Molecular characterization of durum wheat backcross inbred lines derived from *Triticum turgidum* var. *dicoccoides*

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Abstract

Wild species of *Triticaceae*, 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, resistance to insects and fungal diseases, quality and quantity of grain storage protein. The mapping populations currently used are derived from interspecific crosses between cultivated and wild species accessions because of the low polymorphism at the DNA level among wheat cultivars. In order to investigate the genetic basis of important qualitative and quantitative traits and to introduce new, useful alleles into durum wheat, a set of backcross inbred lines (BILs) was developed from the cross of durum wheat cv Latino and one accession of *Triticum turgidum* var. *dicoccoides* (MG29896) with agronomically important traits. The BILs were tested in replicated field experiments for grain protein content and grain yield components and a subset of 28 BILs was selected for molecular marker characterization. The introgression of several segments of *Triticum turgidum* var. *dicoccoides* donor genome located on the durum wheat chromosomes was detected.

Introduction

Recently, some breeding programs have adopted advanced backcross inbred line (BIL) populations as a method for the identification and the introgression of useful genes from wild relatives or unadapted germplasm with the potential to improve the agronomic performance of elite cultivated lines (Fulton et al. 1997; Bernacchi et al. 1998; Talamè et al. 2004). The potential benefits of these lines versus more...
balanced populations (e.g. F2, F3, BC1, etc.) depends on the possibility of reducing the introgressed chromosomal regions and fine mapping loci linked with quantitative traits (QTLs). Epistatic QTLs or QTLs with gene actions ranging from recessive to additive can be detected in BILs and each QTL is treated as a single Mendelian factor (Fulton et al. 1997).

The application of the backcrossing method to the improvement of quantitative traits has been limited mainly because of the low heritability of these characters and the difficulty of simultaneously transferring a relatively large number of genes. In contrast to more traditional procedures of wheat breeding, the backcross inbred line method, first described by Wehrhahn and Allard (1965), produces BILs that can be tested in replicated trials over environments prior to selection. BILs are characterized by the low proportion of the donor parent in each of the population members and therefore are ideally suited for mapping interspecific variation (Eshed and Zamir 1995).

The wild tetraploid wheat *Triticum turgidum* var. *dicoccoides* shows particular promise as a donor of useful genetic variation for several traits, including disease resistance, drought tolerance, grain protein quantity and quality. Despite this, little is known at the genetic level about the introgression of DNA from wild relatives into cultivated wheat. However, wild germplasm contains both favourable traits and many commercially unacceptable characteristics. Information about segregation and recombination frequencies could expedite the utilization of this important germplasm resource.

Methodologies for the identification and mapping of genes underlying quantitative traits in plants have advanced considerably in recent years since the advent of marker-saturated linkage maps (Tanksley 1993). The developments in marker technology were not accompanied by corresponding progress in population structures.

In the present paper, we describe a backcross population capable of facilitating the utilization of wild germplasm in wheat breeding. Molecular markers (microsatellites) were used to follow the transmission of introgressed segments from var. *dicoccoides* into more adapted and agronomically acceptable durum wheat germplasm. A map for each line was then obtained and the proportion of the *dicoccoides* genome introgressed was compared with the theoretically expected value.

**Materials and methods**

**BIL population development**

The recurrent parent, the durum wheat cv Latino, is a semidwarf, high-yielding commercial cultivar with poor grain quality characteristics. The donor parent was the wild tetraploid wheat *Triticum turgidum* var. *dicoccoides* accession MG29896. One hundred and ten F1 plants were backcrossed three times to the recurrent parent and each line was then selfed for seven generations by single-seed descent method, resulting in a population of backcross inbred lines (BILs). No intentional selection
was imposed during population development, but some lines were lost during backcrossing and self-fertilization program. The BC$_3$F$_7$ generation contained 92 BILs. On the basis of phenotype evaluation (Blanco et al. 2004) a subset of 28 BILs was selected and characterized with molecular markers.

**Microsatellite marker assay**

DNA was extracted from leaves of individual plants from the two parents (cv Latino, var. *dicoecoides* accession MG29896) and each of the 28 selected BILs, grown in the field, using a modified DNA extraction method (Sharp et al. 1988).

Microsatellites markers GWM (Röder et al. 1998; Röder et al. unpublished data), GDM (Pestsova et al. 2000) and WMC (Prasad et al. 2000; Gupta et al. 2002) were used for BILs characterization.

PCR reactions were performed in a final volume of 25 µl in a Perkin-Elmer thermocycler. The reaction mixture contained 250 nM of each primer, 0.2 mM of each deoxynucleotide, 1.5 mM of MgCl$_2$ and 1 Taq polymerase unit. The development of the microsatellite markers, primer sequences, chromosome location and the annealing temperature are presented by Röder et al. (1998) for GWM, by Prasad et al. (2000) for WMC and by Pestsova et al. (2000) for GDM markers.

The allelic frequency at each locus and the ratio of Latino/*dicoecoides* genomes for the 28 BILs were calculated with QGENE (Nelson 1997). The observed ratio was compared with the expected ratio for a BC$_3$. The frequencies of Latino and *dicoecoides* alleles, homozygous genotypes and heterozygosity at each marker locus were also calculated for the selected 28 BILs.

**Results and discussion**

In order to cover the entire wheat genome searching for chromosomal regions introgressed from the wild parent, a total of 235 microsatellite markers were preliminarily selected on the basis of published maps (Röder et al. 1998; Korzun et al. 1999). The objective was to identify up to eight polymorphic markers for each of the 14 chromosomes of the A and B genomes and to have one marker every ca. 20-25 cM. The 235 microsatellite markers were tested on parental lines and primers that detected polymorphisms were used to characterize the selected 28 BILs. The percentage of polymorphism between Latino and var. *dicoecoides* was 52.7%, a value much higher than that found among cultivated wheat (25-30%) because of genetic distance between the two parental lines.

In order to obtain the introgression maps of each BIL using the software QGENE (Nelson 1997), the map distances estimated in a RIL population from durum wheat cv Messapia x var. *dicoecoides* accession MG4343 were used (Korzun et al. 1999). The analysis of 138 polymorphic markers used to reveal the presence of *dicoecoides* chromosome segments retained into the 28 BILs indicated an average
genome contribution of 93.1% from the recurrent parent. The distribution of Latino genome in the 28 BILs was quite variable (Figure 1).

![Percentage of Latino genome in BC3F7 lines obtained from the cross Latino × var. *dicoccoides*. The mean value is indicated by an arrow.](image)

The percentage of the introgressed *dicoccoides* genome ranged from 26.3% (max) to 0.9% (min) with an average of 6.3% (Tab. 1). This value is very close to the expected one (6.25%), thus indicating that the genome of *dicoccoides* can be easily introgressed in Latino and there is full compatibility between the two genomes.

**Table 1.** Percentage of recurrent parent (cv Latino) and donor parent (var. *dicoccoides*) and mean values in 28 BC3F7 BILs analyzed with 138 microsatellite markers.

<table>
<thead>
<tr>
<th></th>
<th>Latino genome (%)</th>
<th>Heterozygous genome (%)</th>
<th>var. <em>dicoccoides</em> genome (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Max.</td>
<td>99.1</td>
<td>1.2</td>
<td>26.3</td>
</tr>
<tr>
<td>Min.</td>
<td>76.5</td>
<td>0.0</td>
<td>0.9</td>
</tr>
<tr>
<td>Mean value</td>
<td>93.1</td>
<td>0.6</td>
<td>6.3</td>
</tr>
</tbody>
</table>

The frequency of the *dicoccoides* allele in comparison with the expected frequency was evaluated for each marker. Some alleles from *dicoccoides* were not introgressed, while most loci had an allelic frequency ranging from 3.5 to 15.0%. Markers showing
a frequency significantly lower than expected (6.25%) could be associated to genes that produce poor viability of gametes and/or zygotes (i.e. distorted segregation; Monforte and Tanksley 2000). The markers Xgwm350-7A, Xgwm471-7A, Xpsp3050-7A and Xgwm577-7B exhibited a frequency of *dicoccoides* alleles higher than the expected one, suggesting an association to genes inducing the competition of wild parental gametes. Each line showed an average of 4.6% homozygous segments and 0.2% heterozygous segments introgressed. The BILs showed from 1 to 14 homozygous segments introgressed, compared with the heterozygous segments ranging from 0 to 1, as expected in a BC3F7 progeny (Figure 2). The minimum percentage value of *dicoccoides* genome introgressed as homozygous for each chromosome ranged from 35% (chromosome 3A) up to 100% for chromosomes 4A, 4B, 7A and 7B.

The number of terminal segments was not higher than the number of interstitial segments. Furthermore, the average length of terminal segments (15.4 cM) was not significantly different from the length of the interstitial segments (18.9 cM). A lower number was expected for introgressed interstitial segments, since terminal segments are introgressed following only one crossing-over event, compared to the minimum number of two crossing-over events required to introgress interstitial segments (Figure 3).
BIL lines with different introgressed *dicoccoides* segments could be used to map genes controlling quantitative and qualitative characters. The introgressed segments were found randomly distributed on the wheat chromosomes, with each *dicoccoides* chromosome almost completely integrated in the 28 BILs analysed. The chromosome 5B is almost completely represented by *dicoccoides* segments in seven BILs (BIL-59, 85, 91, 74, 69, 87 and 29; Fig. 4); with further backcrosses to the recurrent parent and by marker-assisted selection, it will be possible to obtain a series of NILs (Near Isogenic Lines). BILs with a low number of introgressed *dicoccoides* segments, could be used to map genes controlling important traits. As an example, the line BIL-69, characterized by one homozygous segment on chromosome 5B and one heterozygous segment on chromosome 6A, selected for high protein content in previous studies (Blanco et al. 2004), could provide a series of NILs after a low number of backcrosses to Latino; these NILs would be valuable for the detection and the fine mapping of high protein content QTLs. NILs can be used as a permanent mapping source to rapidly map newly cloned genes and are especially useful for the study of quantitative traits (Alpert and Tanksley 1996; Monforte and Tanksley 2000). Moreover, the new allelic combination arising after introgression may cause the appearance of genetic variation in the modern programs of plant breeding. BIL analysis indicates the high fidelity with which segments of DNA from *dicoccoides* can be introgressed in the cultivated wheat. BILs are characterized by the low proportion of the donor parent in each of the population members and therefore are ideally suited for mapping important QTL traits.

![Figure 3. Distribution and size (in cM) of terminal (black bars) and interstitial chromosome *dicoccoides* segments introgressed from the var. *dicoccoides* in 28 BILs.](image-url)
Conclusions

This analysis allowed us to identify recombinant lines with reduced length of introgressed segments so that it will be possible to saturate chromosome regions controlling bio-agronomic traits. Efficient use of the genetic variation available in the wild relatives of crop species depends on the ability to introgress desirable DNA segments from wild germplasm into cultivated varieties. Ample recombination must be available to allow the reduction of linkage drag. Results from this study indicate that high levels of recombination occur between the cultivar Latino and the wild relative var. *dicoccoides* genomes. After three generations of backcrossing, the average length of introgressed segments resemble that predicted from theory for an unselected population. This study indicates that the *dicoccoides* genome can be easily introgressed into the cultivated wheat, thus raising the possibility that the genetic variation found in wild genotype could be harnessed for improving many traits. The analysis of genetic maps obtained for each of the 28 BILs and the identification of introgressed chromosome segments from the wild parent could be useful for the identification and fine mapping of region controlling agronomically relevant traits.

Figure 4. Molecular linkage maps of *dicoccoides* 5B chromosome segments introgressed in BIL lines derived from the cross Latino × var. *dicoccoides* (cM distances are indicated at the left). Bold markers indicate *dicoccoides* regions.
Acknowledgements

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