Identifying the source of new variation seen in synthetic backcross derived bread wheat

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Abstract

The expansion of the bread wheat gene pool can be achieved via the creation of synthetic hexaploid wheats (SHWs) and the backcrossing of these synthetics to elite breeding lines. Resistance to many biotic and abiotic stresses has been incorporated into new bread wheat cultivars using synthetic donors. Sufficient microsatellite markers (SSRs) were used to create a dense coverage of all the chromosomes to assess the genetic diversity present in synthetic hexaploid wheats, their backcross derived families, and their parents (where possible). SSRs were also used to test for the selective advantage of SHWs alleles in backcross families after several generations of selection. The SHWs investigated in this study had a high gene diversity and PIC for all SSRs, but highest for D genome markers. The SHWs clustered with their durum parents, but were clearly separated from bread wheat cultivars in a dendrogram. Gene diversity of the synthetic backcross derived lines (SBLs) for the A and B genomes was higher than that of their SHW and bread wheat parents. Gene diversity partitioned within each SBL family was extremely high. Principal coordinates analysis of the SBLs and their parents showed that lines from each SBL family clustered together and closer to their bread wheat parent than their SHW parent. *De novo* generation of genetic variation was seen in many of the SHWs, which was stably inherited in the SBL families. Non-Mendelian inheritance of alleles favoring the SHW parent was seen in some of the markers in one or more SBL families, suggesting that these genomic regions are being actively selected for.

Media summary

New diversity for important agronomic traits can be incorporated into bread wheat from related species using synthetic hexaploid wheats, and can be quantified using molecular markers.

Key words

Gene diversity, selective advantage, synthetic backcross derived lines, synthetic hexaploid wheats.

Introduction

Hexaploid bread wheat (*Triticum aestivum*) was first cultivated around 10,000 years ago. Although it contains an enormous amount of variation and is grown from Northern Europe to Argentina, there are many environments and biotic and abiotic stresses under which it cannot grow. New variation for resistance traits could increase wheat yields without requiring an increase in inputs of pesticides, fertilizers, and irrigation water. Only a small proportion of the available genetic variation in the primary gene pool of any given cultivated species, and even within the cultivated species itself, has been exploited for crop improvement in modern breeding programs. One successful method for introducing variation from the progenitors of bread wheat (BW) into the elite breeding germplasm is the creation of synthetic hexaploid wheats (SHWs). SHWs are produced by artificially crossing tetraploid forms such as modern durum wheat (*Triticum turgidum,* 2n=4x=28 AABB), donor of the A and B wheat genomes, with *A. tauschii* (2n=2x=14 DD), donor of the D genome. The resulting hybrid is haploid and carries the A, B, and D genomes (2n = 3x = 21, ABD). It is converted to a true hexaploid by using artificial chromosome doubling methods based on colchicine treatment (Mujeeb-Kazi et al. 1996) (Fig. 1).

Over a thousand new SHWs have been produced from more than 600 *A. tauschii* accessions at CIMMYT. These SHWs possess favorable qualitative traits (Kema et al, 1995; Ma et al. 1995; Lage et al, 2001; Mujeeb-Kazi et al. 2001) and desirable quantitative traits (Villareal et al. 2001). However, in general,

SHWs carry a large number of unfavorable alleles and are typically backcrossed to elite BW cultivars to produce agronomically acceptable synthetic backcross-derived lines (SBLs).
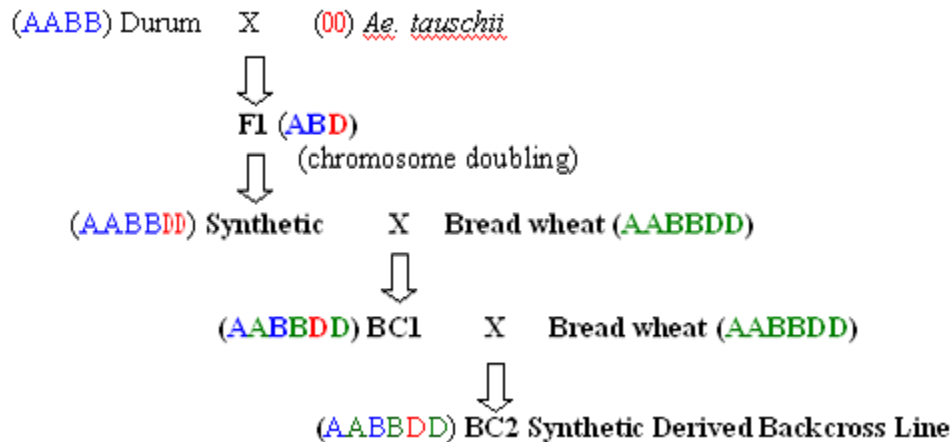

Figure 1. Formation of synthetic hexaploid wheat and synthetic derived backcross lines. Colored capital letters in parentheses indicate genome constitution.

Microsatellites, or Simple Sequence Repeat (SSRs), are PCR-based DNA markers that are highly polymorphic, show co-dominant inheritance, are evenly distributed throughout the genome and are locus-specific (Powell et al. 1996). Previous studies of synthetic derived wheats reveal an increased diversity in the synthetics using AFLP (Lage et al, 2003) and SSR markers (Zhang et al, 2004). Furthermore, the possibility for association analyses exists using molecular markers to characterize the SBLs. In a backcrossing program, it is hoped that only the chromosomal regions carrying genes encoding the traits of interest will be retained from the donor parent after several generations of backcrossing to the recurrent parent. These segments, including the genes and any DNA segments linked to these genes, will occur in the progeny in non-Mendelian proportions because of positive selection, which has been demonstrated in a small study of two synthetic derived families (Zhang et al, 2004). It should be possible, therefore, to locate the chromosomal regions encoding these traits by finding neutral markers that occur in the progeny in ratios that are significantly different than what was expected by Mendelian inheritance.

Materials and Methods

Plant materials

Four types of wheat were fingerprinted in the present study. These included:

1. durum wheats (tetraploid, AABB), used as parents of the original synthetic hexaploid crosses.

2. synthetic hexaploid wheats (SHWs) that were created by crossing the durum wheat parents with *T. tauschii* accessions (the D genome donor) to create haploid ABD plants, which were then treated with colchicine to double the chromosome number and create the fertile hexaploid synthetic wheats.

3. bread wheats (hexaploid, AABBDD), used as the recurrent parents in the recurrent backcrossing with the SHWs to generate the synthetic backcross-derived lines.

4. families of synthetic backcross-derived lines (SBLs).

The *T. tauschii* parents of the original SHW cross could not be fingerprinted, because when the SHWs were created, pollen was bulked from multiple *T. tauschii* plants per heterogenous accession, and the individual pollen donor could not be distinguished from the bulk.

Marker analyses

SSR markers evenly spread across the three genomes are being employed in the present study. SSR markers were either run on small 4.5% (w/v) denaturing (6M urea) acrylamide : bisacrylamide (29:1) gels stained with silver and scored manually or on an ABI Prism 377 DNA Sequencer (Perkin Elmer/Applied Biosystems). Gels were run at constant 3.0 kV voltage, using a 1X TBE buffer (pH 8.3). Each SSR was amplified separately, although multi-loading was applied when possible to increase efficiency.

Statistical Analyses

Alleles from the durum wheat parents that were not recovered in the SHWs, novel alleles in the SHWs not present in the durum wheat parents, and percent of segregating loci by polymorphic SSRs in the SHW were calculated for the SSR markers mapping to the A+B genomes. Polymorphism Information Content (PIC), total gene diversity (H_T), gene diversity within wheat type (H_S) and proportion of genetic differentiation among types (G_{ST}) were calculated according to Nei (1987, p164) for each locus. Genetic distances were computed for all pairs of lines within and between wheat types and were used for cluster analysis, to reveal associations between lines, families or types based on the unweighted paired group method with arithmetic averages (UPGMA) and Principal Components Analysis (PCA). Selective advantage was detected for each marker/family combination by exact test of the parameter P of a binomial distribution, and corrected for significance level using the Sidak test (Dufner et al. 2002). In testing for selective advantage of alleles in the SBLs, a null hypothesis that considered that the bread wheat does have a selective advantage, (H_0: $p \geq 0.75$) was used, with an alternate hypothesis that the bread wheat does not (H_A $p < 0.75$).

Results

The proportion of polymorphic SSR loci, H_S, and the average number of alleles per locus (N_a) of the A, B, and D genomes were higher for SHWs and SBLs than for BWs. In addition, H_S and N_a were significantly higher for the D genome than for the A+B genome, except in the BWs. UPGMA cluster analyses, separately performed for the SSRs on the A+B and D genomes, revealed that both parents of the SHWs were highly diverse.

The modified Rogers' distances between groups were high except for between DWs and SHWs, and between SBLs and BW. UPGMA cluster analysis of DWs and SHWs, based on SSRs mapping to the A+B genomes, showed that SHWs and their corresponding DW parents clustered closely together in almost every case. PCoA based on all SSRs of all genotypes except the DWs revealed that the SHWs clustered separately from the SBL families and BWs. Members of the same SBL family clustered together, and were closely associated with their corresponding BW parents. The SHWs and their corresponding DW parents should have had no allele differences for the A and B genomes. However differences caused by novel alleles present in the SHWs but absent in DW, and by alleles from the DWs that were not recovered in the SHWs, caused a low genetic distance between the two groups.

Allele frequencies in SBL families were consistent with the null hypothesis at most SSRs, as they showed either expected Mendelian ratios or more BW alleles than expected. However, some SSRs in one or more SBL families led to a rejection of the null hypothesis and showed a selective advantage in favor of alleles from the SHWs.

Work to be continued

In order to test for associations between the chromosomal regions, determined in this study to be preferentially inherited from the synthetic wheats, and new phenotypes of interest in the SBLs, the SBLs were phenotyped for the traits listed in Table 1. Association analyses of these traits with the SSRs used in this study pinpointed the genomic location for genes encoding these traits and verified the origin of the traits (i.e. did the trait come from the Ae. tauchii parent or the durum parent, and was it only expressed after recombining the A, B, and D genomes in the newly synthesized ampliploid?).

Table 1. Phenotypes to be scored on the synthetic derived backcrosses for future association analysis.

Seed characteristics	Quality traits	Plant characteristics	Biotic stress resistances	Abiotic stress resistances
Testweight	alveograph W- and P/L values	relative heading date	leaf rust	yield under heat stress
graincolor	grain SDS sedimentation	relative maturity	stripe rust	Yield under drought
grainhardness	glutentype	Yield – irrigated	stem rust	
use type	flour protein	relative height	Septoria tritici	
Thousand kernal weight	HMW-1A, B, and D status	Yield – reduced irrigation	Fusarium head blight	
Test weight	loaf volume			
	crumbstructure			
	mixing time			

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