Euphytica **89:** 27–31, 1996.

Comparative mapping and its use for the genetic analysis of agronomic characters in wheat

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Key words: agronomic characters, QTL, RFLP maps, wheat, *Triticum aestivum*

Summary

The advent of molecular marker systems has made it possible to develop comparative genetic maps of the genomes of related species in the Triticeae. These maps are being applied to locate and evaluate allelic and homoeoallelic variation for major genes and quantitative trait loci within wheat, and to establish the pleiotropic effects of genes. Additionally, the known locations of genes in related species can direct searches for homoeologous variation in wheat and thus facilitate the identification of new genes. Examples of such analyses include the validation of the effects of *Vrn1* on chromosome 5A on flowering time in different crosses within wheat; the indication of pleiotropic effects for stress responses by the *Fr1* locus on chromosome 5A; the detection of homoeologous variation for protein content on the homoeologous Group 5 chromosomes; and the detection of a new photoperiod response gene *Ppd-H1* in barley from homoeology with *Ppd2* of wheat.

Introduction

Although restricted to a certain extent by the lack of polymorphisms for molecular markers, great strides have been made in recent years in the development of a comprehensive genetic map of wheat, particularly through using RFLP techniques (Devos & Gale, 1993). Skeletal maps of all chromosomes are now available, and a large array of characterized DNA probes has been assembled. The parallel development of maps of related cultivated species, barley (Kleinhofs et al., 1993; Graner et al., 1991), rye (Devos et al., 1993) and wild species, for example *Triticum tauschii* (Gill et al., 1991) has also greatly extended knowledge of the wheat map since the cross hybridization of probes has revealed extensive homoeology for DNA sequences. This has enabled a detailed comparison of maps, and these have shown that synteny and collinearity of the maps is so extensive that a generalized map of the Triticeae can be inferred and used as a reference point for wheat mapping.

At the present time, mapping in wheat is moving in three directions. First, technological advances are being made through the development of more informative and user friendly marker systems, for example microsatellites and other PCR based markers. Secondly, map development is being advanced by combining the classical genetic maps of wheat based on major genes with the molecular marker maps. Thirdly, effort is increasing on applying the maps to map agronomically important major genes and quantitative trait loci (QTL). Comparative mapping using landmark probes is an important aspect of the latter approach and will make a considerable impact on the progress of genetic analysis in wheat. It allows comparisons of the nature and type of genetical variation within and between segregating populations, and provides guidance in the search for new genes and allelic variants. This paper discusses the use of these approaches.

Comparisons of allelic variation in different populations

In wheat, as in other species, the location of major genes and QTL can be established by associating marker allele variation with phenotypic variation for the characters of interest in appropriate segregating pop-

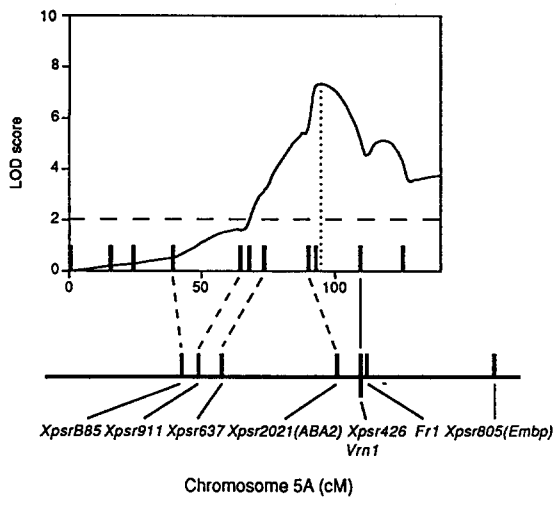

Fig. 1. Comparative genetic maps of chromosome 5A of wheat for different segregating populations, showing location of the vernalization response gene, *Vrn1*. Top: LOD score plot of QTL analysis for flowering time in the segregating population Chinese Spring × SQ1, showing location of a major QTL in the interval *Xpsr1201(ABA2)* – *Xpsr426*; Bottom: location of *Vrn1* by segregational analysis closely linked to *Xpsr426* in the single chromosome recombinant population from the segregating population Chinese Spring (*Triticum spelta* 5A) × Chinese Spring (Cheyenne 5A).

ulations (Hyne et al., 1994). For major genes which can be unambiguously classified in segregating populations, co-segregation with one or more marker loci is sufficient for accurate location. With respect to characters showing continuous variation more complex statistical approaches are required. Several methods are available for doing this varying from simple single marker comparisons using analysis of variance, to sophisticated interval mapping procedures using maximum likelihood or least square model fitting procedures (see Hyne et al., 1994, for example). These analyses can be carried out independently in different segregating populations, but the great strength of molecular marker systems for genetical analysis is that probes which show allelic variation in one population not infrequently show polymorphism in other segregating populations and thus can 'anchor' the respective maps across segregating populations. Thus, it is possible to establish whether variation for a particular character in one population is due to the same major gene or QTL and alleles in another population by reference to landmark loci.

An example from our own work of such an analysis is the location of genes affecting flowering time on chromosome 5A. Using a specialist population of single chromosome recombinant lines, derived from the hybrid between the single chromosome substitution lines Chinese Spring (*Triticum spelta* 5A) × Chinese Spring (Cheyenne 5A), Galiba et al. (1995a) were able to demonstrate discontinuous variation for flowering time which could be correlated with variation for alleles at the *Vrn1* locus determining vernalization response. Further, this variation could be 'tagged' by the different alleles of the RFLP locus *Xpsr426*, which segregated within 2cM of *Vrn1* (bottom map, Fig. 1). It was thus useful to establish how much variation for flowering time in other segregating populations may be influenced by the same locus. To do this a different population derived from the hybrid between Chinese Spring × SQ1 was examined (Quarrie et al., 1995), where flowering time was measured on a set of recombinant doubled haploid lines, derived from the F_1 between the parents, in a spring sown field experiment.

In this experiment, the lines exhibited wide variation in flowering time and no major gene variation could be discerned, since the distribution of means for the recombinant lines exhibited continuous variation. Thus, it was necessary to use QTL approaches to dissect the variation into the effects of individual loci, using the RFLP map of the segregating population which had previously been developed. First, analyses of variance were carried out for each marker in turn, and this revealed that most of the variation in flowering time was associated with allelic variation on chromosome 5A. Furthermore, large differences were associated with RFLP loci *Xpsr2021 (ABA2)* and *Xpsr426*, markers previously shown to map close to *Vrn1* in the Chinese Spring (*Triticum spelta* 5A) × Chinese Spring (Cheyenne 5A) derived population. To confirm this location in the Chinese Spring × SQ1 derived population, the interval mapping procedure of Lander & Botstein (1989) was applied as implemented in the computer package Mapmaker/QTL. The result of this analysis is shown in Fig. 1 and confirms the location of a major QTL linked closely to *Xpsr426*. Thus by comparative analysis this must also indicate a major effect of allelic variation at *Vrn1*, confirming the general effect of allelic variation at this locus on flowering time in wheat, even in spring wheats without a major vernalization requirement.

Evaluation of pleiotropic relationships within segregating populations by comparative mapping

QTL analysis using polymorphisms for different probes dispersed around the genome in a particular population can also establish whether variation for different characters is under the pleiotropic control of the same set of genes. First, QTL analyses can be carried out for each character separately and used to establish the intrachromosomal positions of QTL relative to the available RFLP polymorphisms. Co-location of individual QTL for the different characters implies either the pleiotropic action of the same genes or close linkage of different genes. Distinguishing between these possibilities is difficult. However, if QTL detected at different locations in the genome, or in different segregating populations, demonstrate the same relationships in terms of magnitude and direction of effect on two or more characters, then pleiotropy is the most likely explanation. This can be tested statistically by correlation and regression analysis over all loci of significant effect. In this way, the actions of pleiotropic and independent loci for different characters can be established.

An example of such an analysis is an extension of the work reported above on chromosome 5A. In the segregating population derived from the single chromosome substitution lines Chinese Spring (*Triticum spelta* 5A) × Chinese Spring (Cheyenne 5A), Galiba et al. (1995a) not only mapped the gene *Vrn1* on chromosome 5A but showed that it was closely linked to a locus, *Fr1*, of major effect controlling tolerance of freezing (Fig. 1). Also, in this segregating population, effects associated with other aspects of abiotic stress tolerance such as abscisic acid (ABA) production under osmotic stress (Galiba et al., 1995b) mapped to this chromosomal region. In a different segregating population, Chinese Spring × SQ1, Quarrie et al. (1994) were interested in mapping genes for components of drought tolerance, particularly ABA production using a derived recombinant doubled haploid population. Using the same methods of analysis as described above, QTL analysis in the latter population revealed that a locus of major effect influencing ABA production under drought stress co-segregated with RFLPs closely linked to the location of *Vrn1* and *Fr1*. Clearly, combining these analyses indicates that there is most probably a locus associated with generalized stress responses in this region, of which ABA production, frost tolerance and osmotic stress tolerance are all pleiotropic manifestations. This implies that these effects are all pleiotropic effects of, most probably, *Fr1*, although, of course, an effect of *Vrn1* cannot be unambiguously ruled out.

Location of homoeologous genes within wheat using syntenic probes

One of the major discoveries made during the initial development of the wheat genetic maps was the extensive collinearity in gene order for molecular markers between the A, B and D genomes. Although there are some major translocations between chromosomes, for example a reciprocal translocation between the long arms of chromosomes 4A and 5A, most of the genome is still conserved in terms of gene order.

This has been exploited in developing the maps, since probes which are polymorphic in two or three genomes can be mapped across the genomes to form landmark probes. New probes need only to exhibit a polymorphism in one of the genomes to be mapped relative to a landmark probe, which then establishes the most likely position across the other genomes as well.

This syntheny can also be exploited to search for homoeologous variation for agronomic characters within wheat. Once major genes or QTL of interest are identified and mapped onto a particular chromosome, the known homoeologous regions in the other genomes can be searched for allelic variation. This can be done using the same probes if polymorphisms for these exist on the other genomes, or probes for closely linked loci if the detector loci are monomorphic.

Again, studies of the homoeologous Group 5 chromosomes demonstrate the utility of this type of analysis. Snape & Aitken (unpublished data) studied the influence of the group 5 chromosomes on the genetic control of higher levels of grain protein in certain UK winter wheat varieties. Following a backcross reciprocal monosomic analysis which located variation in grain protein to these chromosomes, they used group 5 probes for mapping the genes concerned in a population of single chromosome recombinant lines for chromosome 5D, using the techniques described above. This analysis established that a major component of the higher level of grain protein of a target variety Avalon was due to a major gene located on the short arm of chromosome 5D. This locus was also closely linked to, or a pleiotropic effect of, the locus *Ha*, determining grain hardness. Attention then turned to

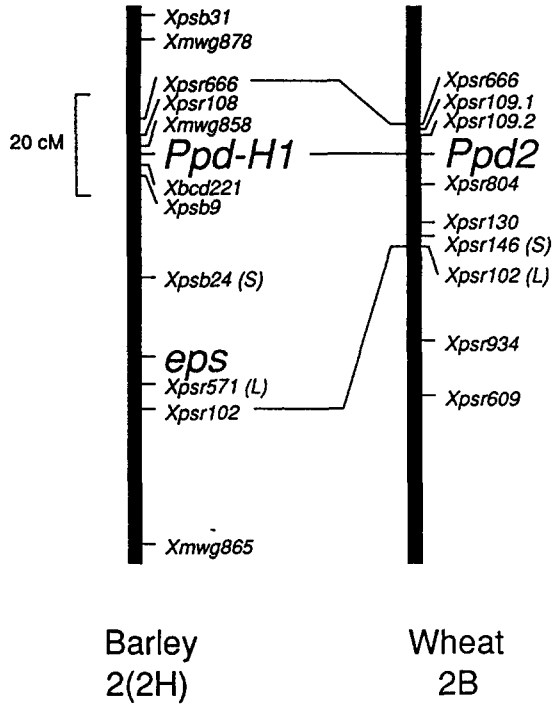

Fig. 2. Genetic maps of parts of chromosome 2 (2H) of barley and chromosome 2B of wheat indicating the homoeologous locations of RFLP loci and the genes *Ppd-H1* and *Ppd2* controlling photoperiod response. The probable location of an earliness *per se* QTL, *eps*, associated with the RFLP locus *Xpsr102* is also indicated on 2H. (S) and (L) indicate short and long arms, respectively.

the study of chromosome 5A. Using landmark probes it was possible to identify homoeoallelic variation on the short arm of chromosome 5A for grain protein, although there was no allelic variation for grain hardness. This suggests that the grain hardness association with protein amount is probably due to linkage rather than pleiotropy. With respect to grain protein levels, a further QTL was detected on the long arm of 5A, although there was no homoeologous variation in the 5D population. Other examples of the utility of this approach in identifying hitherto undescribed genes are also now emerging from studies of flowering time, plant height and other agronomically important attributes.

Comparative mapping using related species

An equally useful approach for detecting homoeologous variation within wheat is to use information on collinearity and homoeology for genes identified from the genetic analysis of intervarietal segregating populations of other species, particularly, barley and rye, and *vice versa*. Indeed, the genetic map of barley is much more extensive than that of wheat due to intrinsically higher levels of polymorphism, but also much greater investment in mapping, particularly in North America through the North American Barley Genome Mapping Program. Presently a great deal of information is emerging from these studies with respect to major genes and QTL detected within doubled haploid populations developed from hybrids between major North American feed and malting barley varieties. However, to be fully exploited this requires that a set of 'anchor' probes for Triticeae species is agreed between the major mapping groups, and used for their respective mapping studies.

To date, one of the best examples of the use of this approach for detecting new genes concerns the location of a photoperiod gene in barley by Laurie et al. (1994). For many years it has been known that the homoeologous Group 2 chromosomes of wheat carry major genes controlling photoperiod response (Welsh et al., 1973; Law et al., 1978), *Ppd1* on chromosome 2D and *Ppd2* on chromosome 2B. However, no homoeologous locus had been reported on barley chromosome 2 (2H), probably because flowering time behaves as a continuously varying character in segregating generations, and no clear discontinuous responses to changes in photoperiod had been observed associated with variation for this chromosome. Indeed, prior to the advent of whole genome QTL approaches such an analysis was difficult without the use of specialist genetic stocks such as single chromosome substitution lines.

By using cDNA and genomic probes derived from both wheat and barley we were able to cross-reference the genetic maps for wheat chromosome 2B and barley chromosome 2 to establish common marker loci, particularly in the region of the short arm of 2B where *Ppd2* had been mapped (Worland et al., 1995) (Fig. 2). Loci polymorphic in a barley doubled haploid population derived from a winter × spring barley F_1, Igri × Triumph, were then used for QTL analysis of flowering time data measured on the population grown under different daylength regimes, 12 hrs and 16 hrs in a specially constructed glasshouse for such experiments. This analysis showed clearly that there are two loci affecting flowering time on barley chromosome 2. In particular, one of these, which indeed had the largest effect on flowering time in this segregating population, interacted with daylength. This locus was linked to the RFLP locus *Xpsr666* on barley chromosome 2 (Fig.

2), an RFLP locus closely linked to *Ppd2* on chromosome 2B of wheat. This locus, now named *Ppd-H1*, is undoubtedly homoeologous to *Ppd1* and *Ppd2*, and has subsequently been shown to be a major component of the difference in flowering time between UK spring and winter barley varieties. The other QTL detected on 2H with effects on flowering time had a much smaller effect than *Ppd-H1*, and this effect was expressed under all day-length regimes, and did not interact with differences in photoperiod or vernalization time. Thus, this appears to be a QTL affecting development rate and can be classified as a so-called 'earliness *per se*' gene. Interestingly, Scarth & Law (1983) also established that a growth rate factor was present on chromosome 2B in addition to *Ppd2*, which Worland (1995) has shown to co-segregate with alleles for *Xpsr102*. Thus, this may also be homoeologous to the barley QTL. Further analysis of other wheat populations is now necessary to establish whether a homoeologous *eps* locus exists on chromosomes 2A, or 2D.

Conclusions

It is now becoming apparent that one of the greatest strengths and advantages of using molecular marker systems, particularly RFLPs, for genetical analysis is the ability to cross-hybridize probes of one species onto the DNA of another. Comparative analysis is fast becoming one of the most exciting and most productive areas of the genetics of all of the major crop groupings. It is probably most highly developed in the Gramineae and a common framework map which merges the maps of all of the major cereals, wheat, barley, rye, rice, oats, maize, sorghum, and millet, as well as sugarcane and forage grasses is emerging. This will enable the information gleaned from genetical studies of the separate species over the last eighty or so years to be considered together in its entirety. This will in turn provide new and novel insights into the biology of our most important food species which can be translated into applications towards providing improved varieties to meet the World's ever increasing food needs.

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