Genetic diversity is a prerequisite for any cultivar development programme. Recent emphasis on hybrid breeding arouses the need for diversity assessment in wheat working germplasm. A variety of approaches – including phenotypic markers, isozymes or storage proteins, and parentage analysis – are used to characterize genetic diversity in wheat. However, phenotypic markers and protein markers are limited in number and affected by the environment. Parentage analysis requires detailed pedigree records of cultivars and is based on certain assumptions that are generally not fulfilled. The development of techniques to detect polymorphisms at the DNA level has revolutionized the molecular analysis of plant genomes, by using a range of molecular techniques. Several molecular marker systems, such as AFLPs (Barrett and Kidwell 1998; Grunberg et al. 2001), RAPDs (Joshi and Nguyen 1993), RFLPs (Siedler et al. 1994; Kim and Ward 2000), STS (Chen et al. 1994; Talbert et al. 1994), ISSRs (Nagaoka and Ogihara 1997), and SSRs (Plaschke et al. 1995; Ma et al. 1996; Bryan et al. 1997; Ahmad 2002), have been used for estimation of genetic diversity in wheat.

Microsatellite markers, also known as SSRs, have been proposed as suitable markers for assessment of genetic variation and diversity among wheat varieties and lines. SSRs are multiallelic, chromosome-specific and evenly distributed along the chromosomes (Roder et al. 1998 a,b). Diversity estimates based on molecular markers and coefficient of parentage (COP) values can predict the level of heterosis (Liu et al. 1999;
Corbellini et al. (2002) and variation among segregating progenies (Burkhamer et al. 1998; Bohn et al. 1999). Currently, a hybrid wheat project is operating at the Punjab Agricultural University, Ludhiana. Under this project, a set of parental lines with their ability to give heterosis in combinations with other lines have been chosen on the basis of qualitative and quantitative attributes. There is a need to estimate genomic diversity among these parental lines. Therefore, the presented study aimed to find genetic relationships among 20 elite wheat genotypes by using microsatellite markers and the pedigree-based approach, and to assess the correlation between 2 diversity indices: coefficient of parentage (COP) and Nei and Li’s (1979) genetic similarity (GS).

A set of 20 elite wheat genotypes, including released varieties and advanced breeding lines, was used for the study (Table 1). Genomic DNA was isolated from a bulk of 5 individual plants of each genotype according to the method of Saghai-Maroof et al. (1984). Twenty-five microsatellite primers (Table 2) were chosen from the microsatellite map of wheat (Roder et al. 1998a). The reaction mixture for amplification contained 10 × PCR reaction buffer, 200 μM dNTPs, 2.5 mM MgCl₂, and 0.5 μM of each primer, 50 ng template DNA, and 1 U Taq polymerase in a reaction volume of 25 μL. Amplifications were performed in an Eppendorf Mastercycler gradient (Eppendorf Netheler-Hinz, Hamburg, Germany). Amplification conditions were: an initial denaturation step at 94°C for 4 min, 35 cycles at 94°C for 1 min, 50–60°C (depending upon primer pair) for 1.5 min and 72°C for 2 min. Amplified products were electrophoresed in a 3.0% agarose-1 × Tris Borate gel at 4 V cm⁻¹.
and visualized after staining with ethidium bromide.

Pedigrees of 20 cultivars were obtained from cultivar descriptions (Zeven and Zeven-Hissink 1976; Martynov et al. 1992) or by personal communications with the breeders. The pedigree trees of each of these 20 cultivars (at the expansion level of 5) were generated using the external pedigree input tool of the International Crop Information System (ICIS) Software (Mclaren and White 1999). The COP values were then estimated using the WCOP function of the International Wheat Information System (IWIS) software, version 2.0.

The presence or absence of each single variant was coded by 0 or 1, respectively, for molecular marker data and scored for a binary data matrix. Genetic similarity (GS) was calculated according to Nei and Li (1979), as follows:

$$\text{GS} = \frac{N_{ij}}{(N_i + N_j)}$$

where $N_{ij}$ = number of bands common to lines $i$ and $j$, and $N_i + N_j$ = total number of bands observed in line $i$ and $j$, respectively.

Polymorphism information content (PIC) was calculated as described by Anderson et al (1993):

$$\text{PIC} = 1 - \sum_{i=1}^{n} \left( P_i \right)^2,$$

where $P_i$ is the frequency of $i$-th allele.

Similarity values were calculated for each pair of lines, and clustering by the Unweighted Pair-Group Method with Arithmetic Averages (UPGMA) was done using software package NTSYSpc.2.02e. The dendrogram was generated using the TREE PLOT function of the same software.

The genotypes included in the present study represent a broad base for diversity analysis. A careful perusal of parentage showed that most lines (PBW343, PBW445, PBW493, CPAN4231, HD2687, Chilero, Luan, Sasia, Tia.2/Kauz, Munia/Chen//Altar, F12.71/Coc//Cno79/3/Kauz, F6.74/Bun//S15/3/Vee#7 and Kauz*2/MNV//Kauz) carried the germplasm base from dwarfing wheats from the 1960’s, developed at CIMMYT, Mexico and further got diversified by genetic input from Veary wheats, whereas others have traditional Indian wheats in their parentage (PBW442, PBW474). CPAN4231 and Catbird have ancestors of Chinese origin. Therefore, it could be expected that these 20 elite lines are a suitable target for diversity-based approach. Twenty-five SSR primers selected on the basis of their distribution across all genomes (A, B and D) were used to evaluate the genetic diversity of 20 elite wheat genotypes.

A total of 93 alleles were detected. The number of alleles per primer ranged from 2 to 6, with an average of 3.72 and the PIC values ranged from 0.17 to 0.77 with an average of 0.58 (Table 2). Figure 1 is representative of the extent of polymorphism observed among 20 wheat genotypes as revealed by primer Xgwm 249. The similarity matrix based on all possible pairs of lines ranged from 0.47 to 0.91, with a mean of 0.70. The genetic similarity values for all Punjab bread wheat (PBW) genotypes ranged from 0.62 to 0.84, which indicated more than 60% similarity in their genomes.

The UPGMA-based dendrogram grouped genotypes into 2 clusters. Cluster I shows that 5 genotypes of the PBW series, namely PBW343, PBW445, PBW459, PBW474, PBW493, form a distinct group (Figure 2a). Cluster II, with 14 genotypes, included closely related pairs of genotypes Kauz*2/MNV//Kauz and TJB 368.251/Buc/Turaco (91% similarity), Luan and TJB 368.251/Buc/Turaco (89% similarity), as well as Tia.2/Kauz and Kauz*2/MNV//Kauz (85% similarity). Genotype PBW442 appeared as unique, having no commonality with other genotypes.

Figure 1. SSR profile of 20 wheat genotypes using the primer Xgwm 249. M = molecular weight ladder; lanes 120 = wheat genotypes as listed in Table 1
COP values estimated for 190 pair-wise combinations of 20 genotypes ranged from 0.0 to 0.53, with an average of 0.115. One tenth of COP values (19) indicated no genetic similarity among the pair of cultivars. The majority of pair-wise combinations showed COP values of 0.01 to 0.1, indicating that fewer than 10% of germplasm under consideration were identical by descent in any pair of cultivars. Cluster analysis of 190 pair-wise COP values resulted in a dendrogram with less distinct patterns of grouping than the dendrogram based on SSR data. However, the genotypes PBW343, PBW445, Kauz*2MNV//Kauz and Tia.2/Kauz showed high similarity values and clustered in group I. Group II reflected complex nesting of genotypes, indicating lack of genetic resolution detected by pedigree analysis among these cultivars (Figure 2b). Similarly to SSR clustering, the highly divergent nature of PBW442 from remaining genotypes was also evident from parentage analysis. This could be due to the modern breeding scheme, which is focused mainly on extensive intercrossing among selected genotypes, resulting in intermingling wheats of different origins. The correlation among similarity values based on pedigree and SSRs was found to be low but significant ($r = 0.285$, $p < 0.05$). A weak correlation among molecular and pedigree data were reported in earlier studies on wheat and other crops (Barbosa-Neto et al. 1996; Barrett et al. 1998). The low level of genome similarities obtained from parentage analysis could be due to distinct names often given to parents which actually trace back to common progenitors and incomplete pedigree records. In the present study, 3 wheats SASIA, Munia/Chen//Altar and Dacula/Chaguial//Cezo have durum wheats in their parentage. However, their similarity values based on parentage analysis were low. This was mainly because the durum progenitors in these wheats were different accessions and parentage analysis failed to see the commonality. In addition to this, a number of assumptions made in calculating COP (Cox et al. 1985; Messemer et al. 1993) may not be fulfilled in real situations, which is another reason for the disparity between pedigree and molecular approaches. However, the genetic similarity estimates based on molecular marker data were expected to be more accurate, as any polymorphism is a direct outcome of variation at DNA level. In earlier studies, wheat genotypes of the same origin were analysed using a small number of wheat microsatellites (21–24) that produced genetic diversity or similarity within a specific group of genotypes (Leisova and Ovesna 2001; Ben-Amer et al. 2001). In our study, genetic similarities obtained using 25 microsatellite primers were found to be higher than in previous studies (Manifesto et al. 2001; Ahmad 2002). Further clustering analysis failed to show distinct groups except Punjab bread wheat genotypes, which formed a separate group.

In summary, this study using microsatellite markers revealed a considerably high genetic similarity among chosen varieties and advanced breeding lines. However, the parentage analysis indicated a high amount of genetic diversity. This suggests that most of the released varieties and advanced breeding lines developed by crossing exotic materials (introduced from CIMMYT, Mexico) or genotypes derived from exotic materials, followed by selection of superior genotypes, make the gene pool smaller for all wheat cultivars. Therefore there is a need to incorporate new variability into the existing wheat germplasm.

Figure 2. Dendrogram of 20 elite wheat lines clustered in respect of similarity estimates based on: (a) SSR markers and (b) pedigree data
REFERENCES


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