* locus, as described by Börner et al. (1993). QTLs for plant height were already detected by Cadalen et al. (1998), of which three were in comparable positions on chromosome arms 1AS, 1BL and 4BL as discovered here. Of further interest may be the two loci on chromosome arms 4AL and 6AS, where major and minor QTLs were detected in several experiments.

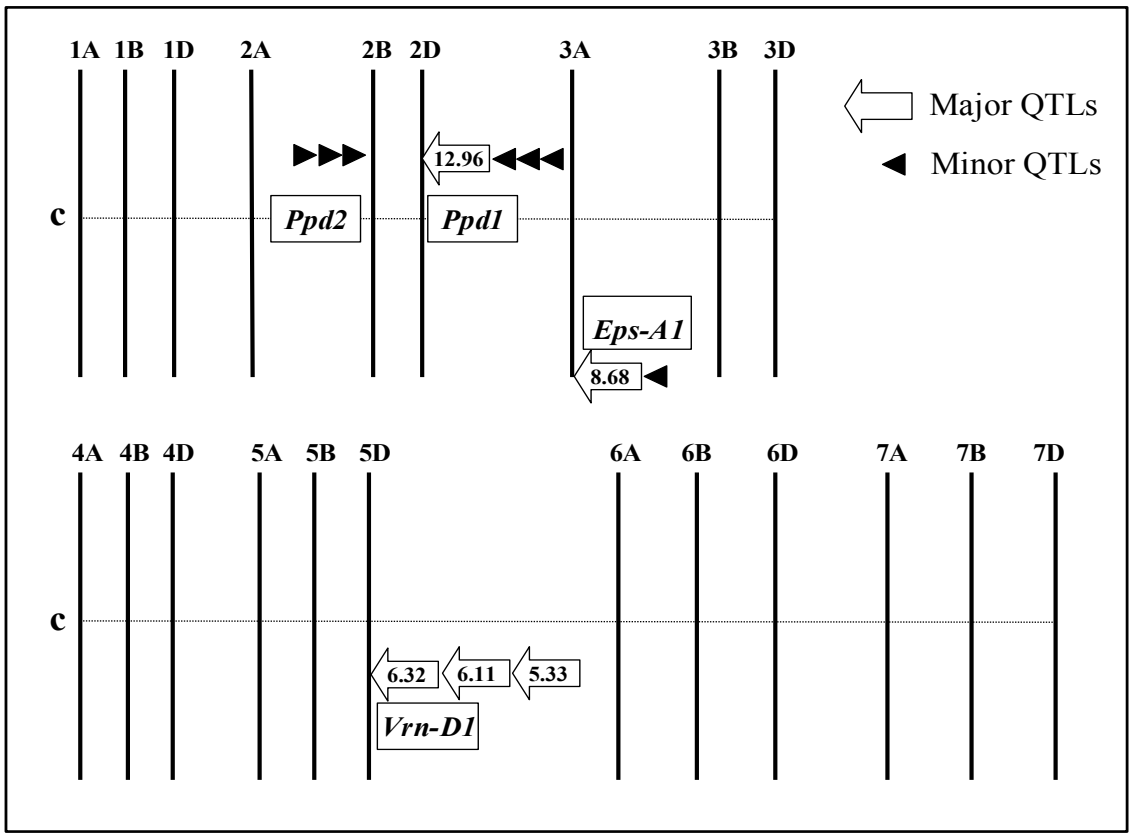

Fig. 2: Map positions of QTLs determining the trait flowering time. The effects contributed by the synthetic wheat and 'Opata 85' are given on the left and right hand side of each chromosome, respectively. Corresponding major genes known from literature are boxed.

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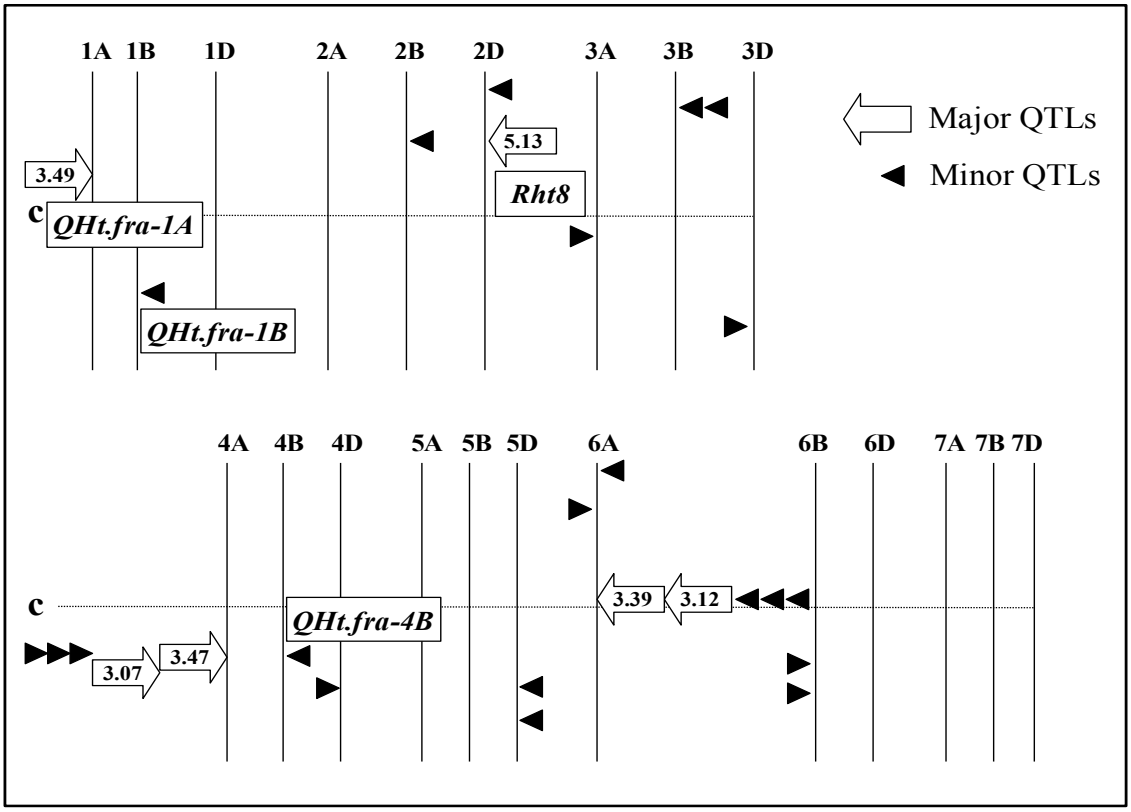

Fig. 3: Map positions of QTLs determining the trait final plant height. The effects contributed by the synthetic wheat and 'Opata 85' are given on the left and right hand side of each chromosome, respectively. Corresponding major genes known from literature are boxed.

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A QTL analysis for earliness under field and controlled conditions in a bread wheat doubled-haploid population

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Introduction

To obtain maximum grain yield it is essential that a crop is well adapted to its environmental growing conditions. In particular, it needs to flower and mature at the most appropriate times. In such a way, earliness can be considered as an adaptive trait (Worland, 1996) and consequently it is a main factor of variation for classical agronomic traits.

Studying tolerance of the winter bread wheat to nitrogen stress, Le Gouis *et al.* (2000) showed that the number of ears per square meters of some late genotypes was more affected by the nitrogen stress. A good way to study the interaction between tolerance to nitrogen deficiency and earliness could be to describe earliness of each genotype precisely. To undertake such a study, the 3 components of earliness, *i.e.* photoperiod sensitivity, vernalisation requirement and earliness *per se*, were measured among a population of recombinant lines derived from the cross between a tolerant and a susceptible cultivar differing also for earliness components. These results were used to detect and locate QTL involved in the control of earliness. The first results of this work will be presented in this paper. Major QTL, controlling photoperiod response and vernalisation requirement, were detected on chromosomes 2B and 2D,.

Materials and methods

Plant material and earliness components evaluation

A population of 241 doubled-haploid (DH) lines was produced from the hybrid between the varieties Arche and Récital. Arche and Récital have contrasting characteristics for both earliness and tolerance to a nitrogen stress (Le Gouis et al, 2000). The DH population together with the parents was studied in field and greenhouse experiments. First, heading date (HD) was recorded over two years (1999-2000) in field sown experiments. Three planting dates (October, March and May) were also used at Estrées-Mons (Lat.: 49°52'44"), INRA northern France. In May, unvernalised and previously artificially vernalised plants (8 weeks at 6.5 °C) were planted. Secondly, in 2001, HD was measured under 2 daylength conditions (9 and 24 hour day) in a specially adapted greenhouse at Norwich, JIC United Kingdom, plants having previously been vernalised for 8 weeks at 5 °C.

With such a design, several criteria could be used to estimate earliness components. **Photoperiod sensitivity** (PS) was measured in 2 ways. First, by considering HD from the October sowing; the normal sowing date for winter bread wheat northern France, since this would reflect mainly differences in photoperiod sensitivity only. Secondly, by comparing HD in the two greenhouse daylength treatments. **Vernalisation requirement** (VR) was characterized by comparing the unvernalised and vernalised plantings in May and also including the results of the partially vernalised March sowing. This last sowing was very useful when ranking genotypes with at least minimum vernalisation needs. **Earliness *per se***

was estimated using HD obtained under the greenhouse long day treatment and also HD observed with the May planting of vernalised plants.

QTL detection and location

The QTL analysis was based on a map constructed through a Genoplante project. A set of 188 microsatellite markers was used covering about 2400 cM (70% of the genome) with an average of one marker each 15 cM. The QTL analysis was performed with the PlabQTL software V1.1 (Utz and Melchinger, 2000) using simple and composite interval mapping.

Results

Earliness components for heading date

Photoperiod sensitivity: Results about heading date and reflecting PS differences showed a clear contrasting behaviour between Arche and Récital and a clear bimodal distribution in the DH population. Arche was more sensitive to photoperiod and headed 11 days later than Récital. In the DH population, one sensitive group of lines (Arche type) and one insensitive group (Récital type) could be easily identified. Two criteria have been computed from the long day (LD) and short day (SD) heading dates registered in the photoperiod greenhouse: the ratio (LD/SD) and the difference (SD-LD). They gave consistent ranking of the genotypes for their PS. Nevertheless, these 2 criteria did not give the same partition of the whole phenotypic variation. Indeed, the variation due to the sensitive group was relatively more important with (SD-LD) than (LD/SD).

Vernalisation requirement: Like the spring wheat Arche, about half of the population headed in the unvernalised May planting. From this planting, the rest of the population behaved like Récital and never reached heading stage. Comparison of HD registered for the March sowing and the vernalised May planting showed that genotypes of the Récital type had a delayed HD due to incomplete vernalization.

Earliness per se: No segregation was observed for earliness *per se.* The 2 parents appeared to be similar for earliness *per se* and a normal distribution was observed in the DH population.

QTL detection and location

Ten QTL with a LOD score up to 3.5 were detected on 8 chromosomes. Six of these QTL controlled PS, 3 of them controlled VR and the last one controlled earliness per se. The 4 QTL explaining the greatest part of the phenotypic variation were located on the 2B and 2D chromosomes. Three of them controlled PS: 2 were located on 2D (P1 and P2) and the third on 2B (P3). The 3 QTL for PS explained jointly about 50% of the whole phenotypic variation for this trait. The fourth one, named V1, controlled VR. It explained only 8% of the variation despite the clear partition of the population. We will now focus discussion on these 4 QTL located on the group 2 chromosomes. The P3 QTL was detected for all the criteria used to measure PS. On the opposite, P1 was detected for October HD and the LD/SD criterion, but not the SD-LD criterion; P2 was detected for October HD and the SD-LD criterion, but not the LD/SD criterion. The V1 QTL was detected for all criteria used to measure VR.

Discussion

QTL controlling photoperiod

First, comparing the results with previous work reported in the wheat literature, and to the major contribution on the subject published by Worland (1996) and Worland *et al.* (1998). The P1 and P3 QTL were located, respectively, in the same region as *Ppd-D1* (*Ppd1*) and in the same region as the QTL (differing from *Ppd-B1* or *Ppd2*) controlling both HD and

earliness *per se*. In addition to these wheat results, we used the barley information provided mainly by Laurie *et al.* (1995). These authors located *Ppd-H1,* a major gene controlling PS, on chromosome 2H; at about the same position as *Ppd-D1*, *Ppd-B1* and P1 detected in the Arche x Récital population. Moreover, they found one QTL (*eps2*) controlling HD but not specifically through photoperiod or vernalisation. It appears that P2 and P3 found in the Arche x Récital population correspond to this particular region. The two cultivars Récital and Arche were studied by Worland *et al.* (1994). They concluded that Récital had *Ppd1* and that Arche was not completely sensitive to photoperiod, its pedigree being however insufficiently documented for more precise conclusion.

The analysis of the 2 criteria (LD/SD and SD-LD) each provided one specific QTL on chromosome 2D. Is this specificity corresponding to the different ways of describing PS through these 2 criteria? Kato and Yamashita (1991) discussed the incidence of choosing one or the other criterion. They found that LD/SD was less correlated to earliness *per se* (measured with HD under LD treatment) than SD-LD, and therefore was better adapted to describe PS. With regard to this correlation aspect, a similar tendency was found in the Arche x Récital data, but the most important thing found was that the correlation between SD-LD and the HD registered under SD was near to 1.

QTL controlling vernalisation

Despite the not definitely excluded candidate barley QTL (*eps2*), no data was really found in the crop literature, in particular in wheat, to explain the V1 QTL. Nevertheless, this QTL can be considered as highly reliable because of the clear segregation observed in the population between spring and winter types. In addition, all criteria used for measuring VR lead to the detection of this particular QTL. More markers are needed to confirm this result and to find an expected major QTL (*Vrn* series) on the group 5 chromosomes.

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QTL analysis using aneuploid and conventionally derived lines for the genetic analysis of grain protein and hardness in wheat

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Abstract

The genetic control of grain protein content and grain hardness were investigated using different types of populations derived from the cross between the varieties Avalon and Hobbit 'sib', which represent parentages of UK varieties with hard textured grain, high protein and good bread-making quality; and soft, low protein, suitable for biscuit making, respectively. Analysis of aneuploid derived lines, backcross monosomic lines and recombinant substitution lines (RSL), indicated the importance of the group 5 chromosomes in the genetic control of both traits. Genetic maps developed on the RSL populations were applied for QTL analysis and detected both major and minor QTL on 5D and 5A for both traits. The major effects were confirmed, and others detected, by further QTL analysis on a recombinant inbred line population derived from the same parents. However this latter analysis failed to confirm the minor QTL found by RSL analysis.

Introduction

The control of grain protein levels and grain texture in wheat is both complex and economically very important. Bakers and millers require consistent crops of high protein, hard-grained, wheat for bread-making. This has often been hard to achieve for UK farmers due to the variability of the summer affecting the performance of each variety. This project was set up to identify novel loci and alleles regulating grain protein and texture in order to find genes which could be used to boost protein levels in UK varieties without causing a subsequent loss in yield. The two parental lines studied here were Avalon and Hobbit 'sib', which represent parentages of UK varieties with hard textured grain, high protein and good bread-making quality (BMQ); and soft, low protein, suitable for biscuit making, respectively. Additionally, they differ only in one high-molecular- weight (HMW) glutenin subunit (Hobbit 'sib' has a null allele at the *Glu-A1* locus and Avalon produces the 1 subunit band). This suggested that they might offer insight into other important factors involved in BMQ. Avalon has also been shown consistently to yield better than would be expected for its high protein content, making it an ideal candidate for investigating yield independent protein alleles.

To carry out genetic analysis for these characteristics, two approaches were used. Firstly, the effects of genes on single chromosomes were studied through the use of reciprocal backcross monosomic (RBM) analysis (Snape and Law 1980) and recombinant substitution line (RSL) analysis. This was complemented by scanning the entire genome for other novel loci using a population of recombinant inbred lines (RIL) derived from the same parents.

Single chromosome analyses

Backcross reciprocal monosomic analysis was carried out using the Hobbit 'sib' monosomic series as female parent for the initial cross with Avalon as the male parent. The monosomic F_1 hybrid plants selected were then backcrossed to the Hobbit 'sib' monosomics both as male and female parents and the families of monosomics once again selected. These families will therefore contain a monosomic chromosome from Avalon or from Hobbit 'sib' each within a

random genetic backcross background of the two varieties (¾ Hobbit 'sib', ¼ Avalon). Comparisons of the two families can yield information about the effects of the single chromosomes without having to go through a lengthy backcross programme to produce single chromosome substitution lines.

Data on mean protein content from each family from micro-plot field experiments showed that only three of the 21 chromosomes (5A, 5BL-7BL, 5D) had a significant effect on protein levels at less than the 5% probability level. These chromosomes were also assessed for yield characters, and the results are presented in Table 1.

Table 1. Results from RBM analysis of the Hobbit 'sib' x Avalon group 5 chromosomes.

Chromosome	% protein	yield/ear (g)	Grain No./ear
Avalon 5A	12.77 *	3.39	79
Hobbit'sib 5A	13.74	3.44	74
Avalon 5BL/7BL	13.05 *	3.43 *	79 *
Hobbit'sib 5BL/7BL	14.25	2.95	65
Avalon 5D	13.11 *	3.36	82
Hobbit'sib 5D	12.11	3.62	84

*= significant differences between reciprocals, P =0.05-0.01

In two out of three cases it was the Hobbit 'sib' chromosome which caused an increase in protein content. However, in the case of the 5BL-7BL chromosome this was associated with a large decrease in both yield per ear and grain number per ear. For this reason only the chromosomes 5A and 5D were chosen to produce substitution lines, and from these, recombinant substitution populations to map the genes involved on the individual chromosomes.

Genetic maps were produced for the RSL populations by genotyping each line using Restriction Fragment Length Polymorphism (RFLP) markers and simple sequence repeat (SSR) markers. The maps were developed using Mapmaker and JoinMap to give the best map order. Markers were then chosen from these maps to remove any which were co-segregating and the remainder used in Quantitative Trait Locus (QTL) analysis using QTL Café (www.web.bham.ac.uk/g.g.seaton), on field data over four growing seasons in both microplot and drilled trials. Three types of QTL analyses were carried out. Single marker ANOVA was performed first to detect effects associated with individual markers. Then, interval mapping and marker regression techniques were applied to the same data to locate the positions of the QTL. Composite interval mapping (fixing known markers-QTL and reanalysing the data) was used in the 5D lines to elucidate minor QTL. This was performed using MapQTL.

The protein and hardness measurements showed more variation in the 5D population than the 5A population, and in the 5D population, hardness could be scored as a discontinuous trait, making it possible to map as a Mendelian trait. The hardness variation also correlated strongly with protein content in these lines (r^2 = .64), but did not do so in the 5A population. The results of the QTL analyses are shown in figure 1.

From the mapping results it is clear that the *Ha*, grain hardness, locus has the major, although not exclusive, effect on hardness in the 5D population, and this also appears to be having a major effect on protein content (the peak of the QTL analysed by interval mapping and by marker regression is at this locus), as would be expected from the high correlation between the two characters. The QTL detected are very consistent over the field trials, including the two on the long arm of 5D, which appear to be having a more minor effect on protein content. The first of these, near locus *Xgwm212* is at a location near to the *Vrn-A1* locus, previously

mapped in this region. The other locus, which seems to have a greater effect, especially in Year 3 (1992 trial), is in a position previously unreported as having an effect on protein content. In all cases, the allele for increased protein comes from the Avalon parent.

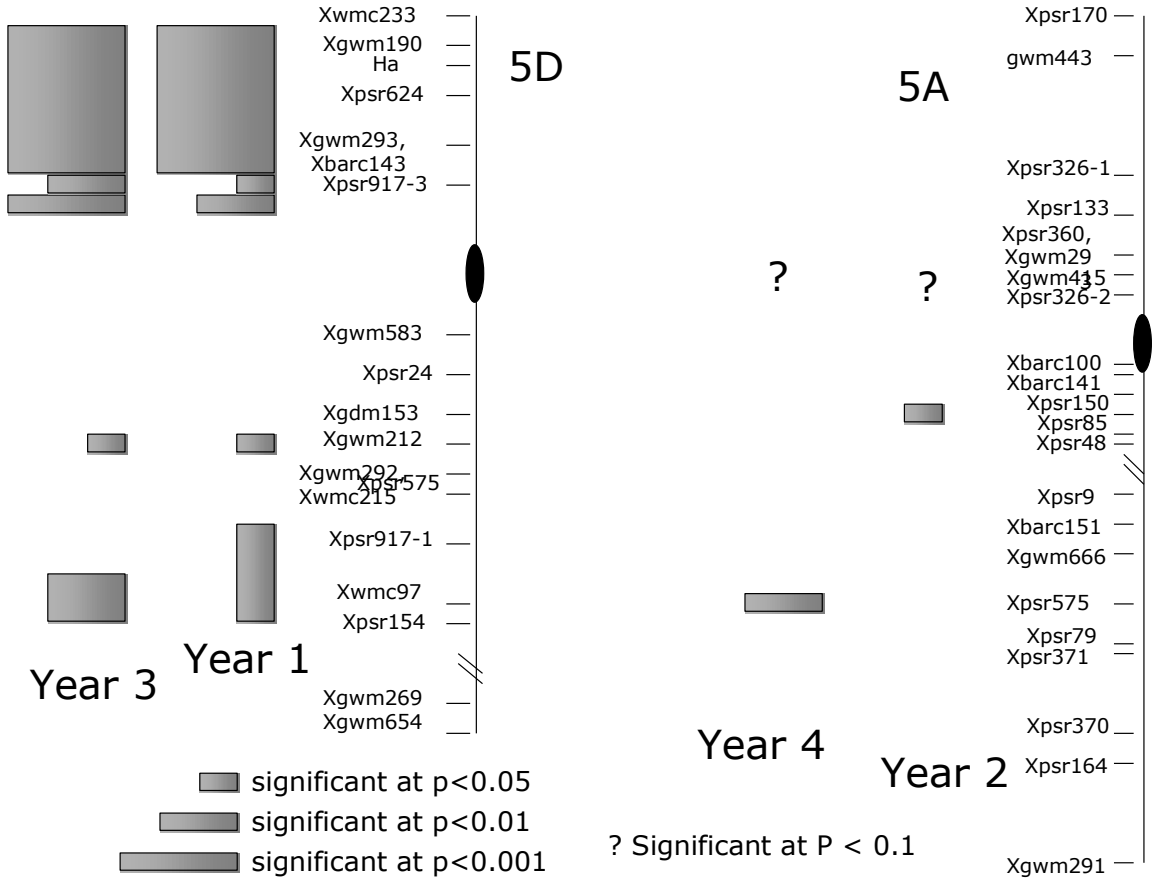

Figure 1. QTL for grain protein content found on chromosomes 5A and 5D in the RSL populations. (data presented from single marker ANOVA analysis).

The QTL for the 5A population are less consistent. This is possibly due to the much smaller variation seen in this population making accurate assessment of genetic effects more difficult in this environmentally affected trait. In Year 2 (1991) two loci were significant, one on each arm. The short arm locus increased protein when the Hobbit 'sib' allele was present, and the long arm locus when the Avalon allele was present. In the following trial in Year 4 (1993), however, this long arm effect disappeared to be replaced by another QTL more distal (at RFLP locus *Xpsr575*) in which the Hobbit 'sib' allele was responsible for the increase in protein. Again in this year, there was a small effect on the short arm with Hobbit 'sib' increasing protein, although this was only significant at $0.1>p>0.05$. Only one QTL for hardness was detected around the centromere (SSR locus *Xbarc100*) in Year 2 (1991) but this was not reproducible. It is possible that the QTL found in Year 4 (1993) are homoeologous to the loci found on the 5D chromosome, but this is impossible to prove without finer mapping.

No QTL for yield differences were detected in replicated drilled plots in any year in either of these populations. Similarly none of the yield variables tested showed any QTL on chromosomes 5A and 5D. This suggests that these protein alleles are indeed yield independent.

Recombinant inbred line analysis

The phenotypic data from the 85 RIL lines showed a higher level of variation than that found in the RSL population, as would be expected if more genes are segregating for these traits in this population. There was a weak but significant correlation between yield and protein content in this population ($r^2 = 0.14$, $p = 0.01$), suggesting that there were yield dependent and yield independent loci determining the total protein content on these lines. A summary of the protein and hardness QTL found in this population is given in table 2.

Table 2. Protein and hardness QTL located in the RIL population.

Trait	Location *	Significance of closest SSR marker	Increasing allele	% protein change between alleles	% protein change between chromosomes in RBM analysis
Protein	1AL, *Xgwm558*	p<0.01	Avalon	0.6	0.5
Protein	2AS, *Xgwm636*	p<0.001	Hobbit 'sib'	1.5	0.6
Protein	2DL, *Xwmc441*	p<0.01	Avalon	0.6	0.6
Protein	3AL, *Xgwm674p*	p<0.01	Avalon	0.5	0.4
Protein	3BL, *Xbarc229*	p<0.01	Hobbit 'sib'	0.6	0.3
Protein	4DS, *Xgdm14*	p<0.01	Avalon	0.4	0.3
Protein	5BS/7BS, *Xgwm234*	p<0.01	Avalon	0.3	0.3
Protein	5D, Ha/ *Xwmc233*	p<0.001	Avalon	0.5	1.0
Protein	7A, *Xbarc108*	p<0.01	Hobbit 'sib'	0.6	0.6
Hardness	1B, *Xgwm264*	p<0.01	Avalon	-	-
Hardness	4B, *Xgwm540*	p<0.01	Hobbit 'sib'	-	-
Hardness	4D, *Xgwm194*	p<0.05	Avalon	-	-
Hardness	5D, *Xwmc233*	p<0.001	Avalon	-	-

*most significant SSR marker given.

The results of the QTL analyses were generally in good agreement with the RBM analysis with a few notable exceptions. The 2A QTL is much larger than the effect seen with the RBM analysis. This is because in 20 of the RILs, it was discovered that there is a deletion of the tip of the short arm of the chromosome, coming from the Hobbit 'sib' parent. Aneuploids of 2A show decreased height, decreased grain weight and decreased ear size in comparison with euploid varieties. All of these characters were shown to be affected by this deletion, and this was probably therefore having a major pleiotropic effect on protein levels in the seed in these lines. There is noticeably no QTL found on chromosomes 5BS/7BS or 5A. Only one marker has so far been found to be polymorphic on the 7B part of this chromosome, and therefore the QTL may remain undetected in this case. The lack of QTL detected on 5A is probably due to a high genetic background effect in this population. It is noticeable that the major 5D QTL is found in the RIL population but neither of the minor QTL are found. As all of the QTL detected in the 5A RSL population were minor, it is therefore unsurprising that this broad analysis has not found them to be significant because of background genetic variation.

Comparisons between the methods of analysis

It seems that in genetic analysis of complex traits such as protein and hardness levels in wheat grains both single chromosome and whole genome genetic analyses have roles to play in understanding completely the genetics behind the phenotype. It is clear that RIL populations will give a good chance to locate important alleles in many different areas of the genome. However, the results from the RSL populations show that this analysis may miss out some of the minor genes which may also be playing an important role in determining the genotype. Backcross reciprocal monosomic analysis has been shown to be a useful tool for identifying chromosomes for study, with its predictions for locations of genes of interest on the group 5 chromosomes being confirmed by QTL analysis. Yield variables have also been analysed on these RILs (data not shown) and it seems from this that some of the protein alleles are indeed separate from any yield-influencing loci. Therefore, in future, it should be possible to breed for high yielding, high quality wheats using some of the loci identified in this study.

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Mutations in wheat leading to enhanced resistance to the fungal pathogen of yellow rust

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Introduction

The isolation and study of plant resistance genes is revealing a story far more complex than the gene-for-gene hypothesis originally implied. In addition to resistance triggered by simple, seedling expressed, race-specific genes are resistances expressed only at later, adult plant growth stages, and although some have been shown to be single, race-specific genes many are polygenic, each individual gene conferring only a partial resistance.

In addition to resistance genes that inhibit pathogen invasion and disease establishment, there are genes that enhance the progress of the disease and have been termed susceptibility or suppressor genes. A number of examples of specific inhibition of seedling-expressed resistance are available in the literature. The introduction of yellow or brown rust resistance genes from diploid wheat accessions into a hexaploid background has often resulted in the loss of the resistance phenotype (Kema et al., 1995, Ma et al., 1995, Assefa and Fehrmann, 2000). Specific suppressors of stem rust resistance genes have been identified in the cultivar Canthatch on chromosome 7DL (Kerber and Aung, 1995, 1999), and for *Lr23* on the 2DS chromosome from *Aegilops tauschii* (Nelson et al., 1997). Susceptibility loci have also been identified in oats, enhancing the spread of crown rust (Wilson and McMullen, 1997).

However, susceptibility genes affecting adult plant resistance (APR) were not identified until Worland and Law (1992) examined APR to yellow rust in the wheat line Hobbit 'sib'. Examination of a monosomic series of Hobbit 'sib' identified chromosomes that contributed to field, yellow rust resistance (i.e. chromosomes 1A, 2A, 4A, 2B, 5BS-7BS, 6B and 2D), but also chromosomes that reduced the level of yellow rust resistance (i.e. chromosomes 3B, 4B, 5BL-7BL, 4D and 5D) (Figure 1). In this paper we report on the characterisation of a number of potential deletion mutants generated in Hobbit 'sib' by Tony Worland, that were selected for enhanced field resistance to yellow rust.

The generation and characterisation of mutants in Hobbit 'sib'

Mutants were generated in the wheat line Hobbit 'sib' using fast-neutron bombardment (M1). These mutants were assessed in the field for yellow rust resistance as adult plants from the M2 generation onwards. All mutant lines are now at the M10 generation and beyond. A number of lines were selected as showing enhanced resistance to yellow rust compared to Hobbit 'sib', and here we present six lines that have given repeatable results in subsequent field and greenhouse tests.

The six mutant lines showed greater resistance to yellow rust than Hobbit 'sib' in both field and greenhouse adult plant tests. The increased resistance in all lines, except one, did not appear to be race-specific, the resistance being maintained against more than one race of *Puccinia striiformis* f.sp. *tritici*.

In addition to the enhanced resistance to yellow rust some of these mutant lines also showed increased resistance to other biotrophic fungal pathogens of wheat. Four mutant lines showed partial resistance to brown rust, while one line showed increased resistance to powdery mildew. Mapping programmes are currently underway to identify the mutant loci contributing to the altered resistance phenotype for each disease, and it will be interesting to see if mutant loci for each disease map to the same location.

As the mutant lines were selected in the field for enhanced resistance to yellow rust, greenhouse seedling tests were carried out to determine whether the mutant phenotypes expressed at seedling growth stages. For three of the mutants enhanced yellow rust resistance could be detected at seedling growth stages.

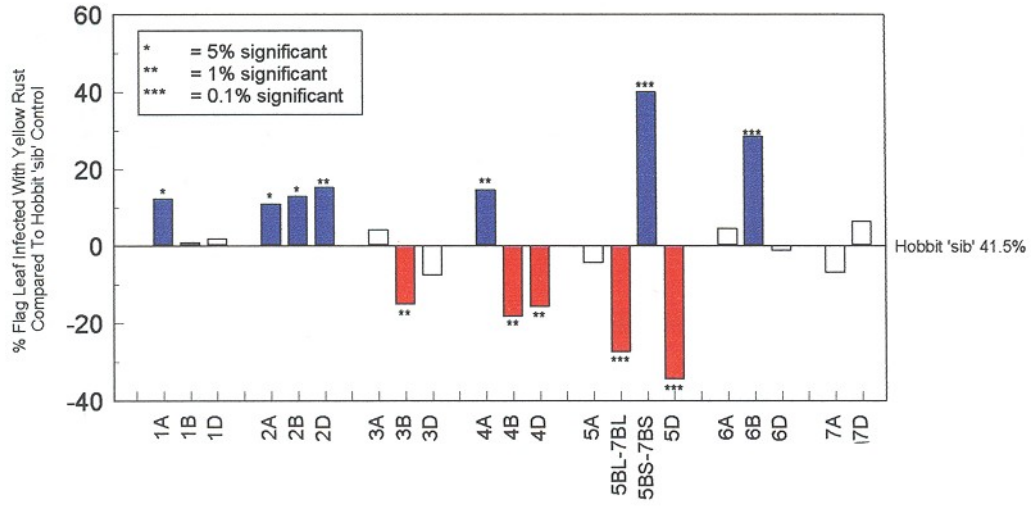

Figure 1. Field resistance to yellow rust in the Hobbit 'sib' monosomic series

Identification of deletions within the mutants

As these mutants were made by fast-neutron bombardment it was assumed that the altered disease resistance phenotypes could be due to deletions in the genome. To identify deletions RFLP markers and RDA clones were isolated and hybridised against genomic DNA from each mutant line by John Howie (1997). Probing with DNA markers identified deletions in all the mutants tested.

Segregation of the mutant phenotype

Crosses were made between three of the mutants and Hobbit 'sib'. F_2 populations were screened with the DNA markers that had identified deletions in each mutant to test for segregation of the deletion with the mutant phenotype. In only one mutant was an identified deletion found to cosegregate with the mutant phenotype. Therefore, these mutants either contain additional, unidentified deletions responsible for the mutant phenotype, or the mutation does not involve a large, readily detectable deletion.

Segregation of the enhanced yellow rust resistance indicated in one mutant line that the phenotype was due to a single, dominant mutation, while in another mutant two or more mutation events were responsible for the enhanced resistance phenotype.

Studies are continuing to map the mutant loci contributing to the enhanced yellow rust resistance, and to study the changes in gene expression between the mutant lines and the parent Hobbit 'sib'.

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Studies into 'durable' yellow rust resistance – revealing the genetics that underlie the phenomenon

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Background

Yellow rust (causal agent *Puccinia striiformis* Westend. f. sp. tritici) can reduce yields by as much as 84% (Murray et al., 1995). The control of this disease is therefore of considerable economic importance.

Some 30 race-specific resistance genes conferring yellow rust resistance have been identified in wheat and used in new cultivars. However the problem with this type of resistance is that the pathogen is able to mutate to a virulent form, often resulting in catastrophic yellow rust epidemics. This occurred in 1996, when the widely used *Yr17* resistance was overcome when virulent *Puccinia striiformis* isolates were found on the widely grown cultivar Brigadier (Bayles and Stigwood, 1996).

Several wheat chromosomes have been identified that contribute a partial resistance and there is much interest in the potential to pyramid these genes into new cultivars and thereby obtain a potentially more 'durable' resistance. Durable, by definition, refers to a situation where a cultivar has been grown for many years over a considerable acreage and maintained resistance, even when other cultivars have been severely infected in epidemics (Johnson, 1992; Gaines, 1976). This kind of resistance is believed to be more difficult for the pathogen to become virulent upon owing to its polygenic nature. Many cultivars have been investigated that fit this description of durability. Boukhatem *et al.* (2002) analysed the Belgian cultivar, Camp Remy, that has remained resistant through 20 years of cultivation. The exploitation of this durability requires an understanding of the genetics of its control as well as the development of markers linked to the genes responsible.

The haploid DNA content of hexaploid wheat is estimated to be some 17,000 Mb in size and comprise of over 30,000 genes (Amuruganathan and Earle, 1991) of which several hundred have been mapped (assigned to a chromosome). The process of mapping is a fundamental tool for genome analysis, where the key is to assign markers to the gene of interest (Williams et al., 1990). The markers currently employed in wheat can be classified into 3 groups (Gupta et al., 1999)

1st generation: Hybridisation based - RFLP

2nd generation: PCR based - RAPDs, SSR (or microsatellites), STS + AFLP

3rd generation: sequencing based – SNP

Much of the mapping of yellow rust resistance has concentrated on single, race-specific resistance genes, such as *Yr15* (Chague et al., 1999) and *Yr17* (Robert *et al*., 2000). Börner *et al.* (2000) however mapped a non-specific resistance gene, *Yrns-B1* to chromosome 3BS close to the SSR marker *Xgwm493*.

Carstens V has specific resistance to many European and North American yellow rust races (de Vallavieille-Pope and Line, 1990) and is known to possess at least one specific resistance gene located on chromosome 2A designated *YrCv* (Stubbs, 1985). Calonnec *et al.* (unpublished) investigated the genetics of its resistance and concluded that there were at least 4 genes contributing toward its resistance. Carstens V has also long been believed to be a

source of durable adult-plant resistance (APR) and is in the ancestry of many wheat cultivars currently covering significant acreage in the UK. One such cultivar is Claire, which has maintained a yellow rust rating of 9 since its release in 1999. This paper discusses the research carried out to determine the genetics underlying Claire and Carstens V resistance with the potential of developing PCR assays for the influential genes.

Approaches

a) Develop doubled haploid (DH) mapping populations with susceptible cultivars
   Claire x Lemhi Claire x Kharchia local
   Carstens V x Lemhi Carstens V x Kharchia local

b) Score DH populations for Yr APR

c) Develop SSR (and AFLP) molecular maps for each DH population

d) Identify APR QTL in the molecular maps

e) Identify seedling (race-specific) resistance in the DH populations

f) Localise the seedling resistance genes on the molecular maps

g) Identify seedling resistance in Buster, Caribo and Carstens VIII from F_2/F_3 populations

Development of mapping populations

A) DH lines have been developed for the following populations:

Code	F1 cross	Number of haploid lines
100	Carstens V x Kharchia local	132
101	Carstens V x Lemhi	67
108	Claire x Kharchia local	98
109	Claire x Lemhi	185

B) F_2 seed has been collected for the following populations:

Code	F1 cross	Number of F_1 plants
102	Carstens VIII x Kharchia local	2
103	Carstens VIII x Lemhi	2
104	Caribo x Kharchia local	3
105	Caribo x Lemhi	3
106	Buster x Kharchia local	2
107	Buster x Lemhi	3

Postulation of seedling resistance

The following cultivars were selected from within the Claire pedigree to screen for seedling (specific) resistance:

Cultivar	Reason (s) for selection
Cappelle Desprez	Source of *Yr16* believed to be present in Claire (P.Fenwick, personal communication) Parent of Caribo
Aquila	Previous market leader bred by Nickersons susceptible to Cv virulent isolates at seedling stage
Galahad	Claire is believed to have *Yr1* resistance that originated from Galahad, (observation made following trials in Nickersons yellow rust nursery) Galahad is also a parent of Wasp
Dynamo	Cultivar developed parallel to Buster, Dynamo is a parent of new Nickersons variety, Deben (which had a NIAB yellow rust resistance rating of 9 in post recommended list trials –2001)
Flame	A parent of Claire
Wasp	A parent of both Claire and Deben
Fresco	Parent of both Consort and Charger; both NIAB rating of 9 on 2001 recommended list Monopol is one of the parents of Fresco and is believed to be the source of *YrCv* in Fresco
Hereward	Has the cultivar Disponent as a parent that is a possible donor of *YrCv* Possibly source of durable resistance as has maintained NIAB rating of 7 for yellow rust resistance for many years
Hunter	Parent of Deben

From the results obtained the following specific resistance genes are postulated to be carried by the cultivars screened

	Gene carried							
	Yr1	***Yr2***	***Yr3***	***Yr4***	***Yr6***	***Yr7***	***Yr8***	***Yr9***
CLAIRE FAMILY								
Claire		✓	✓					
Caribo			✓					
Buster *								
Carstens V *								
Carstens VIII		✓						
NICKERSONS STOCK								
Cappelle Desprez			✓	✓				
Aquila		✓	✓					
Galahad	✓		✓					
Dynamo			✓					
Flame			✓					
Wasp		✓	✓					
PBI STOCK								
Fresco*								
Hereward*								
Hunter*								

Screening for microsatellite polymorphism

Thus far the following polymorphisms have been observed using microsatellites located in regions of chromosomes believed to be influential in the control of APR.

Cultivar	Reason (s) for selection
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Summary

This initial research has been fundamental to further understanding the nature of resistance in Carstens V and has potential to improve and accelerate selection procedures for resistance genes. It is still necessary to produce comprehensive microsatellite maps for the populations of interest and screen for adult plant resistance, before molecular markers can be assigned to resistance genes. However, its hoped that within the time-scale available significant insight will be gained.

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The inheritance of resistance to eyespot at the adult stage in the variety Cappelle-Desprez

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Cereal eyespot caused by *Tapesia yallundae* syn. *Pseudocercosporella herpotrichoides* is one of the most damaging diseases of winter wheat in areas, including Western Europe where mild and damp autumns and winter conditions permit growth and spread of the pathogen.

Moderate resistance to eyespot was first found in the French variety Cappelle-Desprez, (CD) released in the 1950s. The resistance was transferred to other European wheat cultivars and remained durable for about 30 years despite widespread exploitation. As yield losses due to eyespot occurred in cultivars with the resistance of CD, a search for better levels of resistance was undertaken. The best level of resistance in the genus *Aegilops* was identified by Sprague (1936) in the tetraploid species *Ae. ventricosa*. Maia (1967) successfully introduced the gene *Pch1* from this related species onto chromosome 7D of bread wheat. The alien gene *Pch1* was found very tightly linked to the endopeptidase locus *Ep*-D1, so that electrophoretic screening of single seeds could be employed to select resistant genotypes (McMillin et al. 1986). The resistance to eyespot conferred by *Pch1* is higher than that of CD. Therefore it is being actively used both in Europe and the USA by wheat breeders. However, varieties carrying *Pch1* can sustain significant eyespot-induced grain yield losses in situations of severe attack by the pathogen. This is why breeders would like to further enhance the resistance of wheat. A first strategy to achieve this goal is by pyramiding *Pch1* and the genes for resistance in CD. As resistance is a quantitative trait, pooling both resistances in a variety is a laborious and tedious task even if the endopeptidase marker of *Pch1* is utilised. This task should be much easier if the genetics of resistance in CD could be better understood and molecular markers of the genes or QTLs involved are found

Resistance to eyespot in Cappelle-Desprez: current status

The inheritance of resistance to eyespot in CD was investigated only at the seedling stage. Law et al (1975) showed that it was oligogenic, the main factor being located on chromosome 7A.

The gene on 7A was designated *Pch2* by de la Peña et al (1996). These authors suggested later that *Pch2* and *Pch1* are homoeoloci, but the association between *Pch2* and the endopeptidase locus *Ep-A1* is rather loose. They proposed simultaneous selection of two flanking RFLP markers *Xcdo347* and *Xwg380* at a distance of 11 and 18.8 cM from *Pch2* respectively for selecting *Pch2* in breeding materials (de la Peña et al. 1997).

Doussinault (1973) pointed out that resistance to eyespot has three components: contamination probability, resistance of leaf sheath to penetration by the fungus, which is generally called resistance at the seedling stage, and resistance of stem to invasion by the fungus, which is also called resistance at the adult stage. No detailed analysis was carried out to locate the genes involved in this resistance component in CD. Is the resistance of CD at the adult stage conferred by *Pch2* or are other genes involved ? To answer this question, the present report deals with the evaluation of the resistance of the 21 Cappelle-

Desprez(Bezostaya) disomic substitution lines in order to unravel the genetic control of resistance to eyespot in CD.

Evaluation of the resistance among Cappelle-Desprez (Bezostaya) substitution lines

The complete series of single-chromosome substitutions from Bezostaya into CD was evaluated for resistance to eyespot. The set was produced at PBI at Cambridge according to Law and Worland's method (1973). The test at the seedling stage was made according to Macer (1966) and susceptibility was assessed as the percentage of leaf-sheaths infected by the fungus. The substitution lines were, on average, slightly more infected than CD. Only the substitution for chromosome 7A exhibited significant higher levels of infection than CD. Taking into accounts previous results it was concluded that the chromosome 7A of CD carries a major gene for resistance to eyespot at the seedling stage.

To evaluate resistance conferred by genes acting only at the adult stage, the plots were sown in October and were inoculated in February instead of November for assessment of global resistance which combines resistance both at seedling and adult stage. As expected, Bezostaya was much more susceptible than CD. In the first experiment, five substitution lines containing chromosomes 1A, 5A, 2B, 6D and 5D were more infected than CD. The critical lines were tested in subsequent years. Three chromosomes had an effect on adult resistance: the effect of 5A was systematic whereas those of 1A and 2B were significant in two of the four trials.

In 1994, two trials were carried out to evaluate resistance at the adult stage and global resistance. The latter was inoculated in November. The critical chromosomes were 1A, 5A, 2B and 7A. Therefore, it appeared that the gene *Pch2* on chromosome 7A, by acting only at the seedling stage, has a significant influence on the expression of global resistance of CD.

Conclusions

The analyses of the chromosome substitution lines of Bezostaya into CD indicated that, at any stage, no individual substitution line had the level of susceptibility to eyespot of Bezostaya. Thus, the mode of inheritance of resistance to eyespot is polygenic.

Chromosome 7A was confirmed to carry a major gene for resistance to eyespot at the seedling stage as previously reported by Law et al (1975) and de la Peña et al (1996). Following the work of these authors, it was generally admitted that the major gene on chromosome 7A, called *Pch2*, was the main component of resistance in CD. However, this study demonstrates that this chromosome has no effect at the adult stage. Chromosome 5A was shown to carry a major gene for resistance to eyespot at the adult stage that was stably expressed each year of test. Chromosomes 1A and 2B had significant effects in only two years among four.

Chromosome 5A has been shown to carry many genes of agronomic interest, e.g. genes controlling resistance to *Fusarium* head blight (Buerstmayr et al. 1999), plant height and vernalisation response (Korzun et al. 1997). Consequently, a more precise knowledge of the location of the gene(s) from CD conferring resistance to eyespot at the adult stage is needed in order to enable breeders to recombine favorable alleles from different origins or to introduce the two main components of CD resistance, carried by 5A and 7A chromosomes, in other lines. This may be achieved using a 5A recombinant substitution line population derived from the cross between CD and the CD(Bez 5A) substitution line.

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Genetics of resistance to septoria tritici blotch (*Mycosphaerella graminicola*)

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Septoria tritici blotch, caused by the fungus *Mycosphaerella graminicola*, is currently the major foliar disease of wheat in most of Europe, North Africa, South America and several other parts of the world. Until recently, little was known about the genetics of resistance to this disease in comparison to well-studied diseases such as rusts and mildew. New sources of resistance and further knowledge about the genetics of resistance would be of great value to breeders in improving resistance to septoria tritici blotch.

Synthetic hexaploid wheats are a rich source of genetic variation for disease resistance. We showed that Dr E. Sears' Synthetic 6x hexaploid wheat, derived from a hybrid of *Triticum dicoccoides* and *Triticum tauschii*, was resistant to 12 of 13 isolates of *M. graminicola* tested. We located this resistance to chromosome 7D of Synthetic 6x in tests on inter-varietal chromosome substitution lines of Synthetic 6x into Chinese Spring. A gene for resistance to isolate IPO94269 was mapped near the centromere of the short arm of chromosome 7D in a population of single homozygous chromosome recombinant lines and is named *Stb5* (Arraiano et al. 2001a).

The contributions of Bezostaya 1 chromosomes to resistance to septoria tritici blotch were tested on inter-varietal chromosome substitution lines of Bezostaya 1 into two different backgrounds, Hobbit sib and Cappelle Desprez in polytunnel trials of adult plants and whole and detached leaves of seedlings (Arraiano et al. 2001b). Bezostaya 1 chromosome 3A was identified as carrying specific resistance to isolate IPO323. Bezostaya 1 and Cappelle Desprez also appeared to carry components of partial resistance (Arraiano 2001).

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A new source of antibiotic resistance against *Schizaphis graminum* and *Diuraphis noxia*

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The greenbug (*Schizaphis graminum*, Rondani) and the Russian Wheat Aphid (RWA, *Diuraphis noxia*, Mordvilko) are the two most economically important insect pests of wheat and barley throughout much of the Americas. The greenbug is well adapted to the conditions in the Americas, and RWA is the last of the recent introductions into the South Cone of South America. The RWA was first discovered in North America in 1986, and is now widespread in the USA and Canada (Kindler & Springer, 1989; Peairs et al., 1989). The RWA was first found in Chile in 1988 and in 1992 in Argentina (Mendoza) (Ortego & Delfino, 1994); it was found in the main cereal producing region of Argentina, south of Buenos Aires province, in 1994 (Bellone & Almaraz, 1995), and then spread northwards and eastwards infesting barley, bread wheat and pasta wheat in 1995 (Castro et al., 2000, 2001). Parasitoids and chemicals failed to control this pest, possibly because it causes the leaves to curl tightly, which protect the enclosed aphid (Burd et al., 1989). Cereal production of Argentina, Chile and Uruguay shows a loss of 1 million dollars per year provoked by these aphid pests.

Although these aphids can be controlled by the application of pesticides, the breeding of genetic resistance into wheat is a more benign approach and has been used extensively against both species (Castro et al., 1999). Six genes for resistance have been introduced into wheat to control infestation by greenbug, and another 6 genes have been identified against RWA (Worland & Snape, 2000). Studying the above genes in order to determine the allelic relationships and inheritance patterns, Dong et al. (1997) found that in all the possible F_2s tested, resistance to RWA was controlled by qualitative and non-additive genes. RAPD and SCAR markers linked to the RWA resistance gene *Dn2* were developed (Myburg et al., 1998). Recently, two microsatellite markers (*Xgwm111 Xgwm44*) were linked to four dominant RWA resistance genes (*Dn1*, *Dn2*, *Dn5* and *Dn6*) and these shown to be tightly linked to each other on 7DS, near the centromere, linked to *Xgwm635* (Liu et al., 2001, 2002). This new result clearly marked the location of the resistance gene, now designated as *Dn8.* Another marker,*Xgwm642,* (located on 1DL) identified another new gene, *Dn9* (Liu et al., 2001) and flanking markers *Xgwm106* and *Xgwm337* were linked to *Dn4* (located on 1DS) (Liu et al., 2002).

The different resistance mechanisms against aphids were separately studied in wheat (Castro et al., 1999). From a collection of fifty wheat entries only two showed significantly higher resistance against greenbug and RWA collected in Argentina. Synthetic, a hexaploid hybrid (*T. turgidum* x *T. tauschii*) (Law & Worland, 1996) was reported to be the most tolerant cultivar to greenbug and RWA (Castro et al., 1999). The cultivar ´Hope´ was identified as the most antixenotic and antibiotic against both aphid species. Chromosomes involved with antixenosis, antibiosis and tolerance were identified in a set of intervarietal chromosome substitution lines between the recipient 'Chinese Spring' and 'Synthetic' and ´Hope´ as donors (Castro et al., 2001). Different sets of genes determined resistance to both aphid species.

The DHR population (CS/Syn 7D) was characterized for 7D genetic markers, and for their resistance to greenbug and to RWA (Castro et al., 2002). Antixenosis to greenbug was not significantly associated with any of the 7D markers. However, antixenosis to RWA was significantly associated with a marker locus on 7DL. Antibiosis against greenbug was studied for aphid developmental time and was associated with several marker loci on 7DS and on 7DL. This antibiosis component for RWA resistance was also linked to several loci. The total fecundity of greenbugs hosted on the DHR lines was not associated with any genetic markers on 7D. Conversely, the total fecundity for RWA was linked to several markers on the 7DS and 7DL arms. Greenbug and RWA longevities were significantly linked to markers on the 7DS or 7DL arms and three QTLs were mapped. Antibiosis against RWA was explained by association with two or three molecular markers, although antibiosis against greenbug is more complex and it is based on genes located on more than one chromosome.

In order to identify new resistance genes of antixenosis and antibiosis, a new set of DHR lines from CS/Hope 1B was developed and characterized for their resistance against greenbug and RWA.

Antixenotic mechanism of resistance

There were highly significant differences between DHR lines for antixenosis against greenbug and RWA. However, no recombinant line showed as high antixenosis against greenbug as the 1B substitution line, but several of them were not significantly different from the donor parent (´Hope´). For RWA, 28% of the DHR lines showed higher antixenosis than the 1B substitution line. Similar results have been previously reported in the 7D set of recombinant lines (CS/Syn 7D), (Castro et al., 2001). This suggests that other genes located on a different chromosome could be involved with this type of resistance.

Antibiotic mechanisms of resistance

Antibiosis of greenbug and RWA hosted on the DHR lines, showed highly significant differences by means of aphid fertility in a period equal to their developmental time (M_d), total fecundity and longevity of both aphid species. Greenbug developmental time (d) also showed highly significant differences, but this trait for RWA was not significantly affected.

Developmental time (d) of greenbugs reared on 80% of DHR lines was significantly higher compared to that determined in those aphids hosted on both parents and on the 1B substitution line. Only 28% of recombinant lines conditioned longer d for the RWA that they hosted. Since the duration of the immature period determines the distance between two subsequent generations, a longer d, reduces the increase of aphid population, and higher antibiotic resistance.

M_d of those greenbug and RWA reared on DHR lines were significantly reduced compared to that recorded on both parental cultivars and on the 1B substitution line. The M_d of greenbugs hosted on 40% of DHR was reduced to one third of the values recorded on aphids hosted by parental lines or the substitution line. The M_d recorded in greenbugs hosted on 10% of DHR lines were half the values determined on the resistant parent. RWA reared on 76% of DHR lines showed M_d values lower or similar to that recorded on aphids hosted by the resistant parent. Nonetheless, only 24% of DHR lines conditioned as low an M_d as the substitution line. No DHR showed as high M_d as that recorded on those aphids reared on CS.

Total fecundity of those greenbugs hosted on the DHR lines was significantly reduced compared with those values recorded on the aphids hosted on both parents and in the substitution line. Most of the DHR reduced greenbug fertility to half of that determined on aphids hosted either by the resistant ´Hope' or by the 1B substitution line; another group of DHR reduced aphid fertility to 75-80% of that recorded on ´Hope´ and the 1B substitution line.

RWA fertility of those aphids reared on 76% of the DHR lines showed values similar to that recorded for aphids hosted by ´Hope' or by the substitution line. Another 23% of DHR lines conditioned RWA fertilities in between those recorded in the resistant parent and the 1B line. In the rest of the DHR, the RWA fertility was as high as that determined on aphids reared on the susceptible CS.

Greenbug longevity was significantly shorter in those aphids hosted in most of the DHR lines, compared to that determined on ´Hope´ and the 1B substitution line. No DHR line conditioned as high longevity as that recorded on aphids hosted by CS. RWA longevity was reduced for aphids reared in 33% of DHR lines - the rest of DHR lines conditioned RWA longevities similar to those recorded on ´Hope´.

Conclusions

Antixenosis against greenbug was explained from the study of the 1B double haploid recombinant lines. This is the first time that a mechanism of resistance has been associated with a particular chromosome. This could be exploited in improving wheat resistance.

Antibiotic resistance to greenbug was significantly associated with DHR lines for every trait studied. Antibiosis to RWA was also significantly expressed in the 1B DHR lines.

The identification of molecular markers linked to these types of resistance has the potential to accelerate the identification of major genes or QTL of interest and once they are mapped could also be exploited together with the other important genes already located on chromosome 7D.

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Genetic and cytologically based physical mapping of traits effecting cold hardiness in wheat

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Vernalization requirement and frost resistance has long been regarded as traits being under multigenic control. The location of genes controlling these complex traits is now possible by the application of marker-mediated techniques. This is achieved by exploiting precise genetic stocks, such as recombinant substitution lines (RSLs) recombinant inbred lines (RILs) and deletion lines. Since common wheat (*Triticum aestivum* L.) is a hexaploid its vital genes are replicated. This permitted Sears (1958) to develop series of chromosome substitution lines. By comparing chromosome substitution lines with the parental lines it was possible to determine which chromosomes carry gene loci for freezing tolerance and vernalization requirement (Galiba 2002). More recently, Endo and Gill (1996) developed deletion stocks that have homozygous deletions of different lengths for each chromosome arms. That allows the physical localizations of traits affecting cold acclimation (Sutka et al. 1999). Due to recent research efforts several comprehensive genetic maps of different traits, affecting abiotic stress adaptation, are now available through the application of molecular marker techniques (Galiba et al. 1997a; Snape et al. 2001; Cattivelli et al. 2002)

Mapping genes for vernalization requirement and frost tolerance on the homoeologous group 5 chromosomes of wheat

Major genes controlling sensitivity to vernalization, the *Vrn* genes, determine the control of the spring /winter growth habit in wheat. Winter types require vernalization to initiate flowering and spring types do not. The lack of vernalization requirement is generally dominant. Five loci are known to control spring/winter differences and the chromosomal locations of four of them have been established using chromosome substitution line analysis, namely *Vrn-A1* (on chromosome 5A), *Vrn-D1* (on chromosome 5D), *Vrn-B1* (on chromosome 5B) and *Vrn-B4* (on chromosome 7B) (Law et al. 1991). Studies have shown that alleles at the *Vrn-A1* locus appear to be predominant in reducing vernalization requirement (Snape et al., 1985).

In addition, although studies have shown that the genetic control of frost resistance is complex and can be regarded as a polygenic trait (Sutka 1981), major genes have been mapped on chromosomes 5A and 5D using molecular markers (Galiba et al., 1995; Snape et al., 1997). The major question is, therefore, whether vernalization response and cold tolerance are pleiotropic effects of the *Vrn* genes or determined by separate loci. To answer this question requires precise mapping of the loci involved in both traits, using a marker-mediated approach.

To map genes affecting flowering time and frost resistance on chromosome 5A, a population of single chromosome recombinant lines, derived from the cross between the single chromosome substitution lines Chinese Spring (*Triticum spelta* 5A), which is vernalization insensitive (spring habit) + frost sensitive, and Chinese Spring (Cheyenne 5A) which is vernalization sensitive (winter habit) + frost tolerant, was developed. The flowering time of each line was characterised in growth chambers under a 16/8 h day/night regime at temperatures of 22 and 15°C, respectively, without prior vernalization. Frost resistance was assessed using low temperature treatments varying from –10 to –15 °C following hardening. Discontinuous variation for flowering time and frost responses was observed when the phenotypes were assessed under these conditions. RFLP mapping techniques were applied to the lines to develop and anchor the genetic map, and this showed that *Vrn-A1* and *Fr-A1* (formerly *Fr1*, Galiba et al. 1995), the locus for frost resistance, were separate, but closely linked loci, on the long arm of chromosome 5A. Both loci were closely linked to the RFLP markers *Xpsr2021(ABA2*), *Xwg644* and *Xpsr426*, distally on the long arm, but proximal to the 5A/4A translocation.

The analysis of chromosome 5A has been extended to examine if there are homoeologous loci for vernalization and frost resistance, on chromosome 5D (Snape et al., 1997). A recombinant substitution line population was developed from the cross between Chinese Spring, which is vernalization insensitive, and the single chromosome substitution line Chinese Spring (Cheyenne 5D), which is vernalization sensitive. Each line was characterised phenotypically for flowering time and frost resistance as described above. *Vrn-D1* was located distally on the long arm of 5D, closely linked to the SSR markers *Xgwm212* and *Xgwm292*. QTL analysis for cold response revealed that the locus of the *Fr-D1* gene (formerly *Fr2*, Snape et al., 1997) is also situated on the long arm separate from *Vrn*-D1, since the maximum likelihood position was about 6 cM proximal to *Vrn-D1*.

Using the mapping population derived from Chinese Spring (Cheyenne 5B) and Chinese Spring cross, *Vrn-B1*, affecting vernalization response and *Fr-B1,* affecting frost resistance were recently mapped (Tóth, Galiba, Sutka and Snape unpublished). The *Vrn-B1* locus was mapped on the distal portion of the long arm of chromosome 5B, to a region syntenous with the segments of chromosome 5A and 5D containing *Vrn-A1* and *Vrn-D1* loci, respectively. *Fr-B1* was mapped on the long arm of chromosome 5B 40 cM from the centromeric marker. The position of this gene is not orthologous to the other *Fr* genes mapped either on 5A or 5D.

Physical mapping of the *Vrn-A1* and *Fr-A1* Genes

Recently, 436 wheat chromosome deletion lines were generated in the cultivar Chinese Spring using the gametocidal chromosomes of *Aegilops cylindrica* (Endo and Gill 1996). These deletions were used for physical mapping. Analysis of variance for flowering time revealed significant differences between the deletion lines grown at 20°C. They could be divided into two groups. The difference in flowering between group I and II was 14 days (Sutka et al. 1999). The division between the group of deletion lines for flowering time occurred between breakpoints 0.68 and 0.78 indicating, that the *Vrn-A1* locus was situated between them. The frost resistance of the deletion lines could also be divided into two groups. Group II had an average survival of 40%, while for group I the average survival was 27%. This 13% difference in survival between groups was statistically significant (Sutka et al., 1999). The division of the two groups happened between breakpoints 0.67 and 0.68, so *Fr-A1* must be located in this interval. Thus, *Fr-A1* is situated closer to the centromere than *Vrn-A1*, confirming the results published by Galiba et al. (1995).

The role of 5A chromosome in the regulation of carbohydrate content in the course of cold hardening

The frost resistance and endogenous carbohydrate contents of the plants were measured every 8 days in the course of cold treatment (Vágújfalvi et al., 1999). There was a continuous rise in the total water-soluble carbohydrate (WSC) content and in the fructan content as the hardening period proceeded. The increase in concentration was greater in the frost-resistant variety Cheyenne and in the substitution lines Chinese Spring (Cheyenne 5A), Chinese Spring (Cheyenne 5D) and Chinese Spring (Cheyenne 7A) than in the sensitive genotypes Chinese Spring and Chinese Spring (*Triticum spelta* 5A). A significant positive correlation was found between frost resistance, WSC and fructan content after 19 days of cold treatment. A significant correlation was exhibited between the fructose and sucrose contents and frost resistance after 43 days of cold treatment. In the chromosome substitution lines the accumulation of carbohydrates began after 11 days of cold hardening and reached a maximum after 35 - 43 days.

The sucrose and fructan contents in recombinant lines arising from a cross between the substitution lines Chinese Spring(Cheyenne 5A) and Chinese Spring(*Triticum spelta* 5A) were determined after cold hardening (Galiba et al., 1997b). The *Fr-A1* (frost sensitivity) and *Vrn-A1* (spring habit) alleles originating from *Triticum spelta* did not increase the sucrose and fructan contents, while their concentrations increased significantly in recombinant lines carrying the alleles *vrn-A1* (winter habit) and *fr-A1* (frost resistance). In the 7-3 line, carrying the *vrn-a1* and *Fr-A1* genes (where recombination had taken place between the *Fr-A1* and *Vrn-A1* genes), a large accumulation of sugar was observed, indicating that the allele influencing carbohydrate accumulation was closely linked to the *Vrn-A1* gene.

Mapping of genes affecting cold regulated proteins

Among the wheat plants raised under 18/13°C day/night conditions the accumulation of the barley homologous *cor14b* mRNA was observed in the leaves of frost-resistant genotypes, but not in those of frost-sensitive varieties and lines (Vágújfalvi et al., 2000). At higher temperatures (25/18°C) there was no detectable quantity of *cor14b* mRNA in any of the genotypes. The analysis of single chromosome recombinant lines derived from the cross between Chinese Spring (*Triticum spelta* 5A) and Chinese Spring (Cheyenne 5A) identified two loci with additive effects which are involved in the genetic control of *cor14b* mRNAs accumulation. The first locus was tightly linked with marker *Xpsr911*, while the second one was located between marker *Xpsr2021* and the frost resistance gene *Fr-A1*. It was known that

the structural gene is located on chromosome 2 in barley, but its position on the chromosome is unknown (Crosatti et al., 1996). The RFLP mapping of the structural gene was carried out using a *cor14b* gene cDNA probe on a *Triticum monococcum* mapping population. The locus of the *cor14b* allele was mapped on the long arm of the $2A^m$ chromosome.

We can only speculate on the role of the COR 14 protein. It probably helps to prepare the plants for cold hardening. Its accumulation in both wheat and barley was associated with a well-defined threshold temperature, so it is considered to be a useful biochemical marker for the selection of frost-resistant and frost-sensitive genotypes.

Conclusions

These results indicate that the 5A chromosome carries an "adaptive gene complex" in the region of the *Vrn-A1* and *Fr-A1* genes. The development of this gene family regulating adaptation may have represented an evolutionary advantage. Recombination frequency is low between the genes involved, so that the genes are inherited together to form a relatively large functional unit. As the result of continual selection pressure, imposed by severe winter conditions, this grouping of genes would give a selective advantage to progenies carrying them and also to future generations.

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F_2 monosomic analysis of water-stress induced apical sterility in wheat (*Triticum aestivum*)

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Reduced seed-set in wheat (*Triticum aestivum*) has become a common problem in many parts of the world particularly in the regions with inadequate water reserves for irrigation. Environmental stresses, particularly drought and extreme high temperatures often result in reduced seed-set in some cultivars of wheat (Rawson 1996). Apical sterility occurs in some wheat cultivars when the terminal spikelets fail to set seed following exposure to high temperatures above 26 ^{o}C or water stress. Dawson and Wardlaw (1989) subjected the line H757 from Afghanistan to high temperature (25-30 ^{o}C) at the booting stage which resulted in sterility in the upper half of the spike. Kainth (1994) found that the number of sterile apical spikelets increased significantly in a Pakistani line (Y-82187) when subjected to water-stress at the growth stages 30, 33, 37, and 45 of Zadoks' scale in comparison to well watered control plants. Shoran and Joshi (1987) studied the inheritance of apical sterility using segregation ratios in F_2 and back cross generations of hybrids between Indian lines with apical sterility and lines with normal spikes. He reported that a single recessive gene controls apical sterility in these lines. This report on the qualitative inheritance of this character implies that the inheritance of this trait can be studied further using monosomic analysis. The most commonly used method, to identify the chromosome location of genes controlling qualitative characters, is the F_2 monosomic analysis (Law et al., 1987). The objectives of this study were to identify the mode of inheritance of water-stress induced apical sterility using several crosses between apical fertile and apical sterile lines and to identify possible chromosomes carrying the gene(s) for this character.

Materials and methods

Crosses between apical fertile lines FM32, FM147 and Chinese Spring (CS) and apical sterile lines Y82187 and Faisalabad-85 from Pakistan were used in this study. Crosses were made between monosomic plants from the 21 monosomic lines of CS with the variety Y82187. Monosomic F_1 plants were identified cytologically and F_2 seeds were harvested from F_1 monosomic hybrids.

Seeds from each F_1, BC_1 and F_2 generation obtained from disomic crosses and F_2 seed from monosomic crosses were grown in 10-cm pots in a green house, where the minimum temperature was maintained at 16 °C and the maximum varied from 22 to 29 °C. When the plants reached meiotic stage (Zadok's 39-41), they were subjected to water-stress treatment by withholding water from the plants for 5 days.

When the seeds had formed, the plants were categorised into two phenotypic groups, apical spikelets fertile or sterile. The Chi square (χ^2) analysis was used to test the observed segregation ratio in each F_2 and BC1 progeny against the expected dihybrid and monohybrid segregation ratios.

Results

F_1 plants obtained from the cross between CS and Y82187 were apical fertile. This result indicates that apical fertility is dominant to apical sterility which agrees with the results reported by Shoran and Joshi (1987). All F_1 and BC1 plants from the cross Faisalabad × Y82187 showed apical sterility, which indicates that the two varieties possess at least one recessive allele for apical sterility in common.

The F_2 populations obtained by crossing FM32, FM147 and CS with Y82187 segregated in a ratio of 9:7 plants with fertile and apical sterile spikes, while the F_2 population of FM147 × Faisalabad segregated in a ratio of 3:1 plants with normal and apical sterile spikes. The segregation ratio in the BC_1 plants derived from the cross (FM147 × Faisalabad) x Faisalabad did not differ significantly from the expected monohybrid ratio. Segregation in the BC_1 population derived from (FM147×Y82187) x Y82187 fitted the expected dihybrid segregation ratio. These results indicate that the number of genes responsible for apical sterility is not the same in the varieties Y82187 and Faisalabad. These results suggest that Faisalabad-85 is homozygous recessive at one locus determining apical sterility, whereas Y82187 is homozygous recessive at two loci.

The segregation in the F_2 progeny from the crosses between the CS monosomic lines and Y82187 did not deviate significantly from the expected 9:7 disomic ratio for most of the lines. However the F_2 progeny from the cross with the CS line monosomic for chromosome 3A were all apical sterile. The segregation in F_2 progeny from the crosses with the monosomic lines 3D, 5D and 6D deviated significantly from the expected 9:7 ratio because of a deficiency in plants with fertile spikes. This deviation may have been due to the limited number of F_2 seed produced by the F_1 monosomic hybrids.

The F_2 fertile plants from each of these three lines were progeny tested and the F3 plants were subjected to a controlled water-stress treatment. A few apical sterile F_2 plants were progeny tested to ensure that the water-stress imposed was adequate to induce apical sterility. Both the fertile and apical sterile F_2 plants from the cross with the line monosomic for 3D all bred true for apical sterility. The fertile F_2 plants from crosses with the lines monosomic for 5D and 6D either segregated in a ratio of 9 fertile: 7 apical sterile or bred true for fertile spikes. The F3 progeny from the F_2 apical sterile plants tested from these two lines were all apical sterile.

Conclusion

The 9:7 ratios in crosses between apical fertile lines and Y82187 indicate dihybrid segregation with complementary interaction between the two dominant alleles responsible for tolerance to water-stress induced apical sterility. If the alleles at either of these independently inherited loci are homozygous recessive the plant will be susceptible to water-stress induced apical sterility. The monohybrid segregation in the cross between Faisalabad-85 and Y82187 indicates that Faisalabad has dominant alleles at only one of the loci, which is not sufficient to make the genotype tolerant, but both of the loci are recessive in Y82187.

The results from the crosses with the CS monosomic lines indicated that chromosomes 3A and 3D of Y82187 each carry a recessive allele responsible for water-stress induced apical sterility. The deficiency of F_2 apical fertile progeny in the crosses involving chromosomes 5D and 6D, may be due to the limited number of F_2 plants grown or to a greater susceptibility to water-stress of monosomic plants in these lines. Since the clear expression of these genes is dependent on an adequate level of water-stress, there may be modifier genes on other chromosomes conferring physiological tolerance to water-stress, which may mask the expression of the recessive alleles identified.

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The *Ph1* locus of wheat: an enigma soon to be solved?

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In the plant kingdom, polyploids need to promote homologous and limit homoeologous association between chromosomes in order to survive. Through this practice, recombination is thus only permissible between homologues. In wheat, this status is maintained by the presence of the *Ph1* locus, located on the long arm of chromosome 5B (Riley & Chapman, 1958). Its absence is characterised by the presence of multivalent and univalent chromosomes during meiotic metaphase 1 of F_1 hybrids with diploid relatives such as rye or *Aegilops spp.*

Fluorescent *in situ* hybridisation, or FISH, using fluorescently labelled genomic DNA as a probe revealed that in such instances, the degree of pairing possible between wheat and alien chromosomes had been previously underestimated (Miller *et al.*,1994).

The development of CCS1 (Cereal Centromere Sequence 1) (Aragón-Alcaide *et al*., 1996, 1997a) permitted visualisation of centromeres for the first time, and revealed that the *Ph1* locus influenced their appearance during meiosis. At metaphase 1, centromeres in squash preparations were more discrete in the presence of *Ph1*, and were even stretched between the poles at telophase 1. With the adoption of 3-D confocal laser microscopy applied to immature anther sections, it was evident that wheat associated its homologues before the traditional meiotic interphase (Aragón-Alcaide *et al*., 1997b; Martinez-Perez *et al.*, 1999). Miller *et al*., (1998) suggested the term 'Paratene' in recognition of this unique situation.

Further 3-D studies revealed that this pre-meiotic centromere association does not extend to the diploid members of the *Triticeae*, but is delayed until the onset of leptotene, preceding the association of telomeres. However, it is not a unique feature of 4x and 6x wheats which carry *Ph1*, since some polyploid *Aegilops spp.* also exhibit pre-meiotic association (Martinez-Perez *et al.*, 2000)

The examination of somatic tissue in the roots of wheat showed that *Ph1* was effective here also (Martinez-Perez *et al.*, 2001). As roots grow, xylem cells expand and undergo cycles of replication, eventually becoming partially aptotic as they merge to form the familiar tubular structure of mature xylem tissue. Centromeres were seen to associate during these cycles of mitotic division, with a characteristic reduction in these homologous associations when the *Ph1* locus is absent. It was concluded that *Ph1* is involved in the specificity of any associations, rather than their induction.

Although much has been established about the role of the *Ph1* locus, little light had been shed upon the gene(s) involved. No allelism exists, so segregation cannot be generated. Sears (1977) produced a deletion, but its size at around 70 Mb meant that characterisation and localisation was still not possible. Thus, it was still not possible to establish whether it is one gene, many, or even an epigenetic effect.

Any proposed investigation to broaden knowledge of this *Ph1* effect has been hampered by several problems. To screen a mutagenised population in order to obtain, say, five novel deletions, would mean a cytological analysis involving 150,000 Pollen Mother cells (PMCs). Bread wheat has a genome size five times that of humans, much of it repeated, and the advent of molecular biology did not immediately provide a solution. A breakthrough came in 1999 when Roberts *et al*. adopted a two-pronged approach in the generation of five new novel deletions involving this *Ph1* locus. Firstly, they selected fast neutron irradiated populations for reduced fertility, and similarly irradiated specially constructed segmental hemizygotes. They then developed a multiplex PCR assay to screen these populations for alterations inside the 70mb deleted region delineated within the Sears' line, and coupled this with blind cytological scoring of PMCs from control and candidate plants selected by the PCR assay.

By reconciling the novel deletions to rice BACs, it became possible to establish an apparently contiguous rice DNA sequence. However, since rice has less than 2.5% the DNA of wheat, it was obvious that this probably would not provide the complete picture. Although rice chromosome 9 is syntenous with part of the 5B chromosome of wheat, it only represents one twenty-fifth of its total content. More detailed analysis revealed that Sears' original 70mb *Ph1* deletion covered only 4mb in rice, but characterisation of the meiotic behaviour of these novel deletions (Roberts *et al*., 1999) enabled the pertinent region at that time to be reduced to less than 400kb.

Further optimisation has continued at the John Innes Centre by cross-referencing of the rice genetic database to those of other plant genera. In this a sequence, which had initially been derived from rice BACs spanning the *Ph1* equivalent region defined by the novel wheat deletions, was used to uncover around 17 candidate genes. At first, it appeared that none of these corresponded to known meiotic genes, but it was subsequently found that some of these genes on rice 9 had been deleted from the wheat 5BL region. However, the remaining candidates have enabled us to continue to characterise the wheat 5BL arm.

With the anticipated completion of a wheat BAC library at the John Innes Centre in Norwich in the very near future, we have already embarked upon new strategies to hopefully complete the characterisation of this enigmatic *Ph1* locus.

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Evaluation and marker-assisted selection of single and multiple alien segment introgressions in durum wheat

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As demonstrated by a large body of evidence, tetraploid durum wheat exhibits a much lower degree of tolerance toward chromosomal and genic imbalances than hexaploid common wheat. Therefore, the incorporation of minimal amounts of alien chromatin represents a particularly essential requisite for stability and consequent practical usefulness of introgression products involving durum wheat, and even more so if the desired goal is to pyramid multiple alien segments. Luckily, however, chromosome engineering is today greatly assisted by the use of tools coming from molecular genetics and cytogenetics, which make for more efficient and accurate transfers of desirable genotypes than in the past (see, e.g., Ceoloni *et al.*, 1998). By exploiting such tools, the simultaneous incorporation of targeted chromosomal segments, originating from different wild and cultivated alien Triticeae species and containing potentially useful genes for the improvement of resistance and quality traits, has been recently attempted in durum wheat.

Characteristics of the recombinant lines with single alien segments

The initial material consisted of durum wheat recombinants carrying separately the *Pm13* gene from *Aegilops longissima*, the *Lr19+Yp* genes from *Thinopyrum ponticum*, and the *Glu-D1* or *Gli-D1/Glu-D3* genes from common wheat. As the following describes, in all cases the alien genes were associated with relatively short chromosomal segments.

a) *Pm13*

The *Pm13* tetraploid transfer line was homozygous for a 3BS/3S^{l}S recombinant chromosome which was first obtained at the hexaploid level (line R1B, see Ceoloni *et al.*, 1988; Donini *et al.*, 1995) before being moved into a tetraploid background by homologous recombination (Ceoloni *et al.*, 1996). Genetic (Donini *et al.*, 1995; Cenci *et al.*, 1999) and FISH-based (Biagetti *et al.*, 1999) mapping of a series of *Pm13* recombinant lines involving the wheat group 3 chromosomes has established that the R1B line carries the shortest 3S^{l}S segment. In physical terms, this segment, distally located on wheat 3BS, turned out to span less than 20% of the recombinant arm (Biagetti *et al.*, 1999).

b) *Lr19+Yp*

A *Th. ponticum* segment of even shorter length has also been transferred into durum wheat. This was selected from a series of secondary recombinants obtained by *ph1c* induced homoeologous recombination between durum wheat and a primary tetraploid recombinant line (Ceoloni *et al.*, 1998 and unpublished). The initial transfer line was shown by GISH (Genomic in situ hybridization) to contain a 7A/7Ag chromosome with the alien chromatin replacing about half of wheat 7AS and the entire 7AL (Ceoloni *et al.*, 1996). Two genes of potential benefit for durum wheat improvement, i.e. *Lr19* (resistance to leaf rust) and *Yp* (yellow endosperm pigmentation), were known to be closely linked on the distal half of the *Th. ponticum* 7AgL arm. Chromosome engineering of the primary recombinant chromosome yielded several products with reduced amounts of 7Ag chromatin. The selected line, R5-2-10, contained a transfer carrying the target genes within a terminal 7AgL segment representing just 23% of the recombinant arm length (Ceoloni *et al.*, 1998). Not only the transmission of the recombinant chromosome appeared to be normal through both germlines, but also plant vigour and fertility. Other relevant agronomic parameters do not seem to be negatively affected in several selected derivatives of line R5-2-10 (Ceoloni *et al.*, 2000 and unpublished). Such a line has been used to introduce into a tetraploid genotype the *Lr19+Yp* genes along with other alien introgressions (see later).

To this end, however, an even better candidate for future crosses has been obtained by the selection of a tertiary recombinant line (named R9-1). This has been obtained recently by homologous recombination in the region shared by two 7A/7Ag chromosomes having complementary patterns of 7AL and 7AgL chromatin. One such chromosome was that contained in line R5-2-10 (23% distal 7AgL), while the other was similar to the primary type (see above), except for an about 10% distal 7AL (line R8-6-4). The resulting interstitial 7AgL segment, which, like the parental 7AgL portions, contains and fully expresses the desired *Thinopyrum* genes, is subterminally located on 7AL and represents about 13% of the recombinant arm length. GISH preceded by a pre-annealing step between the alien and wheat total genomic DNAs (Anamthawat-Jónsson and Reader, 1995) turned out to be particularly effective in distinguishing the interstitial R9-1 segment from the distal R5-2-10 segment in the (R5-2-10 x R8-6-4) F_2 progeny. Homozygous R9-1 plants isolated within this progeny, together with most of the 7AL/7AgL secondary recombinants, are being currently used to develop genetic maps to be compared to their corresponding GISH-based maps. By means of such "translocation mapping" the physical location is therefore being determined for a number of RLFP probes. Other types of markers are also going to be used, in order to have a better coverage, and thus a more detailed map, particularly of the most distal 7AL and 7AgL regions.

c) *Glu-D1* and *Gli-D1/Glu-D3*

For the two tetraploid recombinant lines in which 1D segments from common wheat, containing the *Glu-D1* (1DL) or *Gli-D1/Glu-D3* (1DS) storage protein genes, had been separately transferred (Ceoloni *et al*., 1996; Vitellozzi *et al*., 1997), various types of marker systems have indicated a distal location of the breakpoints on the homoeologous 1A recipient arms. In particular, FISH with the pAs1 highly repeated DNA sequence as probe, showed that the recombinant 1AS arm possessed in a telomeric position the typical 1DS doublet of pAs1 sites, occupying about 20% of the recombinant arm. This roughly corresponds to the length of the transferred 1DS segment, since the adjacent 5S rDNA locus was proved by RFLP mapping (Ceoloni *et al.*, unpublished) to be already included in the proximal 1AS portion.

On the other hand, the FISH pattern of the same pAs1 sequence on the 1AL/1DL recombinant chromosome containing the *Glu-D1d* locus ('5+10' HMW-glutenin subunits) had suggested that the 1DL segment spanned distally approximately 25% of the recombinant 1AL

(Vitellozzi *et al*., 1997). However, a recent re-investigation of the 1AS.1AL/1DL chromosome by means of the pre-annealing-GISH technique (Anamthawat-Jónsson and Reader, 1995) revealed that the 1DL introgression consisted of a shorter (less than 15% of the total arm length), interstitial segment, subterminally located on the recipient 1AL (Ceoloni and Carozza, unpublished). Evidently, as a result of the *ph1c* induced homoeologous recombination (Ceoloni *et al*., 1996; Vitellozzi *et al*., 1997), two cross-over events had occurred between 1AL and 1DL within a relatively small distance. This must be an infrequent phenomenon between homoeologous chromosomes, even though there is a close affinity between the A and D genome chromosomes, and between the two 1L arms in particular (see, e.g., Naranjo *et al.*, 1987).

Both the 1AL.1AS/1DS and the 1DL/1AL.1AS recombinant chromosomes exhibited normal transmission through both germlines (Ceoloni *et al*., 1996; Vitellozzi *et al*., 1997). Moreover, their presence, was found to be associated with a highly positive enhancement of gluten quality of the carrier durum wheat lines (Ceoloni *et al.*, 2000 and unpublished). In addition, as indicated by preliminary, small-scale field tests, there does not seem to be any detrimental effects on the agronomic performance of derivatives of both the 1A/1D recombinant types (Ceoloni *et al.*, 2000 and unpublished).

Multiple combinations of different alien segments

In order to combine in the same tetraploid genotype different wheat-alien chromosome pairs, a number of F_1s have been firstly produced between homozygous plants for the above described recombinant lines. For the genotyping of the F_2 populations obtained from the double heterozygotes, STS markers associated with the 1D and the $3S^lS$ transfers have been used for PCR analysis of half-seed extracted DNAs.

For the former ones, primer pairs were employed which generate specific amplification products for all the critical alleles. In particular, presence/absence of the *Glu-D3* and/or *Glu-A3* genes (S arm transfers) was monitored by means of a primer pair which amplifies a number of chromosome-specific fragments (D'Ovidio *et al.*, 1997). Two 1D-specific, together with two 1A-specific fragments, which were clearly observed in the DNA profiles of the critical populations, not only allowed discrimination of recombinant from non-recombinant types, but also of homozygous from heterozygous 1AS/1DS individuals. To achieve the same discrimination ability within the segregating populations involving the 1DL-derived segment, two primer pairs, one specific for the 1Dx5 component at the *Glu-D1-1d* locus (D'Ovidio and Anderson, 1994) and the other specific for the *Glu-A1-2a* (*=1Ay*) gene (D'Ovidio *et al.*, 1996), were used. The latter was employed to distinguish homo- from heterozygous carriers among individuals which had shown the typical 1Dx5 amplicon.

Presence/absence of the 3BS/$3S^lS$ recombinant chromosome harbouring the *Pm13* gene was established by means of a dominantly inherited, *Ae. longissima*-specific STS marker derived from a RAPD amplicon (Cenci *et al.*, 1999).

On the other hand, GISH on root-tips chromosome preparations has been largely applied to detect the presence as well as the dose of the *Th. ponticum* 7AL/7AgL chromosome. The recent development of a dominant STS marker, turned out to be associated with *Th. ponticum* *Lr19*-containing segments introgressed into common wheat lines (Prins *et al.*, 2001), could further facilitate selection of the F_2s segregating for the *Lr19+Yp* transfer. Only plants turned out to contain the above STS would then be subjected to a GISH-based screening in order to determine their homozygous or heterozygous condition.

So far, of several combinations produced among the different wheat-alien recombinants described above, preliminary results are available on the F_2s segregation of those involving the R5-2-10 (*Lr19+Yp*) line (=RC) and each of the two carrying the 1A/1D transfer

chromosomes (=PS and PL, with S and L indicating the corresponding exchanged arm). In both progenies simultaneous transmission of the two different recombinant chromosomes (7AS.7AL/7AgL together with 1DL/1AL.1AS or 1AL.1AS/1DS) seems to be normal through both germlines. In fact, with a reasonably good fit with respect to the expectation, all the possible 9 genotypic classes have been detected among 23 (χ^2=6.9, P>0.50) and 49 (χ^2=9.9, P>0.25) derivatives of the RC x PS and RC x PL crosses, respectively. In terms of zygotic tolerance, no differential performance has been observed among genotypes at seedling and later vegetative stages. Analysis of fertility (expressed as seeds per spikelet) of a small sample of representative genotypes has also revealed no significant differences associated with the dosage of the two wheat-alien chromosomes.

Conclusions

Although preliminary, the reported observations indicate a good degree of tolerance of the tetraploid wheat genome toward the type of manipulations performed. Such a result can probably be attributed also to the specific characteristics of the wheat-alien transfers with which the combinations were realized. There is no doubt, in fact, that the more finely tuned is the process of developing suitable introgression products, the higher becomes their possibility of being "well accepted" by the recipient genotype. This certainly seems to be the case with the materials described here. Further work is in progress to corroborate the present findings and to further evaluate the feasibility of the approach of multiple alien segment introgression, both in relation to the recipient species and to the transfer method adopted.

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Characterization of a backcross inbred line population and genetic control of loci responsible for powdery mildew resistance

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It is recognized that the improvement of wheat requires a search for new genes in related species and genera, because, in many cases the genetic variability among cultivated wheats has been already exhausted. Wild species of *Triticeae*, as a result of their adaptability to a wide range of environments in the course of their evolution, include populations with a large variability for many characters such as morphological traits, drought and cold tolerance, insects and fungal diseases resistance, quality and quantity protein content of seeds. In order to investigate the genetic basis of important qualitative and quantitative characters and to introduce new useful alleles into a cultivar of durum wheat, a hybridisation program between wild relatives and commercial cultivars of durum wheat has been carried out.

1. Analysis of chromosome segments introgressed from var. *dicoccoides* in durum wheat

In order to investigate the effects of single or few genes deriving from *Triticum turgidum* var. *dicoccoides* in a cultivated variety of durum wheat (*T. t.* var. *durum*) and to introduce new useful alleles into a *durum* cultivar, a population of BC_3F_8 lines (backcross inbred lines, BILs) has been developed. The recurrent parent, the durum wheat cv. Latino, is a semidwarf high-yielding commercial cultivar with low grain protein content and susceptible to powdery mildew, whereas the donor parent was the *dicoccoides* accession MG29896, with high grain protein content and completely resistant to powdery mildew. One hundred and ten plants were backcrossed three times to the recurrent parent and then, each line was selfed for eight generations by single-seed descent method, resulting in a population of BILs (Figure 1). No intentional selection was imposed during population development, but some lines were lost during backcrossing and self-fertilization program. The BC_3F_8 generation contained 92 BILs. The complete series of backcross inbred lines was grown in 3 different locations for two years and evaluated for components of productivity such as, seeds weight/plant, seed weight/spike, weight of 1000 kernels, for seed quality (protein content/seed and analysis of gliadins and glutenins) and for diseases resistance (powdery mildew). On the basis of these analyses a subset of 28 BIL was selected for molecular marker characterisation.

Eighty-nine microsatellites, developed by Röder et al., (1998) and by Stephenson et al., (1998), 7 biochemical and 5 morphological markers were used to reveal *dicoccoides* chromosome segments retained into the 28 BILs. The percentage of *dicoccoides* genome introgressed into Latino has been estimated by using the software Q-gene (Nelson, 1997) and it 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 *dicoccoides* genome could be easily introgressed in Latino and that there is no incompatibility between the two genomes. The number of *dicoccoides* equivalent genomes estimated to be present in all 92 BILs is 5.75, giving a theoretical probability higher than 99,7% to find any *dicoccoides* segment at least in one line. However, the percentage of *dicoccoides* genome not represented in 28 BIL resulted about 27%, higher than the expected value (13.5%). This discrepancy could be explained by the hazard effect due to the reduced number of analysed lines or by the natural selection against particular loci. The number of *dicoccoides* introgressed segments ranged between 1 and 14 and the number of terminal segments introgressed was similar to interstitial ones. The lines

containing the lowest number of *dicoccoides* fragments are particularly appropriated to produce, by a low number of additional backcrosses, a series of NILs having the complete *dicoccoides* genome distributed as single fragment.

In order to identify and to saturate with molecular markers specific segments controlling useful traits, such as protein content, analyses are in progress on F_2 progenies derived from the cross between some interesting BIL lines and the recurrent parent Latino.

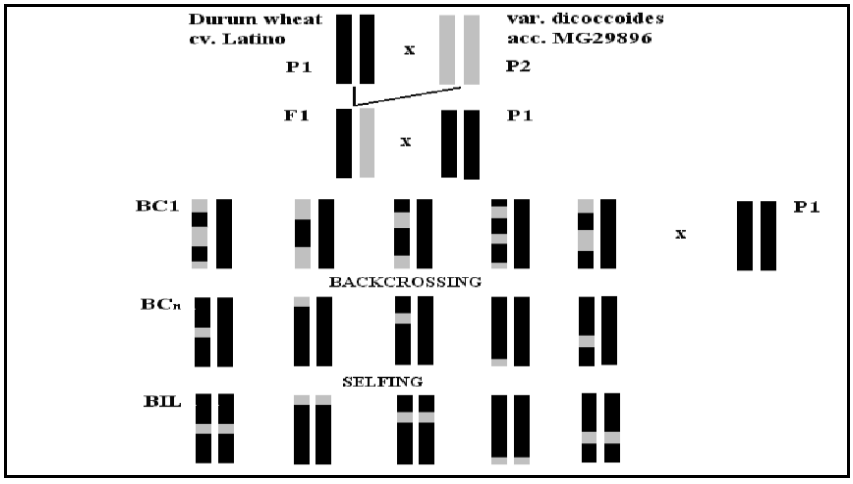

Figure 1. Crossing procedure to obtain BIL population

2. Genetic analysis of powdery mildew derived from wild wheat

Powdery mildew is a common disease induced by the biotrophic fungal pathogen *Blumeria graminis* f. sp. *tritici*, which causes considerable damage to wheat cultivars. A general problem in the fight against diseases is the pathotypes continuous evolution. As a consequence, the resistance against a specific strain of the pathogen usually become ineffective within a very short period. and new sources of resistance are requested.

The wild wheat accession MG5323 of *T. turgidum* var. *dicoccum* resistant to *B. g.* f. sp. *tritici* was crossed with the durum susceptible cultivar Latino. Powdery mildew resistance was valued on F_3 generation and on a derived recombinant inbred line (RIL) population, on adult plant in the field conditions. The populations were assayed on randomised blocks with 3 replications. Segregation analysis data of field resistance suggest a polygenic control. For the bulked segregant analysis (Michelmore et al. 1991) DNA pools were obtained from plants highly resistant or completely susceptible to powdery mildew. About 200 microsatellite primer pairs have been tested and 47% showed polymorphism between Latino and MG5323. Two microsatellite markers resulted polymorphic also between the resistant/susceptible bulks, both for F3 and RIL populations. Segregation analysis of these microsatellites is in progress on F3 and RIL individuals.

The *T. t.* var. *dicoccoides* accession MG29896, highly resistant to powdery mildew, was crossed to the durum susceptible cultivar Latino and resistance segregation data were analysed at seedling stage in greenhouse with a single isolate. The F_2 resistant:susceptible segregation fitted the 13:3 ratio. The hypothesis of interaction between one dominant and one recessive resistance gene was also supported by the analysis of F_3 family.

From the same parental lines a backcross inbred line (BIL) population was developed. Greenhouse and field resistance was scored for 92 BIL. Two classes of resistant lines were detected: 5 lines showed complete seedling greenhouse resistance but were susceptible in the field conditions; two of these (i.e. BIL74 and BIL91) were crossed to the susceptible parent (Latino) and the greenhouse resistance segregation data in the F_2 generations were consistent with a dominant inheritance. Five other BILs exhibited partial seedling resistance and complete resistance in the field trials. The resistance segregation was analysed in the F_2 progeny from the cross between one of the field resistant BILs (i.e. BIL29) and Latino: the resistance resulted to be inherited as a single dominant character. From this F_2 population, resistant and susceptible individuals were selected to produce DNA bulks and PCR screening was carried out using microsatellite and AFLP markers. One AFLP marker was individuated to be associated to the resistance locus at 1 cM. Additional screening is in progress to find new linked markers.

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Production and identification of wheat-barley hybrids and translocations using GISH, FISH and SSR markers

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Bread wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) are two of the most important cereal crops worldwide. Hybridization between these species makes it possible to transfer desirable traits (e.g. earliness) from barley into wheat. The prerequisite for the successful transfer of chromosome segments is pairing between the chromosomes of the different species in the hybrids, which may result in the development of recombinants. The first successful hybridization between wheat (*Triticum aestivum* L.) and barley (*Hordeum vulgare* L.) was reported by Kruse (1973) and not much later a set of wheat-barley addition lines was produced (Islam et al. 1978). The addition lines were produced from the Chinese Spring wheat and Betzes barley varieties, which have high crossability in intergeneric crosses but are unsatisfactory from the agronomic point of view. For the successful transfer of useful genes from barley into wheat it is essential to produce hybrids with genotypes which carry agronomically important characters. However, very few hybrid combinations have been reported from wheat × barley crosses involving barley varieties with good agronomic characters (Wojciechowska and Pudelska 1993; Jauhar 1995; Taketa et al. 1998) and in most cases no backcross progenies were developed.

Fedak (1977), Sethi et al. (1986) and Jauhar (1995) assumed that homoeologous pairing occurred between the chromosomes of wheat and barley on the basis of the chromosome pairing frequency data observed in the hybrids. Islam and Shepherd (1988) observed pairing between barley telocentrics and wheat chromosomes at a frequency of 1.2-4.5%. Wheat-barley chromosome pairing can result in the development of recombinants between the chromosomes of these species. Until now only a few wheat-barley translocations have been developed (Islam and Shepherd 1992, Koba et al. 1997, Endo et al. 1998; Sherman et al. 2001). In Martonvásár wheat-barley translocation lines were produced from hybrids regenerated from tissue culture (Molnár-Láng et al. 2000a).

The main objectives of the experiments were to produce new wheat-barley hybrids and addition lines between agronomically useful genotypes, and to detect wheat-barley chromosome pairing at meiosis in the hybrids with the aim of assessing the frequency of wheat-barley recombinants and of analysing the translocations produced in Martonvásár using cytogenetic and molecular genetic methods.

Wheat-barley chromosome pairing detected by GISH in the new winter wheat (*Triticum aestivum*) × winter barley (*Hordeum vulgare*) hybrids produced in Martonvásár

Fourteen winter barley and two spring barley cultivars were tested for use as pollinators for wheat (*Triticum aestivum* L.) × barley (*Hordeum vulgare* L.) hybrid production. Hybrids were successfully produced with three different barley genotypes: Igri (German two-rowed winter barley variety), Osnova and Manas (Ukrainian six-rowed winter barley varieties) (Molnár-Láng et al. 2000b). Mv9 kr1 × Igri, Mv9 kr1 × Osnova and Asakazekomugi × Manas hybrids were produced besides the Chinese Spring × Betzes hybrid created earlier (Molnár-Láng and Sutka 1994). The seed set was extremely low even in the successful combinations.

No seed set was observed when wheat was pollinated with the other thirteen barley varieties tested.

The new winter wheat × winter barley hybrids were multiplied in tissue culture because of the high degree of sterility and then pollinated with wheat to obtain backcross progenies. Meiotic analysis of the hybrids Mv9 kr1 × Igri and Asakazekomugi × Manas and their *in vitro* regenerated progenies using the Feulgen method revealed 1.59 chromosome arm associations per cell in both initial hybrids. However, among the *in vitro* progenies, the number of chromosome arm associations increased to 4.72 and 2.67, respectively, in the two hybrid regenerants. Genomic *in situ* hybridization (GISH), with total genomic DNA from one of the parental species as a probe, allows chromosomes of different parental origins to be "painted" in different colours in the nuclei of interspecific hybrids (Schwarzacher et al. 1989). GISH is an excellent method for detecting intergeneric pairing in wheat-alien hybrids. GISH was carried out according to Reader et al. (1994). According to the GISH analysis, wheat-barley chromosome arm associations made up 3.6% of the total in the initial Mv9 kr1 × Igri hybrid and 6.6 % and 16.5% of the total in *in vitro* regenerated progenies of the Asakazekomugi × Manas and Mv9 kr1 × Igri hybrids, respectively. This demonstration by GISH of wheat-barley chromosome pairing in the hybrids and especially in *in vitro* regenerated progenies proves the possibility of producing recombinants between these two genera, and thus of transferring useful characters from barley into wheat. *In vitro* conditions caused an increase in chromosome arm association frequency in both combinations and in fertility in some regenerants (Molnár-Láng et al. 2000b).

Production of winter wheat cv. Mv9 kr1/ winter barley cv. Igri addition lines

Mv9 kr1 × Igri hybrid regenerants muliplied *in vitro* were backcrossed with the wheat line Mv9 kr1; after pollinating 4606 flowers with wheat, nine embryos could be dissected, and six BC_1 plants were grown to maturity (Molnár-Láng et al. 2000b). The BC_1 plants were backcrossed again with the wheat line Mv9 kr1 and 24 BC_2 seeds were obtained, 16 of which produced self-fertile plants. The chromosome number of the BC_2 plants ranged between 43 and 46. On the BC_2 plants 735 seeds developed after self-pollination. Plants with 43 and 44 chromosomes were selected after Feulgen chromosome counting from the selfed BC_2 seeds. Meiotic analysis will be employed to decide whether the plants with 44 chromosomes carry an additional pair of barley chromosomes or two different ones. So far no plants have been identified with 22 bivalents showing up as disomic additions; however the meiotic pairing behaviour of only 12 of the 24 plants with 44 chromosomes has so far been analysed.

Wheat x maize crosses were also used to produce disomic additions from monosomic additions. Twenty plants with 43 chromosomes were pollinated with maize (cv. Seneca), from which 22 embryos were dissected. Fourteen embryos started to germinate and 6 plantlets developed. The six plants are now being grown in the growth chamber at present and their chromosome numbers will be checked later on.

The expected transmission ratio of the alien chromosome from the monosomic plants is higher through the female gametes than through the male gamete. It is hoped that different additions can be recovered after colchicine treatment on haploid plants carrying one alien chromosome in the wheat background.

Production and identification of wheat-barley translocations using GISH, FISH and SSR markers in the progenies of wheat-barley hybrids multiplied in tissue culture

Wheat-barley translocations were identified by genomic *in situ* hybridization (GISH) in backcross progenies originating from wheat × barley (Chinese Spring × Betzes, Mv9 kr1 ×

Igri) hybrids regenerated *in vitro*. The regenerated hybrids were pollinated with the wheat line Martonvásári 9 kr1. All were single breakpoint translocations with the relative positions of the breakpoints ranging from the centromere to about 0.8 of the relative arm length.

Five wheat-barley translocations in a wheat background were characterized through the combination of cytogenetic and molecular genetic approaches. The wheat chromosome segments involved in the translocations were identified using sequential GISH and two-colour FISH with the probes pSc119.2 and pAs1. The barley chromatin in these lines was identified using SSR markers. A total of 45 markers distributed over the total barley genome was selected from a recently published linkage map of barley (Ramsay et al. 2000) and screened on the translocation lines. The following translocations were identified: 2DS.2DL-1HS, 3HS.3BL, 6BS.6BL-4HL, 4D-5HS and 7DL.7DS-5HS. Wheat-barley disomic and ditelosomic addition lines for the chromosome 3HS, 4H, 4HL, 5H, 5HL and 6HS were used to determine the correct localization of 21 markers and the position of the centromere. An ancient intragenomic rearrangement between chromosome arms 1HL and 5HS was detected in barley. Physical mapping of the SSR markers on chromosomes 1H and 5H was carried out using the intragenomic and interspecific translocation breakpoints and the centromere as physical landmarks.

Four of the five translocation lines have good fertility and are being multiplied in the nursery. The translocation lines show differences in a number of morphological traits: such as ear type, the length of the ear, awns, plant height, earliness, etc. The agronomic traits of these lines will be studied in more detail when more seeds are available.

Conclusions

New winter wheat × winter barley hybrids were produced using three different winter barley cultivars with good agronomic characters. The production of these new hybrid combinations makes it possible to transfer agronomically useful characters from barley into wheat, which was not the case when using the model barley variety Betzes. The callus culture of intergeneric hybrids could be used not only to increase the number of hybrids when crossability is a problem, but also to induce more frequent intergeneric pairing, which could result in the production of recombinants. The effect of callus culture in increasing hybrid fertility could also be used to obtain backcross progenies from completely sterile hybrids. According to the present observations the increased number of restitution nuclei in the regenerant hybrids could be one reason for the higher fertility of some regenerants. The increased number of wheat-barley chromosome arm associations observed in the regenerated wheat × barley hybrids by means of GISH demonstrates the possibility of creating recombinants between these two genera.

Five wheat-barley translocations were produced and identified with GISH, FISH and SSR markers. The translocations could be useful for both wheat improvement and genome mapping. The intergeneric translocation breakpoints detected by GISH could serve as physical landmarks on the chromosomes to facilitate the physical mapping of the chromosomes. These lines could significantly improve the accuracy with which markers and genes can be assigned to chromosome regions in the barley genome. Furthermore, the translocation lines will contribute to extending the genepool available for wheat improvement.

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EWAC heritage- waiting for new applications

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It is now clear that GENOMICS will achieve a new level of knowledge in current biology, genetics and breeding and will help greatly in understanding the structural and functional organisation of living organisms.

A number of methods has been applied by GENOMICS today. These can be divided into three major groups:

- structural genomics (including genome sequences and **e**xpressed **s**equences **t**ags (**EST**s));
- functional genomics (gene expression analysis, reverse genetics, proteomics, metabolic profiling etc.);
- comparative genomics.

Structural genomics is growing rapidly as a result of the introduction of new technical platforms for high throughput sequencing. In 1999, for example, the public databank (NCBI) contained only eight entries of ESTs for wheat (*Triticum aestivum* L.) but more than 170,300 sequences have been deposited here since June 2002 (http://www.ncbi.nlm.nih.gov/dbEST/dbEST-summary.html). Sequencing the entire genome has become a reality in *Arabidopsis* and rice and may become possible for more complex plants such as maize or wheat. These days, the main area of research applications in molecular biology has moved from structural genomics to functional genomics. Nevertheless, between both groups, tight links exist which will be extended in the near future.

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

Comparative genomics helps to exchange the knowledge from one species to another, saving time and resources.

The results, tools and methods achieved by GENOMICS will become of value in plant breeding studies. There is no doubt that biotechnology has already made a major impact on agriculture and this impact will grow rapidly in the near future. People quickly recognised that it is not the technology or the machinery that limit advances in GENOMICS, but the plant resources themselves. These are often the crucial factors in making new discoveries of the structure and function of plant genomes.

It should be emphasised here that during the last three decades, monosomic series have been developed in more than forty wheat varieties along with a wide range of intervarietal chromosome substitution lines, alloplasmic lines and single chromosome recombinant lines etc.

These precise genetic stocks have been successfully used for many purposes:

- gene and trait localisation and mapping;
- study of the effects of single genes and their different alleles on different traits like plant development, yield components or resistance to various diseases;
- analysis of interactions between different genes in the same genetic background;
- cytogenetic studies on chromosome structure and chromosome pairing;
- alien gene transfer and gene expression;
- study of tissue culture response;
- localisation and mapping of molecular markers.

The GENOMICS era has already opened up many new possibilities for further applications of this genetic material. These could be:

- high resolution trait and gene mapping;
- chromosome sorting and development of chromosome specific libraries;
- gene cloning;
- genetic modification;
- gene expression.

The utilisation of precise cytogenetic stocks with GENOMICS research will lead to the better understanding of the genetics and physiology of crop plants and will contribute greatly to continuing progress in crop breeding and, more generally, throughout agriculture.

Poster

Microsatellite development from genomic and EST libraries for the improvement of wheat genetic mapping

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Microsatellites have demonstrated their great efficiency for wheat genetic mapping. The present project aims at developing microsatellites to establish saturated linkage maps for crosses of agronomic interest.

Three microsatellite enriched libraries were produced using as DNA source the wheat diploid ancestors: *T. urartu* (A library*), Ae. speltoides* (B-genome library) and *T. tauschii* (D-genome library). Four thousand and five hundred clones were sequenced leading to 466 polymorphic primer pairs. A total of 404 microsatellite markers were finally used for mapping resulting in 533 loci. Primer pairs obtained from the A and D-genome libraries gave the best results with respectively 65% and 60% polymorphic products. This contrasts with those developed from the B-genome library (40%). Eighty five percent of the loci produced from the D-genome library mapped on the D genome, 74% of those from the A-genome library mapped on the A genome, while only 65% of those from the B-genome library mapped on the B genome. This may be considered as further information about the distances between the genomes in the diploid and hexaploid conditions.

Simultaneously, 115 additional primer pairs were developed based on the analysis of 1500 ITEC EST sequences containing a microsatellite motif. Eighty four revealed an amplification product, 34 showing polymorphism between the 4 reference cultivars. Finally, 31 microsatellite loci were mapped on either the ITMI or Courtot x Chinese Spring populations. Twenty eight additional monomorphic loci were physically assigned to chromosome arms using the Chinese Spring aneuploid lines.

Finally, this work provided a microsatellite map covering 60% of the wheat genome with a mean coverage of 1 marker every 6,5 cM.

The relationship between the genetic and physical maps in bread wheat: characterization of deletion lines using microsatellites and their use for ESTs mapping

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Molecular-functional physical maps are the prerequisite for the candidate gene approach to identifying and cloning genes underlying quantitative trait loci. One way to achieve this goal would be to define the relationships between physical and genetic maps in order to assign ESTs or genes to particular regions of the genome. Deletion lines are ideal materials for physically allocating loci to chromosomal regions in bread wheat.

We recently received from Dr. B. Gill (KSU) a set of 84 deletion lines covering the 21 chromosomes of wheat. A comparison of genetic– deletion map relationships was compiled from published data (2552 markers, 73 and 37 bridges for ITMI and CtCS maps, respectively). A preliminary characterization based on 320 microsatellites combined with C-banding and spike morphological evaluations, was undertaken to check this material and to establish 106 connections between the deletion and genetic maps. For this purpose, ITMI and Courtot x Chinese Spring genetic maps were considered as reference. This is exemplified for the chromosomes of homoeologous group 5 with the creation of 'deletion bins' (chromosomal region between two deletions characterized by molecular markers anchored to a specific region of the genetic map).

A first molecular-function map was constructed in wheat for genes operating during water stress, in order to test the candidate gene strategy based on the deletion mapping system. One hundred and thirty one clones revealed a significant homology with the Swiss-prot and TrEMBL protein sequences involved in abiotic stress (heat shock, dehydrins…) These were assigned using RFLPs and a set of wheat aneuploid lines. More than 570 bands were recorded, and 404 could be unambiguously assigned to specific chromosomes or chromosome arms. This revealed the major involvement of chromosomes from homoeologous group 5 (91 bands), followed by chromosomes from group 4 (71 bands).

These results give the first comprehensive picture of wheat chromosomal regions involved in water stress. Putative functional gene markers will be sought in related plant species (e.g. maize, barley, rice) using synteny relationships.

Chromosome location of a gene controlling the trait "lowering leaf" in common wheat Gostianum 88 (*Triticum aestivum* L)

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Introduction

For the majority of varieties cultivated in Kazakhstan, the flag leaf occupies a horizontal position after spike emergence. Such spatial orientation of leaf prevents solar radiation from penetrating to the lower leaves. The large flag leaf of Gostianum 88 (G88) has an area 25-30 cm^2. Before ear emergence it has a vertical orientation, but following emergence it drops to an angle of 180º and nestles close to the stem. This change of flag leaf orientation allows solar radiation to get to the lower parts of the plant, and consequently promotes longer functioning and participation not only of the flag leaf but also the lower leaf layers in grain filling. Such spatial orientation reduces competitive ability of plants in phytocenosis and this positively influences productivity (Donald, 1968, Sears, 1998). G 88 has a dense productive spike, coarse grains with 1000 grain weights of 49-52 g. In crosses with various cultivars, the F_1 hybrids shows heterosis involving those basic traits participating in yield formation (Bogdanova, 1992). In the literature, we have not found any research information about this trait. Oservations conducted over many years have shown, that under the conditions of the Almaty region, characterised by moisture deficiency during grain filling, lines having the "lowering leaf" trait are more productive.

The purpose of this work is to determine the chromosomal location of the gene controlling the trait "lowering leaf" in the common wheat line G88.

Materials and methods

In our work we used the common wheat line isolated from cv. G 88 obtained by Dr. E. D. Bogdanova, and the series of monosomic lines of Saratovskaya 29 (S29) developed by O. I. Maystrenko (1971). Plants were grown at the experimental field of Kazakh Institute of Crop Production. For chromosome localization of the target gene, the F_1 and F_2 hybrids were obtained from crossing the S29 monosomic lines with G88. Chromosome counting was carried out using temporary acetocarmine preparations according to standard procedures. The trait was scored visually 14 days after ear emergence. The results of segregation were analysed by the χ^2 test.

Results and conclusions

The cross of Gostianum 88 with S29 and its monosomic lines produced F_1's, all of which showed the phenotype "lowering leaf". This indicates the dominant nature of this character. In the F_2 we observed segregation of plants with lowering and horizontally orientated leaves. Data are presented in Table 1. As can be seen, the disomic F_2 hybrids of the control cross combination (S29 x G88) segregated to give a 3 : 1 (lowering leaf : horizontally oriented leaf), indicating the monogenic and dominant inheritance of the trait (χ^2 =0.66). This 3:1 segregation was found for all the F_2 monosomic hybrids except F_2 mono 2A S29 x G88. In this case we observed 147 plants with lowering flag leaf and 1 plant with horizontally

oriented flag leaf having a nullisomic phenotype (χ^2 =46,71). This proves that the gene controlling the target trait is located on chromosome 2A. Thus, we have identified and localized a new gene controlling the trait "lowering leaf". The symbol *Ll* has been given to this gene.

Table 1. Segregation of plants for the trait "lowering leaf "in F_2 monosomic and disomic populations

Population F_2	Segregation			χ^2 3:1
Mono S29 x G88	Lowering leaf	Horizontal leaf	In all	
1A	128	40	168	0,54
2A	147	1	148	46,71*
3A	116	31	147	1,34
4A	125	37	162	0,42
5A	134	33	167	2,40
6A	160	45	205	1,01
7A	146	37	183	2,22
Disomic	100	28	128	0,66

* - P>0,99

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Transfer and chromosomal identification of genetic material of *Triticum timopheevii* introgressed into the genome of common wheat

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Common wheat is one of the most important food crops in the world. Susceptibility of wheat varieties to plant pathogens results in significant reductions of yield up to 50%. The cultivation of resistant wheat is considered to be the most economically and environmentally safe method to minimize yield losses. One strategy in breeding for disease resistance is the development of varieties employing resistance genes both from cultivated varieties and from wild relatives of wheat. Many specific resistance genes against plant diseases are known and among them some have been introgressed into wheat from wild relatives.

The tetraploid wheat *Triticum timopheevii* Zhuk (2n=28, A^tA^tGG) possesses a unique gene pool conferring resistance to many diseases including leaf rust. The transfer of genetic material from *T. timopheevii* into *T. aestivum* makes it possible to establish and maintain a number of resistance genes assembled in a collection of introgression lines and to use these lines in future breeding programmes. Examples of the transfer of disease resistance genes from the *T. timopheevii* species to hexaploid wheat are resistance to stem rust (*Sr36*, *Sr37*), powdery mildew (*Pm2*, *Pm6*, *Pm27*) and leaf rust (*Lr18*) (Friebe et al 1996; Järve et al 2000; Yamamori 1994). However, *T. timopheevii* also possesses additional genes determining the resistance to these pathogens. In previous studies we reported that a collection of cytologically stable hexaploid wheat lines (2n=42) containing leaf rust resistance genes introgressed from *T. timopheevii* has been developed (Russian patent 2138155 at 19.03.1998; Budashkina 1988; Budashkina and Kalinina 2001).

The main object of this study is the chromosomal location of these leaf rust genes using molecular markers.

Materials and methods

Studies were performed on 24 introgression lines developed by crosses between *T. timopheevii* and the hexaploid wheat varieties Saratovskaya 29, Novosibirskaya 67, Skala, Irtishanka and Tcelinnaya 20. After the single backcross of F_1 progeny to the initial variety, hybrid lines were analyzed for cytological stability and resistance to leaf rust in the F_4B_1 – F_7B_1 generations. The Mains-Jackson international scale of immunity was used. The following marker approaches were carried out for the identification and location of the resistance genes transferred from *T. timopheevii* : the use of biochemical markers (Kalinina et al 1987), the study of C-banding (Badaeva et al 1991) and the application of microsatellite markers (Röder et al 1998, Leonova et al 2001).

Results and discussion

Resistance of the introgression lines was assessed at the flowering stage for plants in the field subjected to a population of leaf rust. Resistance has been repeatedly confirmed in F_7B_1-$F_{15}B_1$ generation and scored 0 or 1, whilst that of the initial varieties of common wheat was scored as 4. Most of the lines were resistant to individual testers of leaf rust and powdery mildew under artificial infection (personal communication of Dr. F. Zeller).

Genetic analysis of the introgression lines showed that primarily 1-2 genes with different types of interaction may provide resistance. It was also demonstrated that resistance genes in the lines were not allelic to known *Lr* genes used in breeding (*Lr9*, *Lr19*, *Lr23*, *Lr24*). Introgression of the *T. timopheevii* genetic material influenced morphological traits also. Thus, lines developed in the awnless Saratovskaya 29, had awned spikes and were speltoid. The leaves of some lines are hairy, a characteristic of *T. timopheevii*. There were also differences in tillering, lodging resistance and length of the vegetative period.

Microsatellite markers were used for more precise identification of the *T. timopheevii* genetic material in the chromosomes of common wheat. 202 wheat microsatellites (GWMS) specific for the A, B and D wheat genomes were chosen for the analysis, overall from 2 to 18 markers per chromosome arm. Marker analysis demonstrated that genetic material of *T. timopheevii* introgressed mainly into chromosomes of homoeologous groups 2 and 5. Practically all introgression lines had *T. timopheevii* fragments in either the 2A or 2B chromosomes. The size of the introgression fragments in chromosome 2B was the same for all of the lines studied and involved both chromosome arms. In 15 lines out of 24 an introgression of *T. timopheevii* occurred into the long arm of chromosome 5A.

From the results of the genetic and molecular analysis, it may be concluded that the hybrid lines, we developed by crossing hexaploid bread wheat with the tetraploid wheat *T. timopheevii,* contain new genes for leaf rust resistance. The use of these lines in breeding programmes may contribute significantly to wheat improvement.

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Distribution of alleles at the locus *Xgwm261* marking the dwarfing gene *Rht8* in Ukrainian hexaploid wheat varieties

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Introduction

Winter wheat is the most important grain crop in the Ukraine, occupying over the past 10-15 years an average 19% of all arable land and contributing from 50% to 60% of the total quantity of annually harvested grain. The winter wheat areas fluctuates between 4.5 to 8.0 million ha in absolute terms (Litvinenko et al. 2001). Scientifically based wheat breeding programmes started in the Ukraine about 80 years ago. Over this period of time at least seven principle wheat variety changes have happened and grain yield has increased from an average of 2.73 t/ha in 1912-1922 to 6.74 t/ha during the 1980s (Litvinenko et al. 2001). The major features of the wheat varieties cultivated during each period are as follows: (I) 1912-1922 - landraces; varieties, selected from local populations; (II) 1923-1947 - very tall varieties derived from inter-varietal crosses; (III) 1948 - 1959 - tall, semi-intensive varieties; (IV) 1960-1967 - medium tall varieties of intensive type for the forest-steppe zone; (V) 1968-1975 - medium tall varieties of intensive type for the steppe zone; (VI) 1976-1980 - semi-dwarf varieties developed during the first breeding phase; (VII) 1980-present - short stem and semi-dwarf varieties developed during the second breeding phase (Litvinenko, 1998). Plant height decreased in each phase by 6.2 cm (6.6%) on rich superior soil conditions and by 5.0 cm (4.7%) on poorer soils. The decrease of plant height was correlated with a yield increase (Litvinenko, 2001). Several types of dwarfing genes were used in the breeding programmes of the Ukraine. The first type, represented by the winter wheat genotypes, Krasnodarsky karlik I and its allelic mutants, the dwarfing genes *Rht3* and/or *Rht5* were involved (Bessarab and Zhirov, 1975). The gene *Rht8* was included in the second type. The source of *Rht8* was the genotype Bezostaya I used in Ukrainian breeding programmes. The sources for the third type are spring wheats from CIMMYT, USA or the Ukraine, carrying genes *Rht1, Rht2* or *Rht3,* or combinations of these genes. The fourth type of dwarfing source is the mutant genotype selected from the variety Odesskaya 16 after chemical mutagenesis. The gene causing dwarfism in this case is not allelic to previously identified genes and has been designated *Rhtx* (Litvinenko, 2001). For the gene *Rht8* on chromosome 2D, a close linkage to the microsatellite WMS261 was found (Korzun et al. 1998). Worland *et al.* (1998; 2001) have shown, that the presence of the 192bp allele at locus *Xgwm261* is correlated with a decreased plant height (by 7-8 cm), and is neutral in its effects on other agronomic characters. The 174bp allele was neutral with respect to plant height, whilst the 165bp allele was correlated with an increase of 3-4 cm in plant height, again having no influence on other agronomic characters.

Materials and methods

Ninety-seven wheat varieties bred in the Ukraine between 1912 to the present were chosen. Seeds were obtained from the Institute of Plant Breeding and Genetics (PBGI; Odessa, Ukraine), the State Commission for Testing and Protection of Plant Varieties of the Ukraine and the gene-bank of the Institute for Plant Genetics and Crop Plant Research (Gatersleben,

Germany). For each variety, DNA was isolated from individual seeds as described in Plaschke *et al.* (1995). DNA was pooled from five seeds. The primers for the microsatellite locus *Xgwm261* are described in Korzun *et al.* (1998). The investigations were performed at the Institute for Plant Genetics and Crop Plant Research (IPK) in Gatersleben using an ALF and ALF-express sequencer as described by Röder *et al*. (1998). Randomly selected varieties were screened for the presence of GA insensitive dwarfing genes according to Börner (1991).

Results and discussion

In our investigation, it was shown that wheat varieties grown in the different agro-climatical regions of the Ukraine from 1912 until 1959 mostly carried the allele 174 bp at locus *Xgwm261*. The 165 bp allele was found in varieties originating from local populations in the 20s-30s. The 214 bp allele occurred in old varieties of the South. This allele does not appear in wheat varieties from Europe (Röder et al. 2002) but occurs in Argentinean wheat varieties (Manifesto, personal communication). From the 60s when the variety Bezostaya I was used extensively in breeding, the 192 bp allele appeared in high frequency. Almost 98% of modern wheat varieties of the southern region bred at the Plant Breeding and Genetic Institute (Odessa), carry the 192 bp allele at locus *Xgwm261*. We found a high frequency of this allele also in wheat varieties from Donetzko-Dneprovskiy and the southwestern regions. A number of varieties were heterogeneous at locus *Xgwm261* carrying both the 174 bp and 192 bp alleles. Comparing the allelic frequencies of *Xgwm261* in the Ukrainian (0.603; n=97) and European (0.250; n=500) gene pools (Röder unpublished data) revealed significant differences ($P<0.001$). In addition to the Ukraine, higher frequencies of the 192 bp allele were detected in sub-sets of the varieties from Greece (0.621; n=29), Italy (0.656; n=34), Yugoslavia (0.736; n=50) and Bulgaria (0.962; n=26) compared to the European average (Röder et al, unpublished data). From the middle of the 70s, beside *Rht8*, the GA insensitive Norin 10 dwarfing genes were used for plant height reduction, very often in combination with the former.

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Use of intervarietal chromosome substitution and tetrasomic lines for the investigation of the response to shading in *Triticum aestivum* L.

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The genetic control of flowering time in plants has received much attention. Thus, 80 loci that affect flowering time have been identified in the model plant *Arabidopsis* (Levy, Dean 1998). For agriculturally valuable crops like common wheat (*T. aestivum* L.), many investigations have been focused on photoperiod response (*Ppd*) genes determining the response of the plant to photoperiod (Worland, 1996), while variability and inheritance of the light intensity response in wheat, as far as we are aware, have only been little studied. However, light intensity may be a more variable parameter of natural light than day length in all ecoclimatic areas because of meteorological factors such as cloudiness and atmospheric transparency. The incoming radiation also varies within a plant canopy (Morelli, Ruberti 2000). In response to a decrease in the amount of light reaching plants, growth elongation is stimulated by means of internode extension, flowering is retarded in sensitive genotypes and, as a result, maturation is delayed and as a consequence sown crops often lodge (Evtushenko 1998). We have demonstrated that there is genetic variability in the response to light intensity in common wheat cultivars (Evtoushenko, Chekurov 2000). Here, we present our results of assessing homoeologous groups of chromosomes controlling the response to shading in common wheat.

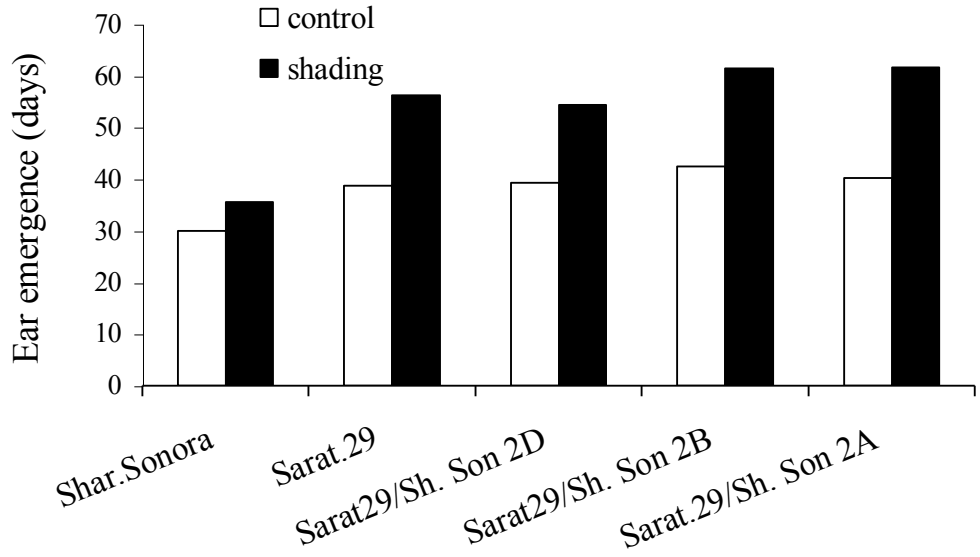

Figure 1. Mean days to ear emergence of the wheat intervarietal substitution lines and varieties under different light regimes

The plants used were Sharbati Sonora (weakly responding to shading), Saratovskaya 29 (strongly responding to shading), and substitution lines Saratovskaya 29/Sh. Sonora 2A(*Ppd3)*, Saratovskaya 29/Sh. Sonora 2B(*Ppd2)*, Saratovskaya 29/Sh. Sonora 2D(*Ppd1)*. They were grown under natural illumination supplemented with artificial light to extend day length (control) and under shading (with illumination about 1500 lux) in the greenhouse. Day length was about 19-21 hours. Experiments were repeated three times. The means for ear emergence time in the varieties and substitution lines are given in Figure 1. The flowering

time under shading for the substitution lines was consistently longer than in Sharbati Sonora. The differences in days to ear emergence between the substitution lines and Saratovskaya 29 were insignificant (Fig.1).

This suggests that genes different to *Ppd* control the response to shading. It should be noted that Sharbati Sonora behaves as a typical shade tolerant genotype: its rapid transition to flowering is associated with a decrease in yield. Saratovskaya 29 and substitution lines are more sensitive to shading, they showed a smaller reduction in the number of spikelets and grains of the main ear under shading.

We further studied the tetrasomic lines derived from Chinese Spring 1A, 3A, 3D, 4A, 4B, 4D, 6B, 6D, 5D and 7B. It is known that these chromosomes influence ear emergence time in wheat (Worland 1996). We propose that some of them are involved in the shading response. Twenty grains of each tetrasomic line and euploid control were grown in two replicates under conditions of natural low illumination (during November - February) and long daylength (to 21 h) in the greenhouse under additional shading to 1500 lux. The data for ear emergence time for the unvernalized lines and euploid Chinese Spring (CS) are given in Table 1.

Table 1. Mean days to ear emergence under shading of the tetrasomic lines of Chinese Spring. Significant difference from "Chinese Spring" are indicated

Lines	Days to ear emergence (± s)
Chinese Spring (CS)	66.0 ± 1.3
CS tetra 1A	73.7 ± 1.1**
CS tetra 3A	67.3 ± 1.5
CS tetra 3D	68.5 ± 1.2
CS tetra 4A	67.9 ± 1.5
CS tetra 4B	62.8 ± 1.3*
CS tetra 4D	67.3 ± 1.5
CS tetra 5D	51.6 ± 3.8**
CS tetra 6B	77.7 ± 4.6**
CS tetra 6D	81.7 ± 6.5**
CS tetra 7B	63.3 ± 1.5*

* P 0.05-0.01; ** P 0.01-0.001

Doubling of chromosome dosage produced earlier ear emergence under shading in the tetrasomics 4B, 5D and 7B. It is known that genes on 4B affect earliness *per se* (Law 1998). It is quite possible that low sensitivity of genotypes to shading is one of the reasons of earliness *per se*. The double dosage of 5D produced a pronounced effect. This effect was possibly due to the double dosage of the *Vrn3* allele. However, this effect was also observed following vernalization (39.3 in 5D vs. 47.8 in euploid, $p<0.001$). Converse behaviour was observed in the tetrasomics of chromosomes 1A, 6B and 6D. In these lines, flowering time was significantly delayed compared to the euploid under shading.

These results may be helpful in clarifying the role particular chromosomes have in controlling flowering time in wheat and sensing different aspects of light in the environment.

The authors are grateful to Dr. L.I. Laikova, Institute of Cytology and Genetics, Novosibirsk, for providing the seed samples.

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Characterization of orthologous genes in cereals using sequence analysis

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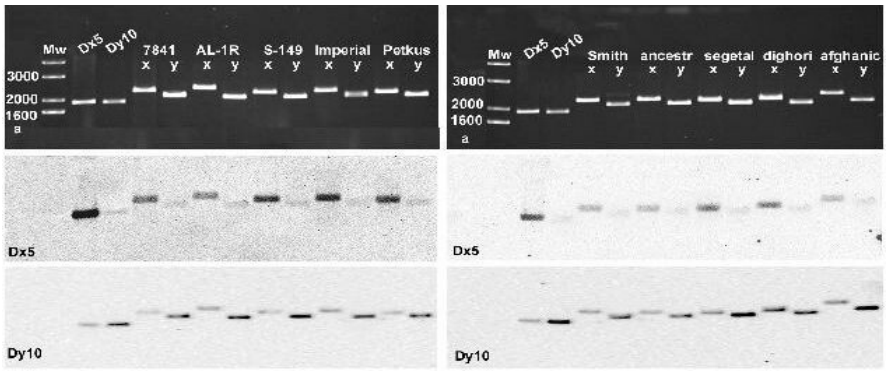
This paper reports the use of primers designed from nucleotide sequences of the *Glu-D1* gene of wheat (AS-PCR for *Glu-D1y10*) that recognise and amplify homologous sequences of the *Glu-R1* gene subunits of cultivated rye, *Secale cereale,* related wild subsp. *ancestrale*, *dighoricum*, *segetale* and *afghanicum*, and *Aegilops speltoides*. Primers directed to amplify *GluD1-y10* allele also amplified products of different size in wheat lines carrying substitutions of chromosome 1R(1D), in rye cultivars and in related wild species. The use of PCR analysis with primers specific not only for particular alleles of a common gene, but also for closely related genes, represents a new tool useful in finding orthologous genes in closely related species. The following primers:L: 5'CAACCAATCTCCACAATC3'R: 5'CTGCAGAGAGTTCTATCA3' were designed and used to amplify the orthologous *Glu1* gene subunits. Cloning and sequencing of PCR products were carried out as described previously (De Bustos et al. 2001). Sequences alignment was performed using Clustal W 1.5 program (Thompson et al. 1994). Phylip program (version 3.5) (Felsenstein 1993) was used to build phylogenetic trees.

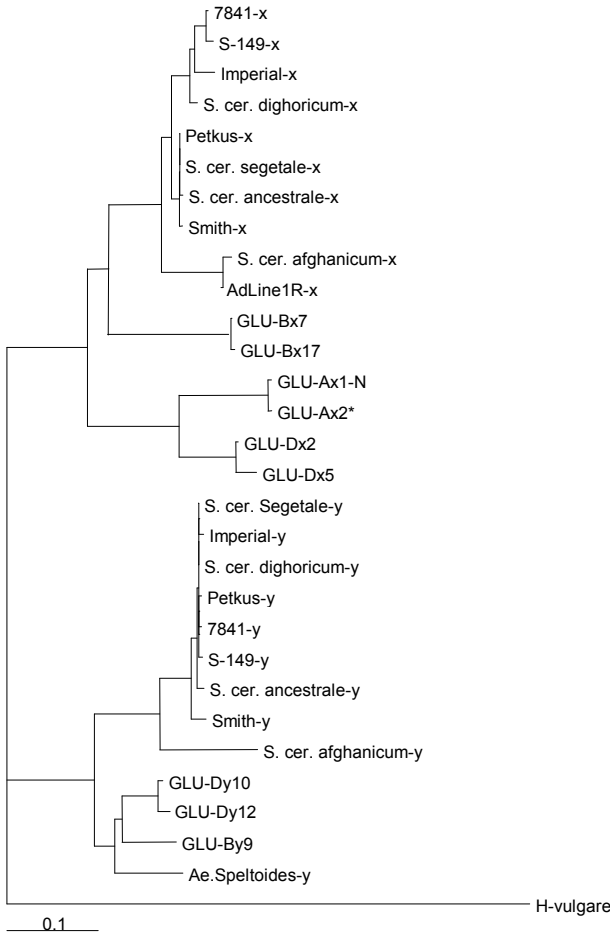
A total of 21 new *Glu1* sequences have been deposited in the EMBL GenBank and DDBJ under accession numbers AJ314767 to AJ314785, AF513640, AF216868 and AF216869.



Relationships of these sequences with x and y-type wheat genes were analysed by Southern experiments (Fig 1). Clones of PCR products were digested using *Nsi*I and *Stu*I. The

recognition site of these restriction enzymes is highly conserved between glutenin genes, flanking the coding region. After digestion, the fragments were separated on agarose gels (a), blotted onto a membrane and finally *Nsi*I and *Stu*I. The recognition site of these restriction enzymes is highly conserved between glutenin genes, flanking the coding region. After digestion, the fragments were separated on agarose gels (a), blotted onto a membrane and finally hybridised using Glu-Dx5 and Glu-Dy10 alleles as probes. Strong signals were obtained in upper bands when hybridised using the Dx5 probe, whereas the Dy10 probe hybridised preferentially with smaller fragments, relating, as expected, to the bigger fragments with x-type glutenins and the smaller fragments with y-type genes.

The coding sequences of x-type genes ranged from 2435 to 2229 bp. For y-type genes, the size of coding sequence ranged from 2211 to 2136 bp. These values are slightly different from those of the wheat glutenin genes. Thereby, x-type rye genes are smaller than wheat genes and y-type rye genes are bigger than wheat y-type. All of them lack introns and show the typical glutenin structure containing the N and C terminal domains and the central repetitive region. The N-terminal region domain starts after the 21-residue signal peptide, almost identical in all glutenins (De Bustos et al. 2001). This region is composed by 86 and 104 amino acids in the x and y-type respectively. However, the C-terminal domain has 42 residues and finishes with the two typical glutenin stop codons in both gene types. The central repetitive region is composed of the basic repeat motifs early described in glutenin genes (Halford et al. 1987). The number and position of cysteines found in rye x-type are identical to those the wheat x-type with the exceptions of an extra cysteine residue found at N-terminal region of subunit present in *S. cereale* ssp *afghanicum*, next to the first cysteine residue. A possible way to test the relation of these proteins with quality would be their use in biotechnological programs, introducing these genes into wheat and analysing the changes in dough quality. Alignment of the sequences characterised with those of the glutenin genes was used to build a phylogenetic tree (Fig 2). As can be observed, orthologous genes from loci Glu A, B, D and R are more closely related than the paralogous genes (x and y-types). This supports the idea that both type of gene were duplicated before the speciation process of Triticeae. This is also supported by a recent work of Anderson *et al*. (2002) for the flanking regions of the HMW glutenin genes.



Acknowledgements

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Winter wheat DH lines with improved intergeneric crossability

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Introduction

Despite all the progress made in biotechnology, molecular biology and DNA technology, culminating in the marketing of transgenic plants, the need for alien gene introgression into cultivated plants by wide hybridisation remains an important task in plant breeding. Success in alien gene transfer depends greatly upon the degree of cross compatibility between cultivated genotypes and the donor species of related genera.

In common wheat (*Triticum aestivum* L.) crossability is genetically controlled by homoeologous genes located on the long arms of the homoeologous group 5 chromosomes. The dominant *Kr* alleles are widely spread in most modern winter wheat cultivars. Recessive *kr* alleles were found in some old spring cultivars and landraces from China, Japan, Iran and Eastern Siberia (Zeven, 1987). However, these primitive wheats are not suitable for alien gene introgression and breeding purposes because of their low agronomic value. For this reason, the transfer of recessive alleles into winter wheat cultivars has been the subject of several research programmes. Molnar–Lang *et al.* (1996) reported the transfer by backcrossing of the recessive *kr1* allele from the spring wheat variety Chinese Spring into the winter wheat Martonvasari 9. Gay and Bernard (1994) improved the crossability of the cultivar Courtot by substituting chromosomes of homoeologous group 5 with corresponding homologous from the highly crossable Japanese cultivars Norin 29 and Fukuhokomugi. Chromosome engineering methods of substitution were also used to replace chromosome 5B of the cultivars Favorit and Bezostaya 1 with its homologue from Chinese Spring carrying *kr1* (Giura, 2001).

Intergenotypic transfers of recessive alleles, either by individual chromosome substitution or by backcrossing are time consuming so that the recipient variety is often out dated and surpassed from the agronomic point of view by other new genotypes. Searching for more crossable genotypes in advanced breeding lines or new varieties is a possible way of overcoming this weakness.

The winter breeding line F 132 developed at RICIC Fundulea showed a good but variable crossing compatibility with rye cultivars. It was supposed that this variation in crossability might be determined by the presence of different alleles at the *Kr* loci. To test this hypothesis, F 132 was crossed with maize to produce dihaploids. More than 100 dihaploid (DH) lines were obtained by this method.

The paper presents the results of cross compatibility tests between 24 DH lines derived from F 132 and *Secale cereale* cv. Harkovskaia.

Material and methods

Wheat dihaploid lines used in this study were developed using the *Zea* system for developing wheat haploids and dihaploids described elsewhere (Giura, 1993). The crossability with rye of 24 DH lines was tested under field condition at Fundulea in 1996 and further analyses were carried out in 1999 using seven DH lines chosen for their high crossability in the previous test. Five spikes of individual DH lines were emasculated and pollinated with *Secale cereale*, cv. Harkovskaia.

Hybrid condition of the resulting seed was checked cytologically by counting the chromosome number in root tips, using the Feulgen staining technique. The plantlets with undetermined chromosome number were transferred to pots and analyzed for spike morphology at heading time. The crossing compatibility was estimated as seed set (number of hybrid seed/100 pollinated flowers) for each spike. Data were analyzed by the means of ANOVA.

Results and discussion

By developing DH lines from the F 132 population, it was possible to make a strict separation of constituent biotypes and to identify in testcrosses with rye those biotypes (DH lines) carrying the homozygous recessive *kr* allele(s).

The F test showed significant differences ($P \leq 0.01$) among the DH lines with regard to mean seed set. However, the dispersion of seed set values, ranging from 5.1% to 29.2%, in the DH lines was unexpected and made it difficult to clearly predict if either the *kr1* or *kr2* allele was present.

According to Lein's (1943) classification of the relationship between crossability genes, a crossability lower than 5% denotes the presence of both dominant alleles *Kr1* and *Kr2*. A seed set from 10 to 30% could be the result of the promoting activities of the recessive *kr2* allele, whereas sets between 30 and 50% might be the result of the activities of the recessive *kr1* allele. Values over 50% would represent the expression of the combined activities of both *kr1* and *kr2*.

On these assumptions, the DH lines tested up to now could be divided into three groups:

- a group of 7 DH lines, having a low seed set (5.1-7.7%),and therefore carrying the dominant alleles at the *Kr* loci;

- a group of 9 DH lines with seed set from 9.0 to 14.7% and therefore carrying the recessive *kr2* allele (crossing ability of this group was to some extent diminished probably due to environmental factors, and not to any accidental heterozygosity in these DH lines);

- a group of 8 DH lines with good crossing ability ranging from 25.5 to 29.2% carrying *kr2* allele. However, in another test carried out in 1999, seven of these crossable DH lines showed a relatively higher seed set (25.4-35.4). According to Lein's classification, a genotype having a seed set over 30% may carry the *kr1* allele. From this point of view, the ongoing genetic analyses using wheat monosomics 5A and 5B will offer conclusive evidence. The source of *kr2* in our material is assumed to be a variety of Chinese origin, Pekin-8, found in the ancestry of the F 132 breeding line and included in the derived DH lines.

From a practical point of view, the availability of complete homozygous crossable DH lines of wheat having very good agronomic performance would enable a more effective way for making intergeneric transfers of useful genes from related species into wheat.

Conclusions

Development of DH lines from a wheat population heterogeneous at the *kr* loci identified lines carrying the recessive allele *kr2* in the homozygous condition by testcrossing with rye. The availability of crossable DH lines in a modern wheat genotype would provide a more effective way for intergeneric transfers of useful alien genes into the wheat genome.

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Fine mapping and origin of a gene for non specific adult plant resistance against stripe rust (*Puccinia striiformis*) in wheat

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Introduction

In general, both specific and non-specific resistance genes may cause plant disease resistance against airborne pathogens of wheat. Specific resistance genes provide an almost complete protection against the disease, albeit of a transient nature, and are expressed usually at all stages of plant development (overall resistance). They are assumed to follow the gene-for-gene relationship (Flor, 1959). 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. Although it was generally expected that non-specific adult plant resistance is inherited quantitatively, 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'. The same mapping population was used to add new wheat microsatellite (WMS) markers known to be located in the region of *Yrns-B1*, in order to saturate the map. In addition, markers expected to be diagnostic for the resistance gene were used to screen 25 appropriate ancestors of 'Lgst. 79-74' and 54 Russian (Siberian) wheat varieties.

Materials and Methods

For fine mapping, an already existing mapping population consisting of 160 F_2 plants that originated from the cross between 'Lgst. 79-74' (resistant) and 'Winzi' (susceptible) was used. The disease scores were performed in F_3 as described by Börner *et al.* (2000). Markers closely linked to *Yrns-B1* were tested in order to find out, whether they may be used as diagnostic ones. Twenty-five old, mainly German varieties, which may contain ancestors of 'Lgst. 79-74', were analysed. Furthermore, 54 Siberian wheat varieties used for diversity studies (Khlestkina *et al*. unpublished) were included. Procedures of WMS analysis and WMS markers are described in Röder et al. (1998). Seven new WMS provided by the company 'TRAIT GENETICS', Gatersleben and known to be located on chromosome 3B were tested for polymorphism between 'Winzi' and 'Lgst. 79-74'. Polymorphic markers were mapped applying the MAPMAKER 2.0 computer programme (Lander et al. 1987) and the QGENE application (Nelson 1997) for the WMS markers and the disease resistance, respectively.

Results and Discussion

Molecular fine mapping of Yrns-B1

For saturating the region of *Yrns-B1* with new molecular markers, the seven WMS kindly provided by the company 'TRAIT GENETICS', Gatersleben were used. Only two of them were shown to be polymorphic between the parents of the mapping population 'Winzi' and 'Lgst. 79-74'. These two markers, *Xgwm3087* and *Xgwm1329*, were used to analyse the whole mapping population. As shown in Fig. 1 both were successfully incorporated into the previous map (Börner *et al.* 2001) by applying the 'MAPMAKER 2.0' programme. The adult plant stripe rust resistance locus was mapped as a QTL. RSq and LOD values calculated for the 3BS chromosome microsatellite markers by the 'Q-GENE' programme are given in table 1. Although all 9 markers have very high LOD score values (> 8.00), one of the two new markers of this map, *Xgwm1329*, was found to have the highest LOD score (20.76). This clearly indicates that *Xgwm1329* is closely linked to *Yrns-B1* (Fig. 1).

Table 1: RSq and LOD values calculated by the Q-GENE programme

Marker	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

Identification and utilisation of diagnostic markers for Yrns-B1

The most closely linked marker *Xgwm1329* was used to screen a collection of wheat varieties (25 lines) which may include ancestors of the resistant parent 'Lgst. 79-74'. From the results obtained this WMS locus seems to be highly polymorphic. This may be the reason, why the 152 bp fragment, characteristic for 'Lgst. 79-74', was not found in any of the lines tested. However, using another linked marker, *Xgwm533*, known from previous studies (Röder unpubl.) to be accessible as diagnostic one, we detected the 117 bp fragment, characteristic for 'Lgst. 79-74', in several varieties (Fig. 2). So far only six of them were tested for adult plant disease resistance. All six, carrying the 117 bp fragment, were found to be resistant in the field.

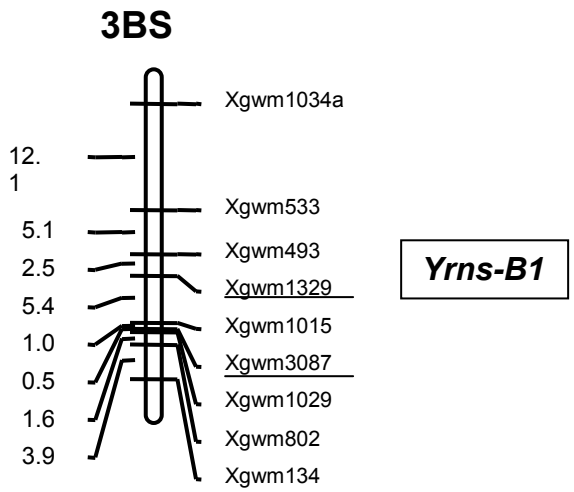

Fig. 1: Molecular marker map constructed by using MAPMAKER 2.0. Underlined markers were newly integrated. The target gene *Yrns-B1* was tagged as QTL by using QGENE.

Screening the 54 Russian spring wheat varieties grown in Siberia with the marker *Xgwm533*, nineteen (about 35 %) were detected carrying the 117 bp allele characteristic for 'Lgst. 79-74'. Most of the Siberian varieties screened with *Xgwm533* are now under field investigation to verify the adult plant disease resistance against stripe rust.

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Possibilities of utilizing GA insensitive dwarfing genes in wheat breeding in Poland based on co-operative research with Tony Worland

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Wheat yields and the area sown to this crop have increased worldwide during the past fifty years. This has been possible due to the introduction of varieties having dwarfing genes originating from 'Norin 10' (Gale and Youssefian, 1985). The dwarfing genes, *Rht1* and *Rht2*, are localized on chromosomes 4BS and 4DS, respectively. They cause a yield

increase of about 20%, mainly through increases in spikelet fertility. Both are insensitive to the application of exogenous gibberellic acid (Gale and Youssefian, 1985; Worland, 1987). The two alleles have also a similar influence (about 20%) on height reduction. Their action can be modified both by the genetic background and by environmental conditions (Gale and Youssefian, 1985; Kertesz *et al.,* 1991; Bőrner *et al*., 1993; Miazga *et al.,* 1993).

Rht3 is also present on chromosome 4B (Morris *et al.,* 1972). Jha and Singh (1977) found that this gene is partially dominant. It reduces shoot height by about 50% (Flintham and Gale, 1983; Gale and Youssefian, 1985). The real source of the gene is not unequivocally determined yet. It is believed to have originated from the variety 'Tom Thumb' or 'Minister Dwarf'. Initially, it was thought that *Rht3* was independent from *Rht1*. At present, scientists accept that both are alleles of the same locus placed on chromosome 4B (Gale and Marshall, 1976). McVittie *et al*. (1978) considers the genes *Rht1* and *Rht3* as homoeoallelic with the *Rht* locus on chromosome 4D.

Worland (1987) found that substitution lines having chromosomes containing dwarfing genes insensitive to gibberellins showed a reduced fertility under greenhouse conditions. It was particularly clear in the case of the substitution line 4B containing *Rht3*. However, a clear increase in fertility was recorded in the field. Moreover, isogenic lines of *Rht1*, *Rht2* and *Rht3* in the varieties 'Bersee' and 'Maris Huntsman' were investigated under controlled environment conditions. The author demonstrated that lines containing GA insensitive dwarfing genes were sensitive towards high temperatures, particularly during the period between the appearance of the flag leaf to heading. It was concluded that the utilization of *Rht1*, *Rht2* and *Rht3* may not be so advantageous in areas where high temperatures occur during critical stages of plant development.

In order to evaluate precisely the influence of *Rht* genes on yield and its components, isogenic lines of the varieties 'April Bearded', 'Bersee', 'Maris Huntsman', 'Maris Widgeon', 'Mercia' and 'Bezostaya' were developed at the PBI, Cambridge and John Innes Centre, Norwich (Gale and Youssefian, 1983; 1985). These lines were tested in several European countries: England (Gale and Youssefian, 1983; Worland, 1987), Yugoslavia (Worland *et al.,* 1990), Hungary (Kertesz *et al.,* 1991) and Germany (Worland *et al*., 1991; Bőrner *et al.,* 1993).

We received isogenic lines of 'Maris Huntsman', 'Maris Widgeon', 'Bezostaya' and 'Mercia' carrying different dwarfing genes from Norwich at the beginning of the 90's. The research was carried out within the 'European Program of Climatic Adaptation of Dwarfing Genes in Wheat' that was coordinated by A. J. Worland. Studies aimed to evaluate the pleiotropic effects of *Rht* alleles on plant height as well as yield and its components and, moreover, to study the climatic adaptation of these genes and the possibilities of using them in Poland (Miazga *et al.,* 1993; 1995a; 1995b).

Kowalczyk *et al.* (1997b) has studied isogenic lines of 'Maris Huntsman' and 'Maris Widgeon' containing the genes *Rht1, Rht2, Rht3, Rht1+2* and *Rht2+3* as well as their controls (*rht*) for three years. They found that the dwarfing genes significantly reduced plant height and showed pleiotropic effects on some yield components. In all the experimental years, they observed decreased 1000-kernel weight. The lowest 1000-kernel weight was recorded in the shortest lines carrying the genes *Rht2+3* and *Rht3*. They found that the genes *Rht1* and *Rht2* increased kernel number per spike and the fertility of the first and second flowers, particularly in the latter two years of the study. In the first year, due to high temperatures during the stage from the beginning of flag leaf appearance to heading, values of these traits were slightly lower compared to the control. Kowalczyk *et al*. (1997b) and Miazga *et al.* (1993) proved that these genes are not only dependant on background genotype, but also on the environmental conditions. Kowalczyk *et al.* (1999b)

has analyzed yield and protein content in isogenic lines of 'Maris Huntsman' and 'Maris Widgeon' containing *rht* (control), *Rht1, Rht2, Rht3, Rht1+2* and *Rht2+3* for three years. They found that the lines carrying *Rht1* and *Rht2* had similar protein percentages in the grain. The shortest lines having strongly wrinkled kernels had the highest protein contents. Yield and protein percentage in grain was dependant on line and experimental year.

Many authors have analyzed the influence of the dwarfing genes *Rht1, Rht2* and *Rht3* on the protein content and yield in common wheat grain. Pepe and Heiner (1975a; 1975b) showed that the genes did not affect either yield or percentage grain protein in F_5 lines. Similarly, Busch and Chamberlain (1981), as well as Feingold *et al*. (1990), found that lower percentage of protein in grain was not strictly associated with dwarfness. However, Gale (1979) studied two series of hybrids originating from crossbreeding between semi-dwarf and tall lines. He revealed that semi-dwarf lines had lower percentage of protein in the grain than the talls.

Gene *Rht1S* is also often used in wheat breeding. It originates from the Japanese variety 'Saitama 27'. This gene is localized on chromosome 4BS and is weakly insensitive to exogenous gibberellic acid. It causes a shortening of stems by about 10% and positively affects grain setting (Worland, 1987; Worland and Petrović, 1988; Bőrner, *et al.,* 1995). The gene is not sensitive to high temperatures at critical stages of growth and, therefore, it occurs in varieties originating in the Mediterranean Sea basin. It is also present, in the Polish variety 'Alfa', along with the gene *Rht8*. Moreover, it has been introduced into Hungarian and Romanian wheats (Worland, 1986). Miazga *et al*. (1997a; 1997b) studied nearly isogenic lines of the two varieties, 'Bezostaya' and 'Mercia', containing *Rht1S*. They evaluated the pleiotropic effects of the gene on plant height as well as yield and its components under the climatic conditions of eastern Poland. It was found that both isogenic lines were significantly shorter compared to the control and had lower 1000-kernel weights. Number of kernels per spike of *Rht1S* lines was similar to that of the controls. Moreover, the short lines had generally higher spikelet and higher fertilities in the first and second florets.

Worland (1986) states that the Hungarian wheat 'Mv 13' contains another dwarfing gene, strongly insensitive to gibberellic acid. Reaction of its seedlings to exogenous GA_3 was similar to that of *Rht3* and the 'Bezostaya Dwarf Mutant' rather than *Rht1*. This gene is often called *Rht 'Krasznodari 1'*, '*Rht1K'* (Bőrner *et al*., 1995) or *Rht1* Bezostaya dwarf (Worland et al., 1995). *Rht1K* arose as a natural mutation in the variety 'Bezostaya 1' and it is localized on chromosome 4BS. This gene is partially dominant and causes a reduction of stem height by about 25% with decreasing grain weight by about 10%. Moreover, it positively affects kernel setting (Worland, 1986; Bőrner *et al*., 1995; Worland *et al*., 1995).

Miazga and Kowalczyk (1996) as well as Miazga *et al.* (1997b) have studied nearly isogenic lines of two varieties 'Bezostaya' and 'Mercia' containing the genes *Rht1K* and their controls (*rht*) for three years. They have shown significant influences of the gene on yield components. Compared to the controls, the isogenic lines carrying *Rht1K* were significantly shorter and had lower 1000-kernel weights. Number of kernels per spike and spikelet fertility depended on the year and variety, and the values were similar or higher than in the controls.

The dominant dwarfing gene *Rht10*, insensitive to gibberellic acid, is localized on the short arm of chromosome 4D (Izumi *et al*., 1983; Gale and Youssefian, 1985; Bőrner *et al*., 1987). It originates from 'Ai-bian 1' variety (Izumi *et al*., 1983). Gale and Youssefian (1985) state that the gene *Rht10* is not allelic to *Rht2* also located on chromosome 4D. Bőrner and Mettin (1988) studied insensitivity to gibberellic acid in 'Ai-bian 1' and its

hybrids with 'Poros' and 'Fakon' varieties, and analyzed the segregation in the F_2 generation. They suggest that *Rht10* is allelic to *Rht2*.

Miazga *et al*. (1995a) studied nearly isogenic lines of 'Mercia' containing *Rht10*. They established that *Rht10* caused shortening of the stem by about 30%. Gale and Youssefian (1985) and Bőrner *et al.* (1987) found that height reduction caused by *Rht10* is larger than that caused by *Rht3* amounting to more than 50%. Miazga *et al.* (1995a) states that number of kernels in the spike and first and second florets, as well as spikelet and first and second floret fertilities in lines carrying *Rht10* were similar to the control. In the first year the authors found significantly lower 1000-kernel weights in the dwarf lines.

Kowalczyk *et al*. (1997a; 1999a) and Miazga *et al*. (1998) performed gibberellic acid tests on Polish varieties and breeding lines of common wheat involved in experiments of COBORU in 1992-1995 as well as the isogenic lines of 'Maris Widgeon' *Rht1* and 'Maris Widgeon' *Rht2*. Coleoptile length and seedling height was measured. Among the varieties and breeding lines, only four lines ('Elena', 'Parada', SMH 1693 and STH 594) as well as five varieties ('Broma', 'Henika', 'Polna', 'Santa' and 'Sigma') was the presence of dwarfing genes insensitive to gibberellic acid found. The response was similar to the isogenic lines of 'Maris Widgeon' containing *Rht1* or *Rht2*.

In order to find out which GA insensitive dwarfing genes occur in the Polish varieties 'Elena' and 'Parada', Kowalczyk (1997a) analysed F_2 hybrids of these varieties from crosses with the isogenic lines of 'Maris Widgeon' *Rht1* and 'Maris Widgeon' *Rht2* as well as 'Maris Widgeon' *rht*. It was found that *Rht2* were present in both the varieties studied.

Due to the small number of short stemmed varieties presently available, and the possibility to adapt *Rht* genes to Polish conditions, the genes *Rht1* and *Rht2* are being introduced into the varieties 'Almari', 'Kamila', 'Oda', 'Rada' and 'Rosa' at the Institute of Plant Genetics and Breeding, University of Agriculture, Lublin. The final aim is to achieve a series of isogenic lines in these varieties(Kowalczyk, 1997b).

Studies performed at the Institute of Plant Genetics and Breeding, University of Agriculture, Lublin within the 'European Program of Climatic Adaptation of Dwarfing Genes at Wheat' revealed that there is a possibility to utilize dwarfing genes, insensitive to gibberellic acid, under Polish conditions. Genes *Rht1*, *Rht2, Rht1S* and *Rht1K* may be recommended for use in breeding programmes due to their good adaptation and positive influence on yield and its components. Reduction of 1000-kernel weight caused by these genes is fully compensated for by higher spikelet fertility, which in consequence, results in greater seed setting in spikes.

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Use of ditelosomic lines of the common wheat varieties Chinese Spring, Saratovskaya 29 and Diamant in genetic studies

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Common wheat ditelosomic lines are widely used in genetic studies for mapping chromosome arms carrying genes for traits involved in plant adaptation. Three series of ditelosomic lines (DT) were obtained, using Chinese Spring as a base (Sears and Sears 1978), in three varieties, Giza 144 (Youssef 1979), Saratovskaya 29 and Diamant (Laikova et al. 1983). Ditelosomic lines for either the short or long arm allow the determination of not only the chromosome arm carrying genes of interest but also their location on the genetic map relative to the centromere and their linkage with other genes. The first molecular-genetic maps were constructed with the use of nullitetrasomic (Nt) and DT lines of Chinese Spring (CS), which are perfect tools for localization of molecular markers on chromosomes in the absence of polymorphisms (Devos et al. 1999).

Ditelosomic lines of Saratovskaya 29 (S29) and Diamant (Dm) were used for constructing monotelosomic lines (Mt, 2n=40+t). The latter were used for constructing substitution lines S29/Janetzkis Probat, Diamant/Novosibirskaya 67, etc. (Maystrenko et al. 1986, 1993). Use of cytologically marked chromosomes helps in constructing lines with inter-varietal chromosome substitutions and eliminates the possibility of univalent shift. Series of DT-lines of S29 and Dm were used to investigate the genetics of mineral nutrition in common wheat. Gamzikova (Gamzikova 1994) identified chromosomes whose genes directly or indirectly affect the uptake and distribution of phosphorus and potassium. It was shown that the response to iron shortage is determined by two genes: *Fe1* on chromosome 7DL of S29 and *Fe2* on 7BS of CS. Ditelosomic analysis allowed the location of the *Pa* gene (auricle pubescence) and the locus *Hl/hl* (dense felt-like leaf pubescence) on chromosome 4BS. Linkage between these two genes, *Hl* and *Pa*, located on the short arm of chromosome 4B, was estimated as 30 cM (Maystrenko 1992). This is only part of our genetic studies with the use of the three series of common wheat DT lines.

Quantitative traits affecting plant productivity (stem height, number of spikelets per ear, grain number, and weight per ear) have been studied in the three series of DT lines. Plants were grown in the field and in the greenhouse. Absence of the standard arm for any chromosome reduced significantly the expression of these characters. The greatest negative effects were noted in lines of homoeologous groups 2, 3, 4, and 6. A significant decrease in grain number per ear was observed in all the DT lines except those of group 5 (Fig. 1). Apparently, the regulation of productivity components depends upon the combined effect of the genotype and growing conditions. Khotyleva *et al*. (1984) obtained similar results in DT lines for the standard arms of Chinese Spring.

We have studied grain hardness, which influences the quality of wheat grain. The gene *Ha* is known to be located on chromosome 5DS (Law *et al.* 1978). Chinese Spring is a soft wheat carrying the dominant gene *Ha*, whereas S29 and Dm are hard-grained varieties. The mean diameter of flour particles (μk) measured with a special device was the main index of grain hardness. In all three series, this index in the 5DL lines was significantly higher than in the control (CS- 130%; S29-29%; Dm-36%). This confirms that the *Ha* gene (soft) is located on the short arm of 5D. The results were confirmed by the two-way variance analysis.

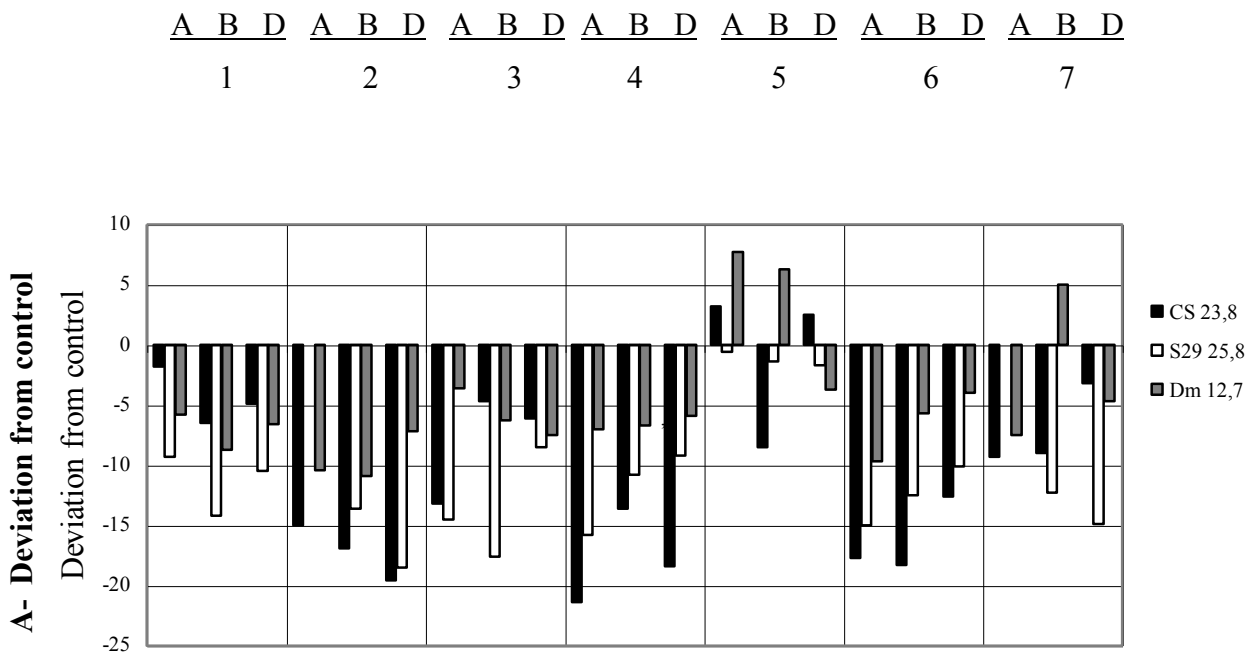

Fig. 1. Deviation of the values of grain number per ear in ditelosomic lines from those in cvs. Chinese Spring, Saratovskaya 29 and Diamant.

A number of quantitative traits have been investigated for the first time in the three DT series of contrasting varieties (Chinese Spring, Saratovskaya 29 and Diamant) under various growing conditions. Similar effects were demonstrated between the three series.

In addition to constant cytological monitoring for univalent shift and the prevention of outcrossing, it is necessary to be aware of possible chromosome aberrations in these experimental DT lines (Devos et al. 1999).

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Comparative analysis of QTLs affecting agronomical traits in rye and wheat

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The extensive conservation of genes both in their expression and position across the *Poaceae* affords the opportunity to study the correspondence between quantitative trait loci (QTLs) affecting common phenotypes in reproductively isolated taxa such as rye (*Secale cereale* L.) and wheat (*Triticum aestivum* L.). Mapping of QTLs for many plant species became possible as high-density linkage maps were developed using molecular markers. Recently, we have described such a genetic linkage map for rye (Korzun *et al*. 2001). However, only few examples of the location of QTLs in rye have been published (Börner et al. 1999, 2000). In the present study we describe the mapping of a number of QTLs in rye, determining several characters of agronomic and biological importance, and compare them with QTLs already mapped in the wheat genome.

Materials and methods

Mapping populations were produced by self-pollinating single plants of F_1 interlineal hybrids. These hybrids were created by diallel crossing the line N6 (cv. Steel), line N2 (cv. Monstrous ear) and line N7 (cv. Vyatka). The N6xN2, N7xN2 and N6xN7 F_{2-3} populations consisted of 90 F_3 lines. The lines were grown and scored for 17 quantitative traits under field conditions in St. Petersburg, Russia. DNA of the three mapping populations was extracted from fresh leaf material cut from seedlings. The procedures for DNA extraction, restriction digestion, gel electrophoresis, Southern transfer, probe labeling and filter hybridization were performed as described by Devos *et al.* (1992). A selection of 51 cDNA and genomic DNA probes from various rye, wheat, and barley libraries was used. The probes were known to be distributed over all seven *Triticeae* chromosomes. A multipoint linkage map was calculated using the MAPMAKER/EXP 3.0 computer programme and QTL mapping based on F_3 means was performed with MultiQTL 2.1.2.

The linkage maps of rye populations

For the three populations, genetic linkage maps were created using a total of 51 RFLP markers. The RFLP loci covering all seven chromosomes were detected by a set of rye, wheat, and barley cDNA and genomic DNA probes. The level of polymorphism was dependant on the source of the clones, with a ranking of rye > wheat > barley. Rye species-specific probes were found to be especially useful for mapping in rye. RFLP markers detected 64 (population N6xN2), 57 (population N7xN2) and 61 (population N7xN6) loci spanning 965.0, 931.1 and 1009.6 cM, respectively. Genetic linkage maps of individual chromosomes consisted of 6 to 11 markers with an average interval length between markers of 16.9 - 18.7 cM.

The highest density (10 - 11 loci) was observed for chromosomes 5R (N6xN2 and N7xN2) and 1R (N7xN6). Chromosomes 3R (N6xN2), 6R (N7xN2) and 7R (N7xN6) showed the lowest density (6) of mapped loci in rye. Regions with skewed segregation ratios were

detected near 12 RFLP markers. Chromosomes 3R and 6R had gaps due to low levels of polymorphism.

Mapping of QTLs in rye

The three parental lines were significantly ($P < 0.05$) different for all quantitative traits except for thousand grain weight. The lines N2 and N7 were most different (significantly different in all traits except for thousand-grain weight). The lines N6 and N7 differed in 14 traits, whilst the lines N6 and N2 were most similar (differing in 11 traits).

For each of 17 traits studied, QTLs were detected in at least one of the three populations. In the N7xN6, N7xN2 and N6xN2 mapping populations, 30, 45 and 26 loci, respectively, significant at $P < 0.05$, were revealed. The number of QTLs found for different traits in each population ranged from 0 to 5. On average for each trait, two loci were found. The greatest numbers of QTLs was found for the traits ear number, plant height and plot yield. The effects of QTL numbers were revealed in two and even three populations.

The percentage of phenotypic variation associated with single QTLs ranged from a minimum of 5.6% to a maximum of 65.2% for population N7xN2, from a minimum of 11.3% to a maximum of 62.5% for population N6xN2 and from a minimum of 7.0% to a maximum of 60.4% for population N7xN6. For each of 17 studied traits 2 - 9 QTLs were detected in the three rye populations.

Comparative mapping of rye and wheat

Detected QTLs for 6 traits (peduncle length, ear length, plant height, grain number, thousand grain weight, grain weight per ear) were compared with those found in the 'International Triticeae Mapping Initiative' wheat mapping population. This consisted of 114 recombinant inbred lines and had been scored for comparable agronomic traits in several environments during four years (Börner *et al.* 2002). Among the three genomic regions associated with QTLs for rye peduncle length (chromosomes 1R, 2R, and 3R), two were associated with peduncle length in wheat (2BL, 2DL, 3A). For the trait ear length the highest number of QTLs in the wheat population (31 loci) was detected, but only a few of these corresponded to the nine chromosome regions associated with ear length in rye (possibly QTLs on chromosomes 1B, 5AL, 5DL, and 6A). For the trait final plant height, many QTLs were detected in both the rye (7 loci) and the wheat (27 loci) populations. In rye, the most important QTL was found in the centromere region of chromosome 3R, which explained 15.4-43.2 % of the phenotypic variation. It may correspond to the 3A QTL in wheat. Additional comparable QTLs were detected on chromosomes 1AS and 5DL. Among the seven genomic regions associated with grain number in rye (chromosomes 2RS, 3RS, 4RS, 5RL, 6R, 6RL), two were associated with wheat grain number QTLs (chromosomes 7AL, 7DL). For the trait grain weight per ear, 2 QTLs were detected in rye (chromosomes 5RL and 6R) and 17 in wheat, however no corresponding genome regions were found. The inheritance of the character thousand grain weight is known to be complex. In the rye populations at least 9 QTLs were detected on chromosomes 3R, 4RL, 5RL, 6R and 7R. Seven genomic regions associated with thousand grain weight in wheat (chromosomes 3AL, 3BL, 5AL, 5DL, 6A, 6B, 7DL) may correspond to the rye QTLs.

The rye genome is considerably rearranged compared with that of wheat. At least eight rye chromosome arms have been involved in translocation events during evolution. In addition, the maps of rye and wheat have rather different marker sets. Further studies are therefore required to establish unequivocally the relationships between the QTLs detected in the two species.

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Pedigree analysis of wheat chromosome 2D

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The short arm of chromosome 2D of bread wheat has been shown in previous studies to carry genes which are important for determining the geographic adaptation of modern wheat varieties. The dwarfing gene *Rht8* and the photoperiodic insensitivity gene *Ppd-D1* are linked to each other and have been mapped to the short arm of chromosome 2D (Worland *et al*., 1998a). The availability of a microsatellite marker, *Xgwm261-2D,* tightly linked to the dwarfing gene *Rht8,* allowed the determination of the distribution of the different alleles of *Rht8* in international breeding programmes. At the same time, it is possible to follow the transmission and descent of *Rht8* from the initial crosses involving the Japanese variety 'Akakomugi', the source of the favoured allele of *Rht8* (Korzun *et al*. 1998). In the current study, microsatellite markers previously mapped on wheat chromosome 2D were used for a retrospective analysis of 59 wheat varieties with known pedigree (Worland *et al*. 1998a) to trace the transmission of the chromosomal region around the genes *Rht8* and *Ppd-D1* through many breeding programmes over time.

Thirty microsatellite loci previously mapped on chromosome 2D (Röder *et al.,* 1998, Pestsova *et al.,* 2000) were checked for polymorphisms between the parental varieties, 'Akakomugi', 'Wilhelmina' and 'Riete'. Twelve of them revealed polymorphisms (Fig. 1) and were used to analyse the 59 varieties. In total, 100 alleles were detected, the number of alleles per microsatellite locus varied from 3 to 12 and the polymorphic information content (PIC) values fluctuated from 0.59 to 0.87 (Table 1).

Eleven varieties out of 59 (18.6 %) were found to be heterogeneous at 1 to 6 microsatellite loci. The presence of two alleles at one locus is probably a result of DNA isolation from several seeds and reflects the presence of unfixed alleles in the varieties. In most cases, the differences between the two alleles in heterogeneous varieties consisted of more than 2 motifs, therefore microsatellite mutations were an unlikely explanation for such differences. We suppose that the main reason for microsatellite heterogeneity is residual heterozgosity due to incomplete inbreeding during variety development or outcrossing events during multiplication and cultivation.

Table 1. Number of alleles (number of unique alleles) and PIC values at different microsatellite loci on chromosome 2D among 59 varieties of bread wheat.

Microsatellite locus	Number of alleles (number of unique alleles)	PIC
Xgwm1099	12 (1)	0.83
Xgdm35	4	0.75
Xgwm702	8 (2)	0.74
Xgwm296	7 (1)	0.70
Xgwm261	3	0.59
Xgwm484	12 (6)	0.77
Xgwm102	6 (1)	0.72
Xgwm1204	12 (4)	0.85
Xgwm1264	6 (2)	0.64
Xgwm349	11 (2)	0.87
Xgwm311	12 (3)	0.83
Xgwm846	7 (1)	0.80

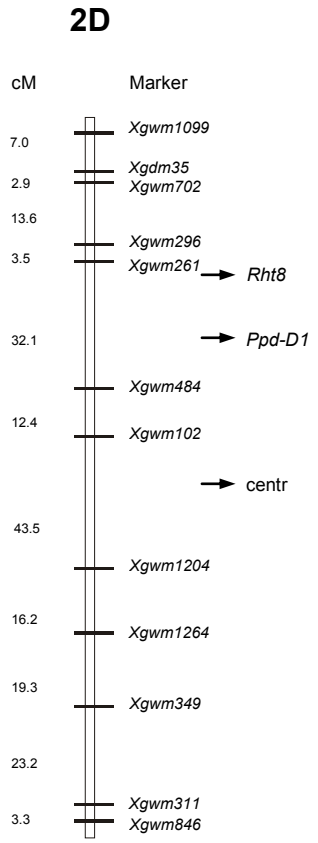

Fig. 1. Chromosomal location of polymorphic microsatellite markers and the genes on chromosome 2D. The short arm of the chromosome is at the top.

All the wheat varieties were divided into two groups based on the analysis of locus *Xgwm261-2D* which is diagnostic for the presence of the gene *Rht8*. The first group consisted of 32 varieties carrying the favoured allele of the gene and the second group included 27 varieties carrying other alleles. For each microsatellite locus, a screen was performed to find the alleles

corresponding to the parental variety ‘Akakomugi’. The comparison of frequencies of 'Akakomugi' alleles between the groups showed a higher frequency of 'Akakomugi' alleles at microsatellite loci neighbouring the gene *Rht8* in the first group, reflecting the fact that selection for the gene was accompanied by linkage drag of 'Akakomugi' alleles. Linkage disequilibrium of 'Akakomugi' alleles was observed for a segment of chromosome 2D which comprised at least 28 cM.

Worland *et al*. (1998b) found a selective advantage for the preservation of the linkage between *Ppd-D1* and *Rht8* in Southern European and the former Soviet Union breeding programmes. A linkage map of chromosome 2D constructed earlier put the photoperiodic response gene *Ppd-D1* 20.9 cM proximal to the gene *Rht8*. Therefore, the gene *Ppd-D1* should be located in the 32.1 cM gap between the loci *Xgwm261* and *Xgwm484.* Additional markers and further investigations will be necessary to confirm, at the molecular level, the preservation of the linkage between *Ppd-D1* and *Rht8* in the breeding programmes.

The comparison of the microsatellite data with known pedigree information allows the investigation of the inheritance of individual microsatellite alleles at different loci on chromosome 2D. We were able to study the transmittance of alleles from parents to progeny for 18 wheat varieties. It was found that 77 % of alleles of traceable alleles were inherited in accordance with the pedigree data whilst the other 23 % represented inconsistent alleles. Maximal number of inconsistent alleles occurred amongst the older varieties. Two possible explanations for the observed inconsistencies are varietal heterogeneity or instability of the microsatellites. As discussed above, a considerable level of heterogeneity exists in varieties. Therefore, the inconsistencies may in the first place be a result of heterogeneities amongst the parental lines used for breeding.

Pedigree analysis showed a diminution of the part of chromosome 2D originating from ‘Akakomugi’ during several generations of selection. Varieties of the early generations seem to carry the complete short arm of chromosome 2D of 'Akakomugi and the varieties of further selections possess only from 1 to 3 'Akakomugi' alleles on 2DS including the diagnostic allele at locus *Xgwm261-2D*.

These results show that microsatellites can be successfully used for studying the inheritance of individual loci as well as chromosomal segments within pedigree breeding programmes.

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The substitution lines for homoeologous group 5 chromosomes of wheat – possible relationship between vernalization requirement and frost tolerance

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The level of frost tolerance of winter cereals is not constant, but it changes during the year. Generally, three frost tolerance stages can be characterised (Gusta and Fowler 1979, Prášil and Zámečník 1991):

1. Hardening (acclimatisation) in autumn, when frost tolerance increases as a result of a gradual decrease in temperature.
2. Maintenance of frost tolerance, which is characterised by fluctuations of frost tolerance and is dependent on internal and external factors.
3. Dehardening - loss of frost tolerance due to the resumption of plant development later in the winter or early in the spring.

Determination of the genetic control of frost tolerance in wheat is difficult, because most probably different genes act at different stages (Roberts 1990, Veiz and Sutka 1989). In hexaploid wheat, at least 12 out of the 21 pairs of chromosomes are involved in frost tolerance control (Fowler and Limin 1997). The chromosomes of homoeologous group 5 have been found to exercise the largest effect on frost tolerance, and the location of the first frost tolerance genes, *Fr1* and *Fr2* , on chromosomes 5A and 5D, respectively, showed them to be closely linked to, but separable from, the vernalization genes (Sutka *et al*.1997). Three main loci involved in vernalization requirement, *Vrn-A1*, *Vrn-B1* a *Vrn-D1*, are located on chromosomes 5A, 5B and 5D, respectively. In spring wheats a dominant allele is present, whilst in winter wheats all three loci occur are recessive, i.e., *vrn*. Košner and Pánková (1998) showed that the different vernalization requirements of winter wheat cultivars are the result of multiple alleles at the *vrn* loci.

Vernalization requirement, which determines the length of the cold period needed by the plants, has been connected with frost tolerance. Recently it became evident that pleiotropic effects of the genes *Vrn* could participate particularly in the regulation of the period when the genes responsible for frost tolerance are active (Fowler *et al*. 1996, Sarhan *et al.*1997). On the other hand, Gusta *et al.* (1997) did not detect any influence of vernalization either on the level of frost tolerance obtained or on the maintenance of its level in wheat seedlings.

Our research is focused on the influence of homoeologous group 5 chromosomes of hexaploid wheat (*Triticum aestivum* L.) on induction, maintenance and loss of freezing tolerance with respect to the presence of genes governing these phenomenon and the probability that these genes are closely linked to the *Vrn* genes. To study this topic, we used two experimental approaches and two sets of wheat substitution lines for homoeologous group 5. In the first experimental approach, we measured the effects of winter frosts on winter survival of plants grown in pots, placed at different heights above the ground over winter (called the provocation method under natural conditions, Prášil and Rogalewicz, 1989). In the second type of experiment, we measured the dynamics of wheat frost tolerance under regulated conditions in growth chambers. Wheat plants were exposed to the laboratory frost test at different time of hardening and de-hardening to evaluate changes in LT50 (the lethal temperature) (Janáček and Prášil 1991).

The first set of substitution lines was created by substituting chromosomes 5A, 5B and 5D from the spring cultivars Zlatka, Chinese Spring and Česká Přesívka, respectively, into three different winter wheat backgrounds (Košutka, Vala and Zdar). This led to the expression of spring growth habit due to presence of the dominant *Vrn* alleles. The winter survival of the substitution lines was significantly lower to that of parental cultivars.

With the second set of substitution lines, we studied the relationship between vernalization requirement and frost tolerance in the reciprocal substitution lines between the two winter wheat cultivars (Mironovskaya and Bezostaya). The substitution lines for chromosomes 5A or 5D of Bezostaya, in the background of Mironovskaya, as well as the one, carrying chromosome 5B of Mironovskaya in the background of Bezostaya, showed a lower vernalization requirement and winter survival than the parental lines. The frost tolerance, following 8 or 12 weeks of hardening at 2^{o}C, was similar to that received after winter survival. However, following hardening for 2 weeks, high frost tolerance was found in all the lines.

From these first results we conclude that the vernalization genes present on the substituted chromosomes most probably affected the maintenance of frost tolerance, which influences the over-wintering (winter survival) of wheat lines. However, they did not affect the induction of frost tolerance at the beginning of cold hardening. It seems that two gene systems affect the dynamics of frost tolerance in wheat, and only one of them is related to the *Vrn* genes.

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Lipoxygenase activity in common wheat lines with substitutions for chromosomes 5A and 5D

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Lypoxigenase (Lpx, linoleate:oxygen oxidoreductase, EC 1.13.11.12) is known to catalyze *in vivo* the oxidation of unsaturated fatty acids resulting in the formation of peroxide and hydroperoxide compounds in plant cells (Siedow, 1991; Grechkin, 1998). It is usually represented by several molecular forms differing in enzymatic and biochemical properties. Three molecular forms of Lpx were found in wheat grains (Hsieh and McDonald, 1984; Didenko *et al*. 2001). Isoforms of Lpx differ in their influence on quality parameters of gluten (Frazier *et al.* 1977; McDonald, 1979; Shiba et al. 1991). The structural genes for wheat Lpx were shown to be localized on homoeologous groups 4 and 5 (Hart and Langstone, 1977; Li *et al.* 1999).

The physiological and biochemical action of Lpx on wheat technological properties is based on the ability of superoxygen radicals for oxidise SH-groups in storage proteins in ripening kernels to form interchain SS-bonds. These stabilize the macrostructure of functional glutenin – the protein base of the structural matrix of gluten (Trufanov, 1994).

Earlier Didenko *et al*. (2001) described the influence of intervarietal substitutions of chromosomes carrying the structural genes for Lpx on enzyme activity in the recipient cultivar Saratovskaya 29 (S29). In the present work, the study of this phenomenon has been continued using intervarietal substitution lines with other donor cultivars. Substitution lines with donor chromosomes 5A and 5D from non-related cultivars Janetskis Probat (JP), Atlas 66 (A66), Novosibirskaya 67 (N67) and Grekum 114 (G114) were used for the investigation. The lines were developed at the Institute of Cytology and Genetics SB RAS by T.T. Efremova and O.I. Maystrenko (1996).

An enzymatically active protein fraction was obtained from the flour of freshly crushed kernels through homogenizing in the cold with a double quantity of 0.1M Tris-HCl buffer (pH 7.5) containing 5mM of EDTA. After centrifuging, the protein content in the supernatant was determined by the Lowry method and Lpx activity (E) – by the formation of conjugated hydroperoxides of linoleic acid - using the spectrophotometer (234 nm). Specific activity was calculated according E quantity on mg of protein in enzyme extracts.

From Table 1 it can be seen that all four donor cultivars (JP, A66, N67, G114) exceeded the recipient S29 in their Lpx activity by 50-60%. However, for the 5A and 5D substitution lines the Lpx activity changed in different ways.

In the S29/JP 5A substitution line specific Lpx activity is 27% higher compared to the recipient but does not reach the level of the donor. At the same time, in the 5D substitution line it is 24% lower than in S29. In the case of the substitution line S29/A66 the 5A Lpx activity increases by 24% and is comparable with the line S29/JP 5A, but in the line S29/A66 5D the effect of substitution was insignificant. Both lines carrying chromosomes 5A and 5D from the donor G114 show an increase of Lpx activity comparable with the donor cultivar. In the case of donor N67 the substitution for the 5A chromosome was insignificant while the 5D substitution led to an increase of 34%.

From the data obtained, it may be concluded that the 5A and 5D chromosomes of different donor cultivars carry *Lpx* genes coding for the molecular forms of enzyme differing in their

activity. This agrees with the results of biochemical investigations. In genetic terms, it means that these chromosomes carry different alleles of *Lpx*.

Table 1. Specific lipoxygenase activity (in % to the recipient) in intervarietal substitution lines involving chromosomes 5A and 5D and in their parental cultivars

Lines and cultivars	LPX activity	Lines and cultivars	LPX activity	Lines and cultivars	LPX activity	Lines and cultivars	LPX activity
S29	100	S29	100	S29	100	S29	100
A66	133**	G114	161***	N67	160**	JP	162**
S29/A66 5A	124**	S29/G114 5A	149***	S29/N67 5A	108	CS9/JP 5A	127**
S29/A66 5D	106	S29/G114 5D	160***	S29/N67 5D	134**	S29/JP 5D	76**

** - $P<0.01$; *** - $P<0.001$

Our interest in wheat Lpx is conditioned mostly by its participation in the processes of forming protein macroassociations in the endosperm, which are characteristic of the gluten of physiologically ripe grain. The study of the genetic basis of Lpx activity regulation in cells is possible by using precise genetic stocks. These investigations are of interest to the development of new wheat high-quality genotypes using methods of chromosome engineering.

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Business Meeting

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Andreas Börner chaired the business meeting and reviewed the proposals made by Tony Worland, Victor Korzun and himself during the 10th EWAC meeting in Viterbo in 1998. The decision was to choose the following three sets of intervarietal substitution lines for further investigation:

- 'Cappelle Desprez/Bezostaya' (responsibility JIC Norwich)
- 'Chinese Spring/Synthetics' (responsibility IPK Gatersleben)
- 'Saratovskaya 29/Janetzkis Probat' (responsibility ICG Novosibirsk, IPK Gatersleben)

Four steps for further exploitation became necessary:

- Multiplication of the stocks
- Verification of the substitution lines
- Development of single chromosome recombinant lines
- Phenotypic evaluation

Multiplication is a continuous process. Whereas the 'Cappelle Desprez/Bezostaya' set have enough seeds at the JIC, the 'Chinese Spring/Synthetics' and 'Saratovskaya 29/Janetzkis Probat' sets need to be multiplied. This has been done at the IPK during the last two seasons.

The results of the verification of the stocks are summarised in the paper of Salina *et al.* (present proceedings). In all the three sets several substitutions were identified as being incorrect. In some cases new backcross programmes have already been initiated, in order to get correct lines.

Single chromosome recombinant lines have been developed for a range of chromosomes in two of the three series. Fourteen sets are available for the 'Cappelle Desprez/Bezostaya' series and six for the 'Chinese Spring/Synthetics'. For the latter, in addition, a set of 85 introgression lines covering the whole D genome have been developed at the IPK Gatersleben (see Pestsova *et al*., present proceedings). All of the introgression lines and most of the single chromosome recombinant lines are well characterised by molecular markers. For the 'Saratovskaya 29/Janetzkis Probat' series at the moment the first two sets of single chromosome recombinant lines (chromosomes 2D and 5D) are under development. All members of the EWAC community are invited to use the defined genetic material for co-operative phenotypic evaluation and gene mapping.

A further point of the discussion was the question, whether it will be necessary to develop new stocks. It was felt that this need only be done in exceptional cases. The focus should be more on the maintenance and validation of the stocks developed in the past. They cover already a world wide range of varieties and, therefore, a large wheat gene pool. Consequently, it becomes necessary to update the inventory of the stocks still available.

For further evaluation of well defined material, Tatyana Pshenichnikova offered to perform quality tests in Novosibirsk for a certain number of lines. Enrique Suarez offered to test interesting material in Argentina for resistance against diseases such as Septoria, Fusarium and the rusts. Further field tests for agronomic characters may also be undertaken in Poland, Spain or Germany.

Finally the place and time of the 13th EWAC meeting was discussed. Katharina Pankova offered to host the next workshop in Prague. All participants agreed to meet in 2005 for the first time in the Czech Republic in Central Europe.