Genetic diversity in conventional and synthetic wheats with drought and salinity tolerance based on AFLP

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Das, M. K., Bai, G-H. and Mujeeb-Kazi, A. 2007. **Genetic diversity in conventional and synthetic wheats with drought and salinity tolerance based on AFLP**. Can. J. Plant Sci. **87**: 691–702. Genetic diversity among 14 drought tolerance (drought accessions) and 27 salinity tolerance (salinity accessions) related conventional and synthetic wheat (*Triticum* sp.) accessions containing different sources of the D genome was assessed using amplified fragment length polymorphism (AFLP). The wheat accessions were analyzed with 20 *Eco*RI/*Mse*I primer combinations. Among 918 fragments scored, 368 were polymorphic across all 41 wheat accessions, 348 were polymorphic among the drought accessions and 310 were polymorphic among the salinity accessions. Similarity coefficients among all accessions based on Jaccard's coefficient ranged from 0.18 to 0.92 with an average of 0.53 ± 0.01; among drought accessions, from 0.16 to 0.79 with an average of 0.43 ± 0.02; and among salinity accessions, from 0.16 to 0.92 with an average of 0.57 ± 0.01. Polymorphic information content (PIC) among all accessions ranged from 0.05 to 0.50 with an average PIC of 0.30 ± 0.01; among drought accessions, from 0.13 to 0.50 with an average PIC of 0.37 ± 0.01; and among salinity accessions, from 0.07 to 0.50 with an average PIC of 0.29 ± 0.01. Cluster and principal component analysis showed distinct groups of accessions both within drought and salinity entries. These accessions possess a substantial amount of genetic diversity and would be very valuable materials for breeding wheat with drought and salinity tolerance.

Key words: AFLP, DNA fingerprinting, genetic diversity, synthetic wheat

Das, M. K., Bai, H-H. et Mujeeb-Kazi, A. 2007. **Diversité génétique des variétés classiques et synthétiques de blé tolérant la sécheresse et la salinité selon la technique AFLP**. Can. J. Plant Sci. **87**: 691–702. Les auteurs ont évalué la diversité génétique d'obtentions de blé (*Triticum* sp.) classiques et synthétiques tolérant la sécheresse (n = 14) ou la salinité (n = 27) et renfermant différentes sources du génome D par la technique du polymorphisme amplifié de la longueur des fragments (AFLP). Les obtentions de blé ont été analysées grâce aux combinaisons d'amorces 20 *Eco*RI/*Mse*I. Sur les 918 fragments évalués, 368 étaient polymorphes chez les 41 obtentions, 348 étaient polymorphes chez les obtentions tolérant la sécheresse et 310 étaient polymorphes chez celles tolérant la salinité. Le coefficient de similarité reposant sur le coefficient de Jaccard variait de 0,18 à 0,92 avec une moyenne de 0,53 ± 0,01 pour l'ensemble des obtentions, de 0,16 à 0,79 avec une moyenne de 0,43 ± 0,02 pour celles tolérant la sécheresse, et de 0,16 à 0,92 avec une moyenne de 0,57 ± 0,01 pour celles tolérant la salinité. Le contenu de matériel polymorphe variait de 0,05 à 0,50 avec une moyenne de 0,30 ± 0,01 pour l'ensemble des obtentions, de 0,13 à 0,50 avec une moyenne de 0,37 ± 0,01 pour celles tolérant la sécheresse, et de 0,07 à 0,50 avec une moyenne de 0,29 ± 0,01 pour celles tolérant la salinité. L'analyse des grappes et des composantes principales révèle l'existence de groupes distincts parmi les obtentions tolérant la sécheresse et celles tolérant la salinité. Ces obtentions présentent une importante diversité génétique et constitueraient du matériel d'une grande utilité pour la sélection de variétés de blé tolérant à la fois la sécheresse et la salinité.

Mots clés: AFLP, identification par le code génétique, diversité génétique, blé synthétique

Abiotic stresses such as drought and salinity are major global constraints to wheat production. Approximately 32% of the wheat-growing regions in developing countries go through some drought stress during the growing season (Morris et al. 1992). In total, around 45 million ha of wheat-producing land are characterized by periodic drought stress (Byerlee and Moya 1993). Soil salinity causes significant reductions in plant productivity, and consequent economic losses associated with reduced grain quality and yield of agricultural crops (Pitman and Lauchli 2002).

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Abbreviations: **AFLP**, amplified fragment length polymorphism; **CIMMYT**, International Maize and Wheat Improvement Center; **DNA**, deoxyribonucleic Acid; **PCA**, principal component analysis**; PIC**, polymorphic information content; **RFLP**, restriction fragment length polymorphism; **SSR**, simple sequence repeat; **UPGMA**, unweighted pair-group method with arithmetic averages.

Genetic diversity is the foundation for genetic improvement of various crops. Genetic diversity of germplasm including those naturally occurring or synthesized can be assessed through pedigree analysis (Cox et al. 1985; Martin et al. 1991) and DNA markers (Autrique et al. 1996; Karp et al. 1997; Barrett and Kidwell 1998; Davila et al. 1998; Soleimani et al. 2002; Sasanuma et al. 2002). DNA markers, however, provide a direct measurement of genetic relationships among samples analyzed based on their genome composition. Also, an unlimited number of markers is available for such analyses. Therefore, DNA markers are a necessary complement to pedigree analysis.

Based on restriction fragment length polymorphisms (RFLPs) in 113 improved cultivars and landraces of durum wheat (*T. turgidum* L.), Autrique et al. (1996) reported a mean genetic distance of 0.21 and 0.31 within the improved lines and landraces, respectively. Using AFLP markers Barrett and Kidwell (1998) studied genetic diversity among wheat (*T. aestivum* L.) cultivars from the US Pacific Northwest, and reported mean genetic diversity estimates ranging from 0.49 to 0.58 for within spring and winter types and between spring vs. winter types. Manifesto et al. (2001) used AFLP and simple sequence repeat (SSR) markers and quantified the genetic diversity among 105 modern and older bread wheat cultivars from Argentina concluding that Argentinian bread wheat germplasm had maintained a relatively constant level of genetic diversity during the last half century. Soleimani et al. (2002) detected a substantial amount of genetic variation between and within cultivars in 13 registered modern Canadian durum wheat cultivars based upon AFLP markers, reporting a mean pair-wise genetic distance of 0.40. Sasanuma et al. (2002) studied genetic diversity of wheat wild relatives from the Near East using AFLP, and reported the existence of potential genetic diversity among the wild relatives in natural populations. Lage et al. (2003) reported genetic diversity using AFLP and agronomic traits among 54 synthetic hexaploid wheats derived from crosses between emmer wheat (*T. dicoccum* L.) and goat grass (*Aegilops tauschii* Coss.). Based on AFLP, they observed clear grouping according to geographical origin for the *T. dicoccum* parents, but no clear groups for the *Ae. tauschii* parents. The hexaploid synthetics also revealed similar clustering as the *T. dicoccum* parent. Based on percentage polymorphic markers, the synthetic hexaploid wheats showed a considerably higher level of AFLP diversity (39%) than normally observed in cultivated wheat (12 to 21%).

Reports on molecular marker studies of genetic diversity among wheat germplasm with drought tolerance are limited. Moghaddam et al. (2005) studied genetic diversity in bread wheat genotypes for tolerance to drought using AFLPs and agronomic traits such as plant height, days to flowering, days to maturity, grain yield and harvest index. Their study included 14 wheat genotypes from Iran and 14 wheat genotypes developed by or obtained by the International Maize and Wheat Improvement Center (CIMMYT). They reported that the genetic basis of drought tolerance of these accessions was different, particularly when comparing Iranian and CIMMYT accessions. No report was available on molecular marker studies of genetic diversity among salinity-tolerant wheat germplasm. However, Lindsay et al. (2004) reported an SSR marker closely linked to *Nax1*, a locus for sodium exclusion that gives salt tolerance in durum wheat, mapped on chromosome 2AL.

Deoxyribonucleic acid (DNA) markers are the most suitable means for genetic diversity estimation (O'Donoughue et al. 1994; Plaschke et al. 1995; Kim and Ward 1997). However, the extent of their utility may depend on the type of the DNA markers, level of polymorphisms they reveal, and their genome coverage. Markers that can detect higher levels of polymorphism between wheat varieties can be utilized more efficiently to estimate genetic diversity. AFLP is such a marker class that can generate high levels of polymorphisms even in low polymorphic species like wheat (Bai et al. 1999). It is a multiplex marker system in which several polymorphic bands can be produced per assay (Vos et al. 1995). In addition, AFLP analysis is highly reproducible making it a suitable marker system for genetic diversity analysis and high-resolution mapping (Zabeau 1993).

Since genetic diversity for traits such as drought and salinity tolerance is limited in conventional wheat, introgression of genes from wild relatives into elite cultivars has been a major wheat-breeding program objective within CIMMYT. *Aegilops tauschii* Coss. ($2n = 2x = 14$, DD), a diploid wheat relative, is a rich source of resistance genes to many biotic and abiotic stresses (Mujeeb-Kazi and Rajaram 2002). Synthetic hexaploid wheats ($2n = 6x = 42$, AABB-DD), which are generated by crossing tetraploid durum wheat ($2n = 4x = 28$, AABB) to various *Ae. tauschii* accessions, are an important bridging material for introgression of desirable genes from *Ae. tauschii* into bread wheat. CIMMYT has developed numerous synthetic hexaploid wheats using diverse durum cultivars and *Ae. tauschii* accessions. These synthetic wheats possess many other important biotic stress traits that can be utilized for wheat improvement (Mujeeb-Kazi 2003a), such as resistance to Fusarium head blight (*Fusarium graminearum* Schw.), leaf rust (*Puccinia triticina Eriks.*), Septoria tritici blotch (*Septoria tritici* Roberge in Desmaz.) (Mujeeb-Kazi et al. 2000), Karnal bunt (*Tilletia indica* Mitra) (Mujeeb-Kazi et al. 2001a), tan spot [*Pyrenophora tritici-repentis* (Died.) Drechs.], spot blotch (*Cochliobolus sativus*) (Mujeeb-Kazi et al. 2001b) and *Stagonospora nodorum* blotch [*Phaeosphaeria nodorum* (berk.) Castellani & Germano] (Cox 1998; Xu et al. 2004). A set of germplasm with high levels of tolerance to drought and saline environments has also been identified (Reynolds et al. 2005). The objectives of the present study were (i) to evaluate genetic diversity of the selected germplasm sets that contain different D genome accessional sources conferring tolerance to drought and salinity based on AFLP and (ii) to identify parents for developing doubled-haploid-based mapping populations (Mujeeb-Kazi 2003b).

MATERIALS AND METHODS

Plant Materials

Wheat accessions in this study include 14 accessions (drought accessions) with different levels of drought toler-

ance and 27 accessions (salinity accessions) with different levels of salinity tolerance. The drought accessions consisted of five conventional wheat cultivars, five synthetic hexaploid wheats, and four durum wheat cultivars that were parents of the respective synthetics (Table 1). Entries D1 to D5 are the synthetics with superior drought tolerance and are currently being used for drought tolerance wheat breeding in CIMMYT. Their advanced derivatives after crosses with bread wheat cultivars have also performed well under reduced irrigation. Among the five conventional wheat cultivars, cv. Opata is a susceptible check and an ideal drought sensitive parent for developing mapping populations. The salinity accessions consisted of 19 conventional wheat cultivars, seven synthetic hexaploid wheats and one durum wheat cultivar (Table 2). The durum wheat (PDW 34) and wheat cultivars Oasis, PBW 343, Galvez S87 and Yecora F70 are salinity-susceptible, while the rest of the accessions are salinity tolerant. The salinity-tolerant accessions have been selected based on potassium: sodium (K:Na) discrimination levels in hydroponics using protocols of Gorham et al. (1987) and Shah et al. (1987). All salinity-tolerant accessions had K:Na ratios over 2.5, where a ratio of close to 1.0 indicates salt sensitivity. Entry S5 (PDW 34), the durum susceptible check had a K:Na value close to 1.0. The synthetic wheats differed not only in sources of D genome, but also in their A and B genome compositions. The latter durum parent in every case was salt susceptible (Pritchard et al. 2002).

AFLP Assays

Genomic DNA was isolated from bulked wheat leaves of two to three seedlings (approximately 10 d old) using the CTAB procedure (Saghai-Maroof et al. 1984). AFLP analysis was performed using protocols described by Zabeau (1993) and Vos et al. (1995). Laboratory optimization and minor modifications for AFLP analysis were made according to Bai et al. (1999). Genomic DNA (300 ng) from each of the wheat entries was double digested with *Eco*RI and *Mse*I restriction enzymes. Following restriction digestion, *Eco*RI and *Mse*I adapters were ligated to the digested DNA fragments. Ligated DNA was diluted 10-fold for pre-amplification. Forty micro-liters of PCR reaction mixture contained 10 µL of the diluted DNA, 4 µL of 10X PCR buffer, 4 µL $MgCl_2$ (25 mM), 1.6 µL dNTPs (5 mM), 0.75 µL *Eco*RI pre-amplification primer (100 ng μL^{-1}), 0.75 µL *Mse*I pre-amplification primer (100 ng μL^{-1}), 0.15 µL *Taq* polymerase and 18.75 µL of deionized water. Pre-amplification PCR was done in a MJ thermocycler (MJ Research Inc., Waltham, MA) with the following thermal profile: 94°C for 1 min followed by 30 cycles at 94°C for 30 s, 56°C for 60 s and 72°C for 60 s. The PCR product was then analyzed on 1.5% agarose gel to confirm pre-amplification.

The pre-amplified DNA was diluted 10-fold. The PCR reaction mixture for selective amplification included 2 µL of the diluted DNA, 1 µL of 10X PCR buffer, 1 µL of 25 mM $MgCl_2$, 0.4 µL of 5 mM dNTPs, 0.35 µL of *Mse*I selective primer (50 ng μL^{-1}), 0.4 µL fluorescence-labeled *Eco*RI selective primer (1 ρmol μL^{-1}) from LI-COR (LI-COR Inc, Lincoln, NE), 0.04 µL *Taq* polymerase, and 4.8 µL deionized water. The PCR thermal cycles were as follows: 2 min at 94°C followed by 13 cycles at 94°C for 30 s, 65°C for 30 s, 72°C for 60 s with the annealing temperature lowered by 0.7 °C after each cycle; then followed by 23 cycles at 94°C for 30 s, 56°C for 30 s, 72°C for 60 s. A final extension was conducted for 5 min at 72°C. Twenty AFLP selective primer combinations were used for selective amplification (Table 3).

The PCR products from the selective amplification were mixed with 5 µL of loading buffer and denatured for 5 min at 95°C before 1 µL of this product from each sample was loaded on each well of a 6.5% denaturing Gel Matrix gel (Li-Cor Inc., Lincoln, NE). The gel was ran in 1X TBE buffer at 1500 V and 40W for 3.5 h in a Li-Cor automated DNA sequencer (Li-Cor Inc., Lincoln, NE). DNA size standard from Li-Cor was used as a reference to calculate molecular size of each AFLP fragment.

Data Analyses

AFLP bands ranging from 70 to 350 base pairs were scored as present (1) or absent (0). Unambiguous fragments were entered as 0.5 in the data matrix. In order to ensure accurate scoring, all markers were scored at least twice. Polymorphism rates were estimated for all possible pairs of lines by dividing the number of polymorphic bands by the total number of bands. Polymorphic information content (PIC) was estimated using the formula used by Anderson et al. (1993):

$$\mathrm{PIC} = 1 - \sum_{i=1}^{n} p_i^2$$

where p_i is the frequency of the *i*th allele. Each polymorphic fragment was scored as a locus with two allelic classes. The maximum PIC value of an AFLP locus was 0.5. Cluster analysis and principal components analysis was conducted using the procedures in the NTSYS-pc software (Rohlf 2000). Genetic similarity between entries was estimated using the similarity coefficients of Jaccard (1908), Rogers and Tanimoto (1960) and Nei and Li (1979). The resulting distance matrices were used for cluster analysis by the UPGMA [unweighted pair-group method with arithmetic averages (Sneath and Sokal 1973)] method. The goodness of fit of the clustering to the data matrix was assessed by cophenetic correlation using the NTSYS-pc software (Rohlf 2000).

RESULTS

Analyses of 41 wheat entries (14 for drought and 27 for salinity) with 20 AFLP primer combinations produced a total of 918 scorable AFLP fragments (Table 3). A partial AFLP image generated from Li-Cor 4200 DNA analyzer showing typical AFLP variation when a single pair of AFLP primers was used for selective amplification is shown in Fig. 1. Of the 918 markers, 368 were polymorphic among all 41 wheat entries (drought and salinity entries combined) studied, 348 were polymorphic among the drought entries, while 310 were polymorphic among the salinity entries. For all 41 entries, the number of polymorphic bands per primer combination ranged from 8 to 33 with an average of 18.4 ± 1.5 (Table 3). For the drought germplasm, the number of poly-

Table 1. Thirteen drought-tolerant entries comprising durum parents, synthetic wheats, and conventional wheat cultivars and a susceptible conventional wheat check used for analysis of genetic diversity based on AFLP

ENTRY	Name (pedigree)	Accession type
D1	CPI/Gediz/3/Go//Jo69/Cra/4/*Ae. tauschii* (208)	Synthetic
D2	Yav 3/Sco//Jo69/Cra/3/Yav79/4/*Ae. tauschii* (498)	Synthetic
D3	D67.2/P66.270//*Ae. tauschii* (257)	Synthetic
D4	Gan/ *Ae. tauschii* (897)	Synthetic
D5	Decoy 1/ *Ae. tauschii* (458)	Synthetic
D6	Nesser	Wheat
D7	Sitta	Wheat
D8	Dharwar Dry	Wheat
D9	Weebill 1	Wheat
D10	Opata (Blue Jay/Jupateco 73)	Wheat
DP1	CPI/Gediz/3/Go//Jo69/Cra	Durum
DP2	D67.2/P66.270	Durum
DP3	Gan	Durum
DP4	Decoy 1	Durum

Table 2. Twenty-six salinity-tolerant and -susceptible conventional and synthetic wheat entries and a susceptible durum wheat check used for analysis of genetic diversity based on AFLP

ENTRY	Name (pedigree)	Accession type
S1	Shorawaki (Land race)	Wheat
S2	WH 157	Wheat
S3	Kharchia 65 (Land race)	Wheat
S4	Lu 26-S	Wheat
S5	PDW 34	Durum
S6	SNH-9	Wheat
S7	KRL 1-4	Wheat
S8	Galvez S87	Wheat
S9	Oasis F86	Wheat
S10	Chinese Spring	Wheat
S11	Ciano T79	Wheat
S12	Yecora F70	Wheat
S13	KRL – 19	Wheat
S14	PBW 343	Wheat
S15	Pericu (Chil/Prl)	Wheat
S16	Mepuchi (Buc/Bjy/Prl)	Wheat
S17	Cochimi (Seri * 3//Buc 'S')	Wheat
S18	Calafia (PFAU//Ald/Pvn/3/Myna/Vul)	Wheat
S19	Oasis	Wheat
S20	Hahn/Parula	Wheat
S21	Chen/*Ae. tauschii* (205)	Synthetic
S22	68.111/Rgb-u//Ward Resel/3/Stil/4/ *Ae.tauschii* (781)	Synthetic
S23	Altar 84/*Ae. tauschii* (224)	Synthetic
S24	Altar 84/*Ae. tauschii* (502)	Synthetic
S25	Altar 84/*Ae. tauschii* (211)	Synthetic
S26	Ceta/*Ae. tauschii* (1027)	Synthetic
S27	Croc 1/*Ae. tauschii* (224)	Synthetic

morphic bands per primer combination ranged from 8 to 30 with an average of 17.4 ± 1.4, while for the salinity entries, the number of polymorphic bands ranged from 6 to 26 with an average of 15.5 ± 1.4 per primer combination. Polymorphism rates were estimated at 40, 38, and 34% for all 41 entries, drought entries, and salinity entries, respectively.

Pair-wise comparisons were conducted between the genotypes based on the AFLP data. Jaccard's similarity coefficient was used to evaluate the genetic diversity among the accessions. Within all 41 entries, the highest similarity coefficient (0.92) was between synthetics S22 and S25 and the lowest similarity coefficient (0.18) was between the drought entry D7 and the salinity entry S5 (the salinity-susceptible durum wheat check). The average similarity coefficient for all 41 entries was 0.53 ± 0.01. The result from pair-wise comparisons indicated that about 37% of the entry pairs had similarity coefficients of 0.50 or less (Fig. 2). Within the drought entries, the highest similarity coefficient (0.79) was between synthetics D2 and D4 although they were unrelated based on their pedigree information. The lowest similarity coefficient (0.16) was between entry DP2 (a durum wheat) and D7 (a conventional wheat). The average similarity coefficient for drought entries was 0.43 ± 0.02. Pair-wise comparisons indicated that more than half of the entry pairs had similarity coefficients of 0.50 or less (Fig. 3). Within the salinity entries, the highest similarity coefficient (0.92) was between synthetics S22 and S25 and the lowest similarity coefficient (0.16) was between the entry S5 (the salinity-susceptible durum wheat check) and S6 (a conventional wheat cultivar), with an average similarity coefficient of 0.57 ± 0.01. About 31% of the pair-wise comparisons had similarity coefficients of 0.50 or less (Fig. 4).

For all 41 entries, PIC ranged from 0.05 to 0.50 with an average PIC of 0.30 ± 0.01 (Fig. 5). For the drought entries, PIC ranged from 0.13 to 0.50 with an average PIC of 0.37 ± 0.01 (Fig. 6), while for the salinity entries, PIC ranged from 0.07 to 0.50 with an average PIC of 0.29 ± 0.01 (Fig. 7). PIC of AFLP in drought entries was high and mainly appeared between 0.40 and 0.50 (Fig. 6), while PIC of AFLP in salinity entries was relatively lower with two peaks appearing in both distribution extremes (Fig. 7).

In general, high cophenetic correlations ranging from 0.873 to 0.944 were obtained (Table 4) where $r > 0.9$ indicates a very good fit; $0.8 < r < 0.9$ indicates a good fit; $r < 0.8$ indicates a poor fit (Capo-chichi et al. 2001). All three methods used in these analyses gave a good fit with the best cophenetic correlation from the Jaccard coefficient (Table 4). No major variations were observed in the dendrogram patterns obtained by the three similarity coefficients; therefore, only the dendrogram obtained based on the Jaccard coefficients is presented. The dendrogram involving all 41 wheat accessions grouped the accessions into three main clusters (Fig. 8). Cluster one consisted of all five durum entries used in this study [one from the salinity entries (S5) and four from the drought entries (DP1 to DP4)]. The second cluster consisted of all conventional wheat entries (D6 to D10) from the drought entries and 15 of the 19 conventional wheat entries and one synthetic wheat entry from the salinity entries. The third cluster had all the five synthetic wheat entries (D1 to D5) from the drought entries and five of the six synthetic wheat entries from the salinity entries. This cluster also contained four conventional wheat entries from the salinity entries. The similarity matrix based on the Jaccard's coefficient was also used as input for principal component analyses (PCA). A plot of the first three principal components for all 41 accessions is presented in Fig. 9.

Table 3. List of AFLP primers used in DNA fingerprinting of 41 drought and salinity-tolerant and susceptible wheat germplasm entries with the total and polymorphic bands amplified for them

Primer pair	Total markers amplified	Polymorphic markers			% polymorphism		
		All entries[z]	Drought tolerant	Salinity tolerant	All entries	Drought tolerant	Salinity tolerant
e-ACT/m-CAC[y]	47	17	17	14	36.2	36.2	29.8
e-ACT/m-CAT	60	26	26	25	43.3	43.3	41.7
e-ACT/m-TGC	60	18	16	18	30.0	26.7	30.0
e-ACT/m-AGTG	55	22	22	19	40.0	40.0	34.5
e-ACT/m-CAGT	60	33	30	24	55.0	50.0	40.0
e-AAC/m-CAC	40	21	19	14	52.5	47.5	35.0
e-AAC/m-CAT	30	11	11	11	36.7	36.7	36.7
e-AAC/m-TGC	35	8	8	6	22.9	22.9	17.1
e-AAC/m-AGTG	35	11	11	8	31.4	31.4	22.9
e-AAC/m-CAGT	20	10	9	6	50.0	45.0	30.0
e-AGT/m-CAC	60	21	20	18	35.0	33.3	30.0
e-AGT/m-CAT	58	16	16	13	27.6	27.6	22.4
e-AGT/m-TGC	45	12	11	10	26.7	24.4	22.2
e-AGT/m-AGTG	35	16	14	13	45.7	40.0	37.1
e-AGT/m-CAGT	65	28	28	26	43.1	43.1	40.0
e-GCTG/m-CAC	41	14	12	10	34.1	29.3	24.4
e-GCTG/m-CAT	50	26	25	25	52.0	50.0	50.0
e-GCTG/m-TGC	45	17	15	16	37.8	33.3	35.6
e-GCTG/m-AGTG	37	24	22	20	64.9	59.5	54.1
e-GCTG/m-CAGT	40	17	16	14	42.5	40.0	35.0
Preamplification primers							
*Eco*RI	5′-GACTGCGTACCAATTC-3′						
*Mse*I	5′-GATGAGTCCTGAGTAA-3′						

[z]Drought and salinity entries combined.
[y]Where e and m represent the selective amplification primers of *Eco*RI and *Mse*I, respectively.

The results from PCA are similar to those obtained by UPGMA clustering (Fig. 8). The five durum entries separated from wheat at the first principal component (PC1), and the second principal component (PC2) contained the cluster consisting mainly of the conventional wheat entries. The third principal component (PC3) contained the cluster consisting mainly of the synthetic wheat entries. The genetic constitution of the synthetics was different from the conventional wheat cultivars studied, thus suggesting that synthetics would add diversity to the drought- and salinity-tolerant germplasm.

The dendrogram for the drought accessions grouped the accessions into two main clusters (Fig. 10). Cluster one consisted of the four durum parents (DP1 to DP4). Cluster two consisted of all five synthetics and five conventional wheat lines. The second cluster formed two sub-clusters that were separated at the 46.5% similarity level with one sub-cluster consisting of the five conventional wheat lines and the other consisting of the five synthetics. Durum parents were distant from both the synthetics and the conventional wheat lines. The plot of the first three principal components for the drought accessions is presented in Fig. 11. The results from PCA are similar to those obtained by UPGMA clustering (Fig. 10). The four durum entries separated from conventional and synthetic wheat entries at the first principal component (PC1), and conventional wheat entries separated from synthetics at the second principal component (PC2). The third principal component (PC3) separated individual accessions within each major cluster. The clustering of the drought entries also indicated that genetic constitution of synthetics was different from the conventional wheat cultivars studied and thus the synthetics would add more diversity to the drought-tolerant germplasm.

The dendrogram for the salinity accessions (Fig. 12) shows that this clustering method grouped 26 of the 27 entries into two major clusters while entry 27 (S5, the salinity-susceptible durum wheat check) remained by itself. The two major clusters merged into one cluster at the similarity level of 50.4% and the susceptible durum check (S5) merged with this cluster at the similarity level of 28%. The durum check lacks the D genome; therefore, it is genetically distant from the synthetics and conventional wheat cultivars. Of the two major clusters, one cluster consisted of six of the seven synthetics and four conventional wheat lines. Another cluster consisted of one synthetic and the remaining conventional wheat lines. The PCA results (Fig. 13) also support the results obtained by UPGMA cluster analysis (Fig. 12). PC1 separated two major distinct groups of wheat, while PC2 clearly separated the durum check (S5) from the two wheat groups. PC3 separated individual accessions within each major group consisting of both conventional wheat cultivars and synthetics.

DISCUSSION

Synthetic wheat lines developed at CIMMYT have many valuable traits including drought and salinity tolerance that can be used for genetic improvement of wheat. The present study was aimed at measuring genetic diversity among several drought- and salinity-tolerant synthetic and conventional wheat lines using AFLP markers and to identify parents

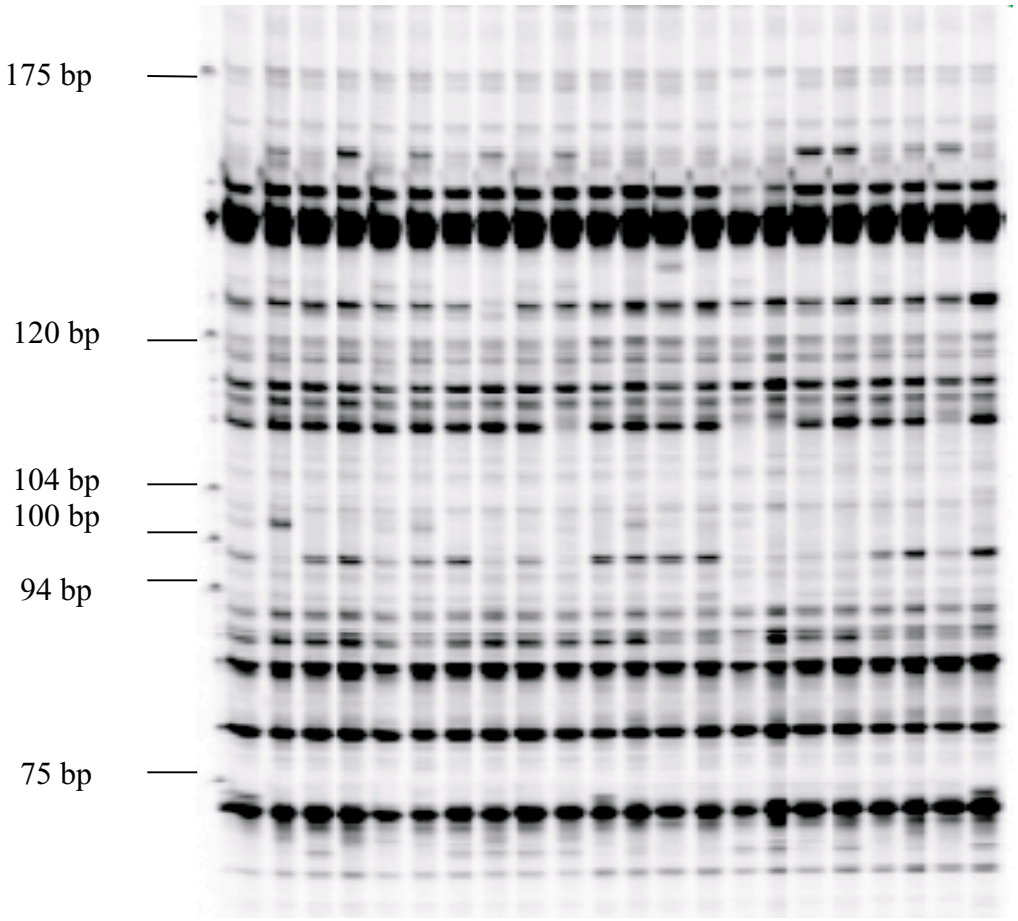

Fig. 1. An example AFLP fingerprints produced by primer pairs *Eco*RI + ACT/*Mse*I + AGTG.

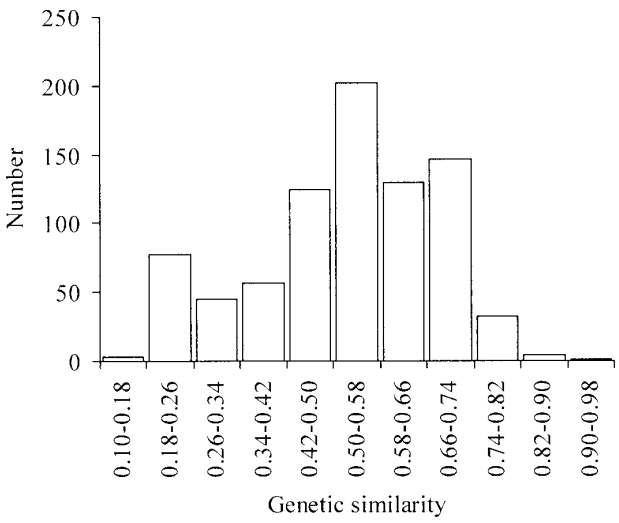

Fig. 2. Distribution of genetic similarities among 41 wheat entries estimated from 368 polymorphic AFLP markers.

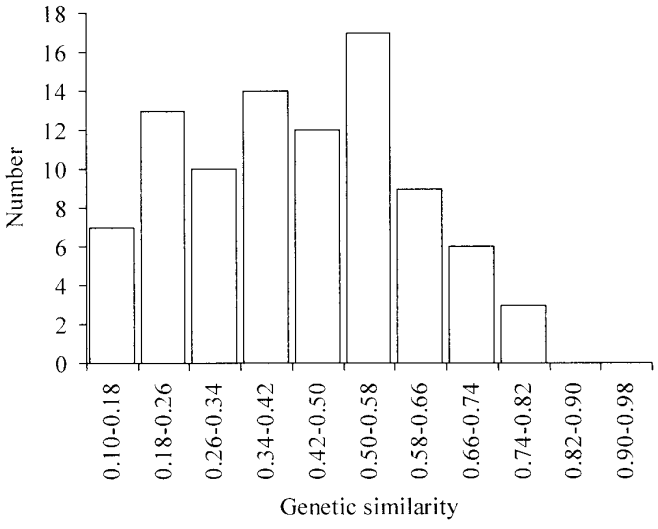

Fig. 3. Distribution of genetic similarities among 13 drought-tolerant wheat entries and a drought-susceptible wheat check entry estimated from 348 polymorphic AFLP markers.

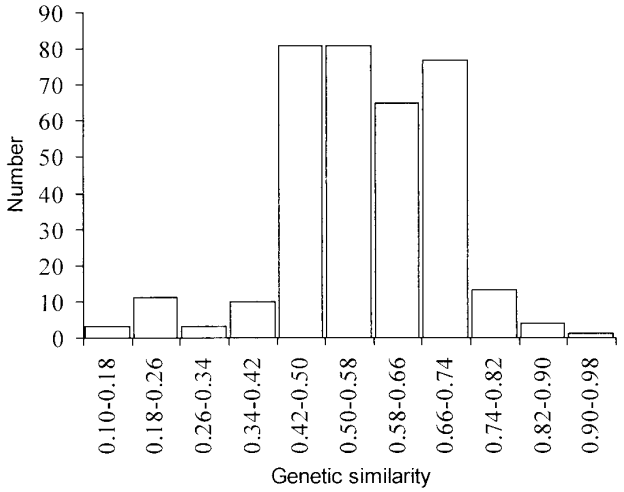

Fig. 4. Distribution of genetic similarities among 26 salinity-tolerant and susceptible wheat entries and a salinity-susceptible durum wheat check estimated from 310 polymorphic AFLP markers.

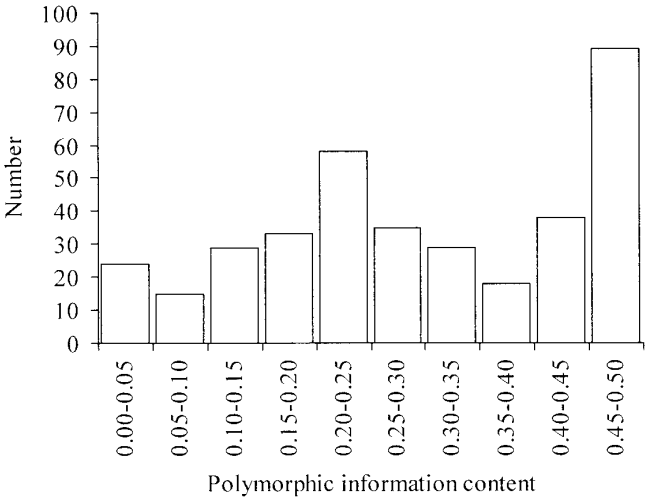

Fig. 5. Distribution of polymorphic information content scores for 368 AFLP markers among 41 wheat entries.

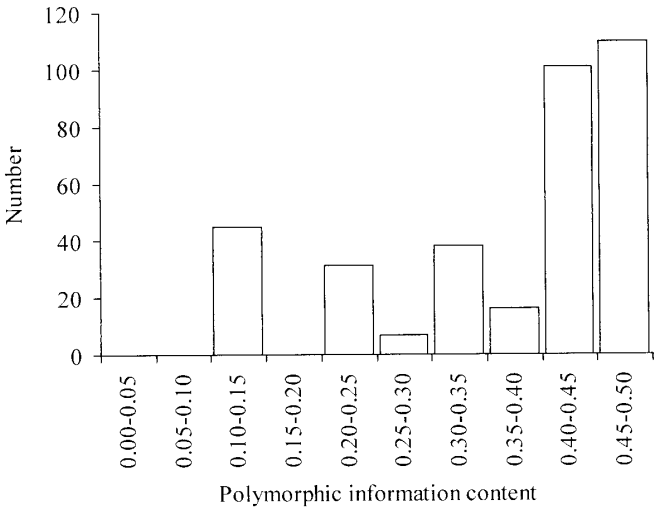

Fig. 6. Distribution of polymorphic information content scores for 348 AFLP markers among 13 drought-tolerant wheat entries and a drought-susceptible wheat check entry.

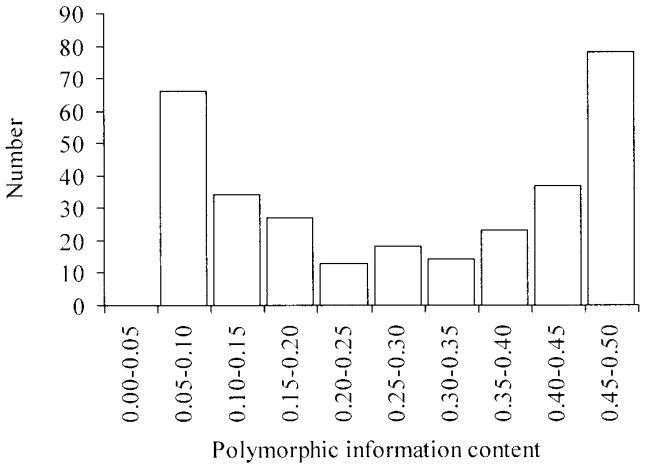

Fig. 7. Distribution of polymorphic information content scores for 310 AFLP markers among 26 salinity-tolerant and susceptible wheat entries and a salinity-susceptible durum wheat check.

for developing doubled-haploid-based mapping populations (Mujeeb-Kazi 2003b). Twenty primer combinations used in this study revealed substantial genetic diversity among the drought-tolerant and salinity-tolerant wheat germplasm.

When all 41 wheat accessions were analyzed together, the average similarity coefficient was 0.53 with the lowest similarity coefficient being 0.18. Within the drought entries, the average similarity coefficient was 0.43 with the lowest similarity coefficient being 0.16. The lowest similarity coefficient for the salinity entries was the same as that for drought entries, but the average was higher (0.57). These results and the distribution of the similarity coefficients indicated substantial amounts of genetic diversity among the drought and salinity entries. Based on 117 polymorphic AFLP markers, Bohn et al. (1999) reported genetic similarity among 11 wheat lines ranging from 0.40 to 0.83 with an average similarity of 0.61. Using AFLP markers Barrett and Kidwell (1998) reported mean genetic diversity estimates of 0.58 for pair-wise comparison of spring vs. winter wheat, 0.53 for within winter wheats and 0.49 for within spring wheats. Soleimani et al. (2002) using AFLP markers reported a mean pair-wise genetic distance of 0.40 for several Canadian durum wheat cultivars.

Our results of the average PIC (0.30 ± 0.01 for all 41 entries, 0.37 ± 0.01 for drought and 0.29 ± 0.01 for salinity entries) were similar to that of Manifesto et al. (2001) who reported an average PIC value of 0.30 ± 0.15 in wheat for AFLP markers. However, the range of PIC values (0.26 to 0.38) reported by Manifesto et al. (2001) were smaller than the ranges of PIC values (0.05 to 0.50 for all 41 entries, 0.13

Table 4. Cophenetic correlation values obtained using three methods to determine similarity coefficients

Entries	Nei and Li (1979)	Jaccard (1908)	Rogers and Tanimoto (1960)
All entries[z]	0.933	0.944	0.911
Drought	0.894	0.920	0.873
Salinity	0.932	0.942	0.942

[z]Drought and salinity entries combined.

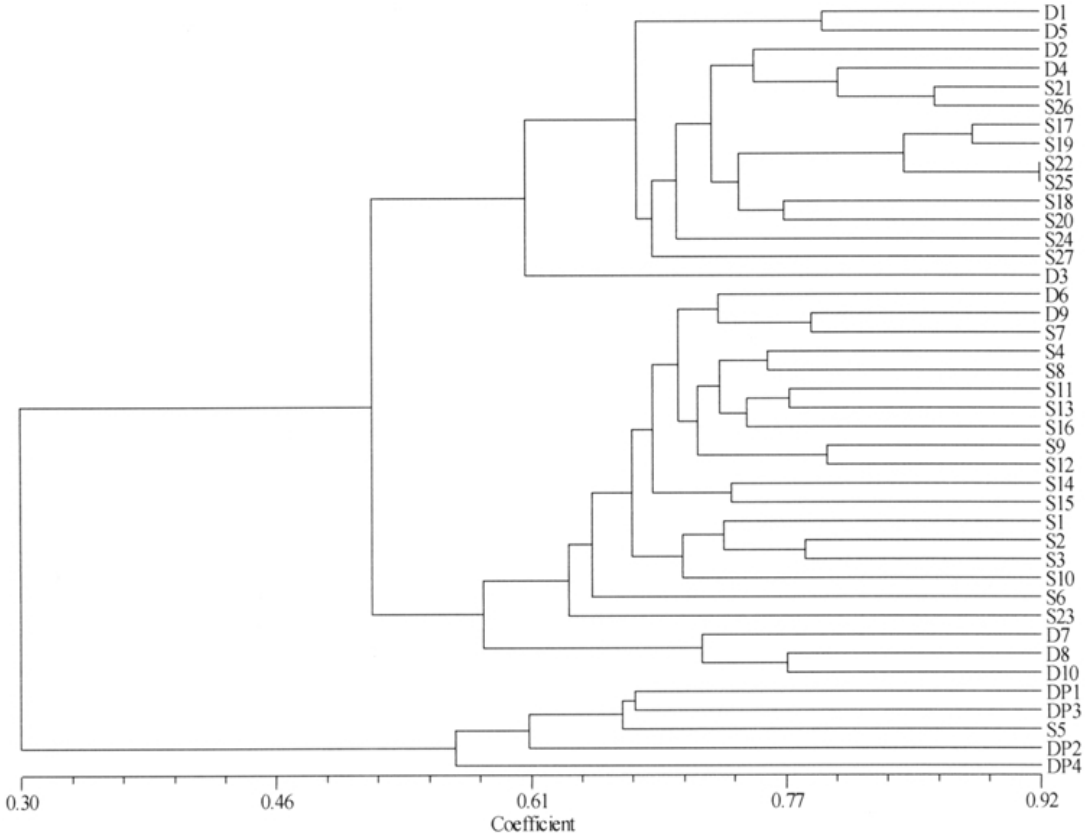

Fig 8. Dendrogram from the Jaccard's similarity coefficients based on 41 wheat entries and 368 AFLP markers. Entries D1 to D5, drought-tolerant synthetic wheat; D6 to D9 drought-tolerant conventional wheat; D10 drought-susceptible conventional wheat check; DP1 to DP4, drought-tolerant durum parents; S1 to S4 and S6 to S20, salinity-tolerant and susceptible conventional wheat; S5, salinity-susceptible durum check; S21 to S27 salinity-tolerant synthetic wheat.

to 0.50 for drought entries and 0.07 to 0.50 for salinity entries obtained in our study. PIC is a quantification of the number of alleles or bands that a marker has and the frequency of each of the alleles or bands in the entries under study. Since a marker with fewer bands has less power to distinguish between entries and alleles present at low frequency also have less power to be distinguished, a higher PIC was assigned to a marker with many alleles and with alleles present at roughly equal proportions in the entries under study. Thus, PIC can be looked as the measurement of usefulness of each marker in distinguishing one individual from another. For AFLP, each polymorphic fragment was scored as a locus with two allelic classes and the maximum PIC of an AFLP locus is 0.5. Therefore, the PIC values in our study indicated that the AFLP markers were reasonably powerful in distinguishing one individual from another. This was in congruence with Manifesto et al. (2001).

Dendrograms (Figs. 8, 10, and 12) obtained by cluster analysis using UPGMA and Jaccard's similarity coefficient show that there was substantial diversity at the DNA level among the drought-tolerant and salinity-tolerant germplasm. When all the 41 entries were used in the cluster analysis, the five durum entries were grouped together as expected. One cluster contained all conventional wheat and only one synthetic wheat line and the other cluster contained mainly synthetics but some conventional wheat entries. This indicated that most of the synthetics were distant from the conventional wheats. These two later clusters contained both salinity and drought entries. This indicated that these drought and salinity entries had enough genetic similarity to cluster together.

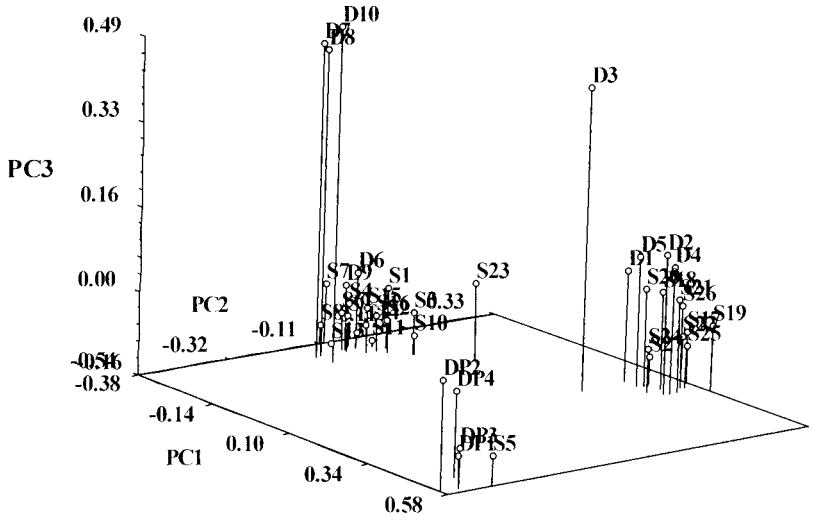

Fig. 9. Plot of first three principal components from the Jaccard's similarity matrix based on 41 wheat entries and 368 AFLP markers. Entries D1 to D5, drought-tolerant synthetic wheat; D6 to D9 drought-tolerant conventional wheat; D10 drought-susceptible conventional wheat check; DP1 to DP4, drought-tolerant durum parents; S1 to S4 and S6 to S20, salinity-tolerant and susceptible conventional wheat; S5, salinity-susceptible durum check; S21 to S27 salinity-tolerant synthetic wheat.

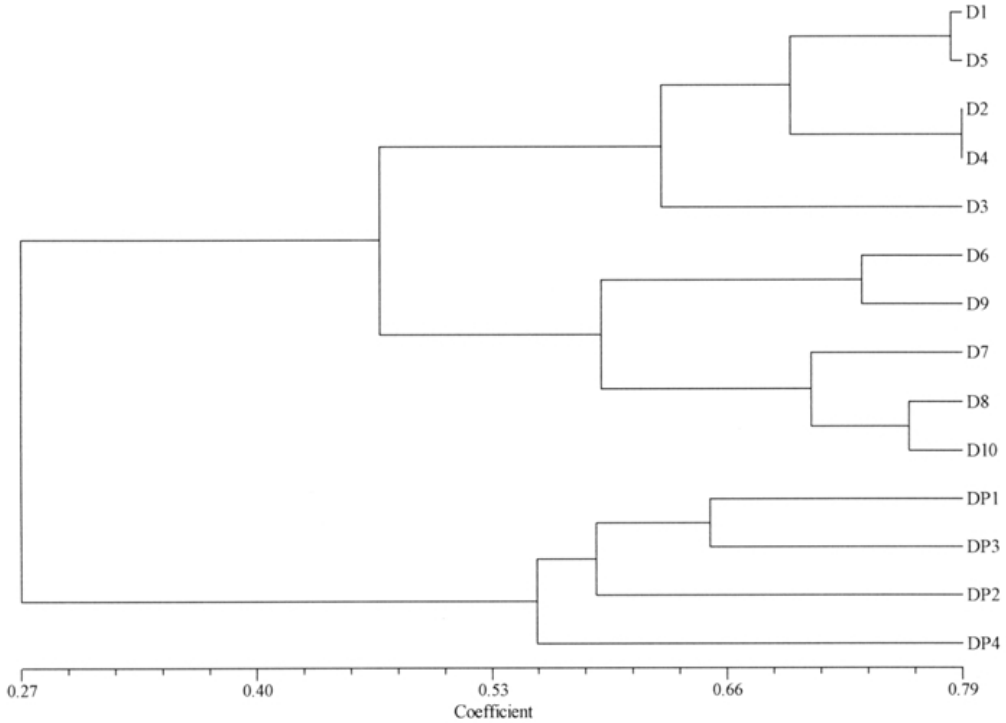

Fig. 10. Dendrogram from the Jaccard's similarity coefficients based on 13 drought-tolerant wheat entries and a drought-susceptible wheat entry and 348 AFLP markers. Entries D1 to D5, drought-tolerant synthetic wheat; D6 to D9 drought-tolerant conventional wheat; D10 drought-susceptible conventional wheat check; DP1 to DP4, drought-tolerant durum parents.

For the drought entries, the four parents were grouped together in both PCA and cluster analysis, that was expected because they were all durum entries with AABB genomes. It was interesting to note that four of the synthetics developed from these four parents by crossing each to a different *Ae. tauschii* accession were closer to each other, suggesting that genetic constitution of different *Ae. tauschii* accessions involved in these crosses could be similar to each other. The two sub-clusters of the drought accessions merged at the 46.5% similarity level indicating that they

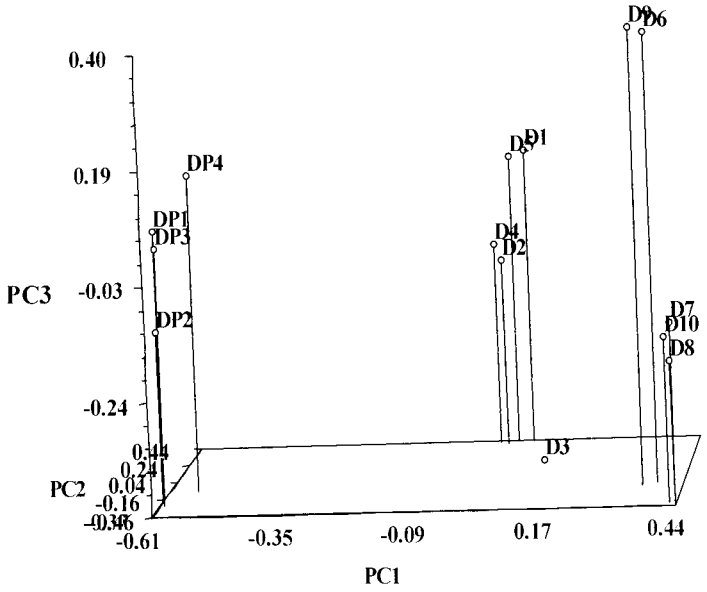

Fig. 11. Plot of first three principal components from the Jaccard's similarity matrix based on 13 drought-tolerant wheat entries and a drought-susceptible wheat entry and 348 AFLP markers. Entries D1 to D5, drought-tolerant synthetic wheat; D6 to D9 drought-tolerant conventional wheat; D10 drought-susceptible conventional wheat check; DP1 to DP4, drought-tolerant durum parents.

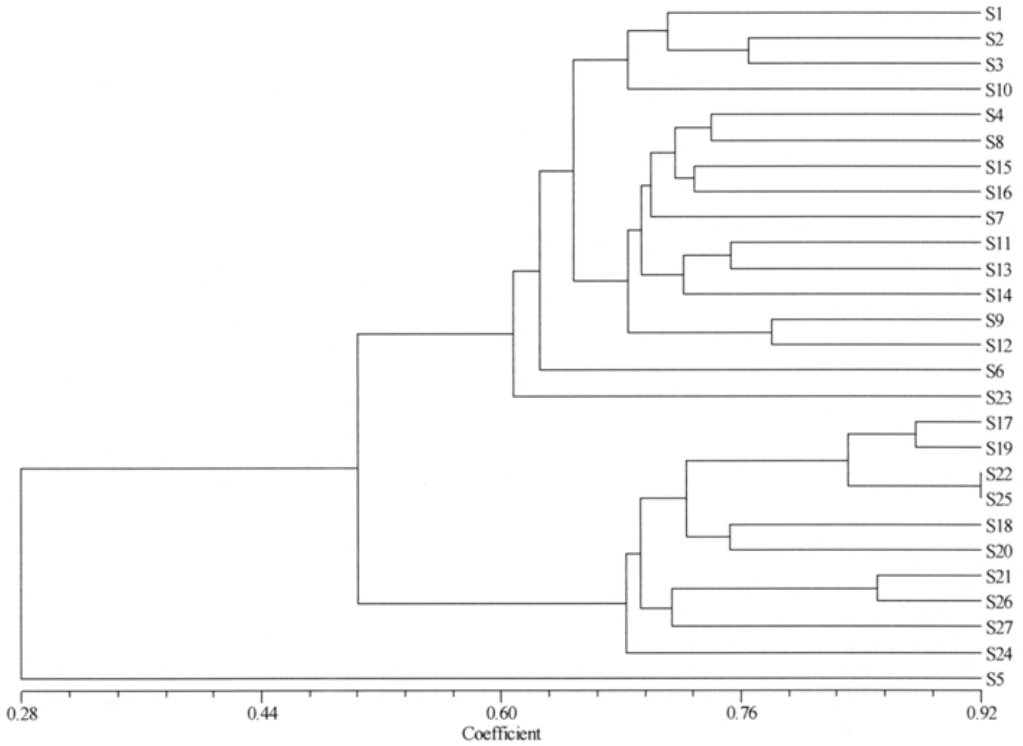

Fig. 12. Dendrogram from the Jaccard's similarity coefficients based on 26 salinity-tolerant and susceptible wheat entries and a salinity-susceptible durum check entry and 310 AFLP markers. Entries S1 to S4 and S6 to S20, salinity-tolerant and susceptible conventional wheat; S5, salinity-susceptible durum check; S21 to S27 salinity-tolerant synthetic wheat.

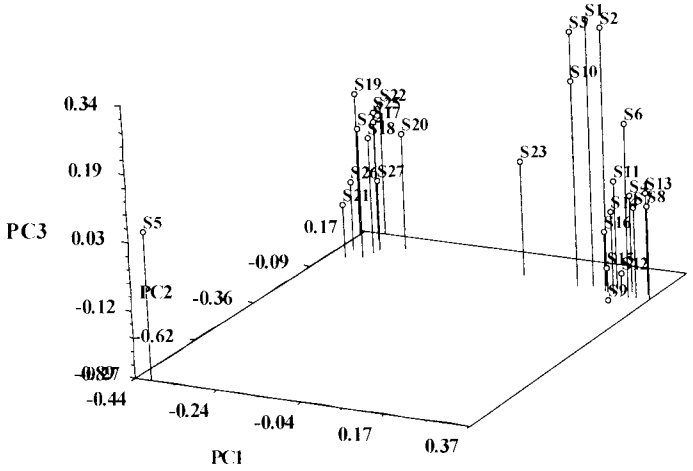

Fig. 13. Plot of first three principal components from the Jaccard's similarity matrix based on 26 salinity-tolerant and susceptible wheat lines and a durum wheat control and 310 AFLP markers. Entries S1 to S4 and S6 to S20, salinity-tolerant and susceptible conventional wheat; S5, salinity susceptible durum check; S21 to S27 salinity-tolerant synthetic wheat.

were distantly related at the genetic level. It was interesting to note that one of these two sub-clusters contained the five conventional wheat lines and the other contained the five synthetics. Although both the conventional wheat lines and the synthetics have the same genome, AABBDD, they exhibited differences due to their earlier conventional and recent synthetic origin. This also indicated that AFLP markers were powerful enough to reveal such genetic distinction. It would be possible to select appropriate drought-tolerant parents from both groups for a breeding program to enhance genetic diversity for drought tolerance. Our results are in congruence with those of Moghaddam et al. (2005) who reported existence of genetic diversity among drought-tolerant wheat entries from CIMMYT and Iran.

The salinity entries formed two major clusters that merged at the similarity level of 50.4%. These two groups were clearly separated by the second principal component. The result indicated a high level of genetic diversity among these entries. Using these entries to breed for salinity tolerance would enhance genetic diversity of salinity-tolerant cultivars. As expected, accession S5, the salinity susceptible durum wheat check stood alone in both the dendrogram and the PCA plot and merged with the other accessions at the similarity level of 28%. This result again indicated that AFLP fingerprinting provided a very accurate measurement of genetic relationship among diverse entries. Both synthetics and conventional wheat entries in each of two sub-clusters indicated substantial genetic diversity within the salinity-tolerant wheat and synthetic accessions.

The synthetic and conventional wheat entries used in this study have shown different degrees of tolerance to drought and salinity. Genetic diversity assessment using AFLP has clearly indicated that there exists ample diversity among these entries at the DNA level and therefore some entries would be very valuable when breeding objectives target drought and salinity tolerance. Some of the entries would be valuable for using as parents for developing doubled-haploid-based mapping populations.

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