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Molecular Markers Based Identification of Diversity for Drought Tolerance in Bread Wheat Varieties and Synthetic Hexaploids

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Abstract
The complexity of the wheat genome has delayed the development and application of molecular markers to this species and wheat now lies behind barley, maize and rice in marker development. However, improvements in marker detection systems and in the techniques used to identify markers linked to useful traits has allowed considerable advances to be made in recent years. To evaluate the genetic diversity 53 genotypes of Richard's selection, were studied at National Agriculture Reseach Center (NARC) Islamabad. The present study found that RAPD analysis is a valuable diagnostic tool. Different sets of RAPD primers were used to study the polymorphism at molecular level. Highest number of amplifications was shown by primer OpG-2 in Richard's material. Coefficient of similarity as well as genetic distances among these three sets of materials was calculated by using Unweighted Pair Group of Arithamatic Means (UPGMA) function (Nei and Li, 1979). The SHs derived genotypes of Richard's selection were highly polymorphic with a polymorphism percentage of 69.70 as compared to NUYT (rainfed) and elite Pakistani bread wheat varieties with a polymorphism of 44.44% and 61.11% respectively. Cluster analysis was done in which grouping of genotypes was done on the basis of genetic distances. Cluster analysis revealed that genotypes of Richard's genotypes are showing high level of among cultivar variation as compared to NUYT (Rainfed) and elite Pakistani drought tolerant bread wheat varieties. These genotypes were also phenotypically evaluated.

Introduction
It is vital for plant breeding programmes to have sufficient diversity available to allow for the production of new varieties that are aimed towards the improvement of crop productivity and able to withstand damage from biotic and abiotic factors. In this respect, efforts have also been made to predict the prospects of developing superior genotypes from a cross by the measurement of a genetic similarity (GS) or genetic distance (GD) between the parents, since the later can be used as an estimation of expected genetic variances in different sets of segregating progenies derived, from different crosses. The availability of genetic variability in elite wheat material is pre requisite for any breeding programme aimed towards the improvement of wheat productivity. Wheat breeding through hybridization also requires the selection of diverse genotypes, irrespective of whether the end product is pure line or a hybrid variety.

Wheat is used mainly for human consumption and supports nearly 35% of the world population. It is nutritious, easy to store and transport and can be processed into various types of food. The demand for wheat is expected to grow faster than any other major agricultural crop. To meet the needs of the growing world population, the forecast demand for the year 2020 varies between 840 (Rosegrant *et al.* 1995) and 1050 million tons (Kronstad, 1998). Due to land limitations, the enhancement of wheat production must come from higher absolute yields, which can only be met by the concerted action of scientists involved in diverse agricultural disciplines and in particular by increased efforts in plant breeding (Braun *et al*. 1998). In addition to continuous investments in conventional breeding methods, the better understanding of the current and expanded genetic diversity and biotechnological tools should be considered for raising the yield frontier in wheat. Pakistan, as a developing country has less share in global wheat production. Its consumption is increasing day by day due to ever increasing population at 3.0 percent per anum Wheat occupies 70% of Rabi (winter season) and 37% of total cropped area in Pakistan. During the year 2005-2006, it has been cultivated on 8.33 million hectares with an annual production of 21.109million tons. (Govt. of Pak. 2005-06). About 20% area of wheat is under rainfed condition, where moisture is the major limiting factor. Drought affects every aspect of the plant growth and the ability to yield well under stress is conditioned by different physiomorphic traits. Considerable efforts have been made in the past, in beeding for improved drought tolerance in field crops. Struggles have been made primarily to the use of empirical breeding approaches by concentrating on yield and components of yield in wheat. These traits are genetically complex and are not easy to manipulate, hence, little success has been achieved to develop drought tolerant wheat varieties over the last 50 years. A number of morphological traits like plant height, pubescence, pigmentation, and especially drought tolerance have been identified in wheat and are relatively simple in inheritance.

The best option for yield improvement and yield stability under soil moisture deficit conditions is to develop drought tolerant crop varieties through molecular approaches e.g application of molecular markers like RAPD (Randomly Amplified Polymorphic DNA). These makers are preferably used to detect polymorphism of genetic material of agronomically important drought tolerant cultivars. Molecular

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markers provide a direct measure of genetic diversity and go beyond indirect diversity measures based on agronomic traits or geographic origin. Simple sequence repeats are highly polymorphic in wheat and, therefore, suitable for the discrimination of genotypes. They are generally genome specific, abundant, codominant, and cover all 21 wheat chromosomes. They have been successfully employed to characterize genetic diversity in seed bank collections of improved wheat germplasm and wild relatives(Li *et al*. 2000 and Hammer, 2000). The germplasm improvement and genetic diversity is the key to durable and sustained production of wheat. The principal aim of this work was to enrich the gene pool of cultivated wheat varieties by tapping the vast genetic resources available in the plant's wild relatives. Priority candidates were A and D genome accessions for primary pool belonging to *Triticum boeoticum, Triticum monococum, Triticum urartu and Aegilops tauschii (Syn Ae. Squarosa, Triticum tauschii)*. Objective of our conducted study was molecular characterization of germplasm by using RAPD and emphasizing primarily on D genome.

Materials and methods

The experimental material comprised of 53 genotypes of Richard's selection (Table 1).This material was evaluated at National agriculture Research Centre (NARC) Islamabad.

Molecular diagnostics

The material was subjected to molecular evaluations for determining their DNA based diversity. This was done by using RAPD primers and the protocol is described below.

DNA extraction

In the growth room 5 to 7 cm long pieces of fresh leaf material were cut from the plants (3 week-old seedlings) and were placed in 1.5 ml eppendorf tubes. The tubes were subsequently dropped in the liquid Nitrogen to rapidly freeze the leaf material. The plant material was then crushed to a fine powder with a knitting needle while still inside the tube. 500 µl DNA extraction buffer (1% SDS, 100mM NaCl, 100mM tris base, 100mM Na_2EDTA, PH: 8.5 by HCl) was added to each eppendorf tube containing the crushed leaf material and was mixed well with the help of a knitting needle. 500 µl

Table 1. Pedigree List of Richard's Selection

Pop#	Pedigree
1	EXCALIBUR/WBLL1
2	TEV2/FRET2
3	CETTIA/WBLL1
4	TC870344/GU1//TEMPORALERA M 87/AGR/3/WBLL1
5	PASTOR/3/KAUZ*2/OPATA//KAUZ/4/CHEN/AE.SQ//2*OPATA
6	PVN/3/PRL/SARA//TSI/VEE#5/4/WBLL1
7	BAV92/3/PRL/SARA//TSI/VEE#5/4/WBLL1
8	PASTOR/3/URES/JUN//KAUZ/4/WBLL1
9	1455/2*PASTOR
10	AUS 4930.7/2*PASTOR
11	KRICHAUFF/2*PASTOR
12	PAM94/3/ALTAR 84/AEGILOPS SQUAROSA(TAUS)//OPATA/PASTOR
13	T. DICOCCON PI94625/AE.SQUAROSA (372)//FRET2/3/2*WBLL1
14	T.DICOCCON PI225332/AE.SQUAROSSA (895)//WBLL1/3/2*WBLL1
15	ATTILA/WBLL1
16	FRET2/3/CHEN/AE.SQ//2*OPATA
17	FRET2/3/CHEN/AE.SQ//2*OPATA
18	FRET2/3/CHEN/AE.SQ//2*OPATA
19	DOY1/AE.SQUAROSA(333)/3/PRL/VEE#6//CHOIX/4/HAHN/PRL//CLMS/3/ HAHN/PRL
20	ARLIN_1/AE.SQUARROSA (1017)//ATTILA/3/ATTILA*2/M10
21	KLCQ/KASO2
22	TUI//2*SUNCO/SA1166/3/TUI/4/FINSI
23	HXL7573/2*BAU//WBLL1
24	GPO8 KAZAKSTAN 6 WM98-99/4/KAUZ//ALTAR 84/AOS/3/ KAUZ/5/ /KAUZ//ALTAR 84/ AOS/3/KAUZ
25	68.111/RGB-U//WARD/3/FGO/4/RABI/5/AE.SQUARROSA (882)/6/ ATTILA/10/…
26	RABE/2*MO88/3/CAZO/KAUZ//KAUZ
27	URES/JUN//KAUZ/3/ALTAR 84/AE. SQ//2*OPATA
28	CROC_1/AE.SQUARROSA (205)//KAUZ/3/ENEIDA/4/FINSI
29	FILIN/IRENA/5/CNDO/R143//ENTE/MEXI-2/3/AEGILOPS SQUAROSA (TAUS)/4/WEAVER/6/…
30	CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUAROSA (TAUS)/4/OCI/5/…
31	MILUN/BERKUT
32	FILI/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUAROSA (TAUS)/4/WEAVER/6/BERKUT
33	FILI/IRENA/5/CNDO/R143//ENTE/MEXI_2/3/AEGILOPS SQUAROSA (TAUS)/4/WEAVER/6/BERKUT
34	FILIN/3/CROC_1/AE. SQUAROSA (205)//KAUZ/4/FILIN/5/VEE/MJI//2* TUI/3/PASTOR
35	FILIN/3/CROC_1/AE. SQUAROSA (205)//KAUZ/4/FILIN/5/VEE/MJI//2* TUI/3/PASTOR
36	PRINIA/BERKUT
37	CROC_1/AE. SQUAROSA (205)//KAUZ/3/PRL/SARA//TSI/VEE#5
38	QT8343//PASTOR*2/OPATA
39	JARU/3/URES/JUN//KAUZ/4/URES/JUN/KAUZ
40	CROC_1/AE.SQUARROSA (205)//KAUZ/3/2*PJN/BOW//OPATA
41	TAN//TEMPORALERA M 87/AGR/3/NG8319//SHA4/LIRA
42	BABAX/3/PRL/SARA//TSI/VEE#5/4/CROC_1/AE.SQUARROSA (224)//2*OPATA
43	JNRB.5/PIFED
44	JNRB.5/PIFED
45	CROC_1/AE.SQUAROSSA (205)//BORL95/3/KENNEDY
46	CNDO/R143//ENTE/MEXI_2/3/ AEGILOPS SQUARROSA (TAUS)/4/ WEAVER/5/2*JANZ
47	D67.2/P66.270// AE.SQUARROSA (320)/3/CUNNIGHAM
48	CROC_1/AE.SQUAROSSA (205)//BORL95/3/KENNEDY
49	CROC_1/AE.SQUAROSSA (205)//BORL95/3/KENNEDY
50	CHEN/AEGILOPS SQUARROSA (TAUS)//BCN/3/BAV92
51	KAUZ*2/BOW//KAUZ/3/W98.6.38
52	SARA/THB//VEE/3/VEE/PJN//2*KAUZ
53	CHEN/AE.SQ//WEAVER/3/ATTILA*2/M10(MUTATED C-306)

phenol: chloroform: isoamylalcohol (in the ratio of 25:24:1) was added and tubes were well shaken until a homogenous mixture is made. Samples were then centrifuged at 5000 rpm for 5 minutes. The aqueous phase (supernatant) was transferred to a fresh tube. To precipitate the DNA 50 µl 3M sodium acetate (pH= 4.8) and 500µl isopropanol was added to the tube and mixed gently. To make the DNA pellet, samples were centrifuged at 5000 rpm for 5 minutes. After pouring the supernatant, pellet was washed with 70% ethyl alcohol. Pellet was dried at room temperature for an hour and was resuspended in 40 µl TE buffer (10mM Tris, 1mM EDTA and PH: 8.0) (Weining and Langridge, 1991). To remove RNA, DNA was treated with 40µg RNAase-A (0.20 µl of commercially supplied RNAase-A purchased from Gene Link, USA) at 37°C for 1 hour. After RNAase treatment, DNA samples were run on 1.0% gel to check the quality of DNA and then was stored at 4°C. To use in Polymerase Chain Reaction (PCR) a 1:5 dilution of DNA was made in doubled distilled, deionized and autoclaved water.

Polymerase chain reaction

PCR reactions were carried out in 25µl reaction containing 50-100 ng total genomic DNA templates, 0.25µM of each primer, 200µM of each dATP, dGTP, dCTP, dTTP, 50mM KCl, 10mM Tris, 1.5mM $MgCl_2$ and 2.5 units of Taq DNA polymerase. The amplification conditions were as; an initial step of denaturation for 1 minutes at 94°C followed by 45 cycles each consisting of a denaturation step of 1 minute at 94°C, an annealing step of 1 minute at 34°C and an extension step of 2 minutes at 72°C. Seven minutes were given after the last cycle to the extension step at 72°C to ensure the completion of the primer extension reaction. GeneAmp PCR system 2700 was used for all amplification reactions.

Gel electrophoresis

For electrophoresis of the amplification products, 1.5 % agarose/TBE gel was used. Gels were visualized by Ethidium Bromide under the UV light chamber and observed using the computer program UVIPhotoMW.

DNA Marker

The GeneRuler^{TM} 1kb DNA Ladder (Catalogue # SM0313, Lot: 00018968, Concentration: 0.1µg/µl) by Fermentas was used for sizing and approximate quantification of wide range double stranded DNA fragments on agarose gel. The ladder was premixed with 6X Loading Dye Solution for direct loading on gel.

Statistical analysis

For statistical analysis of RAPD the scorable bands were considered as a single locus/allele. The loci were scored as present or absent. Bivariate 1-0 data matrix was generated. Genetic distances were calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedure as follows (Nei and Li, 1979).

$$GD_{xy} = 1- d_{xy}/d_x+d_y-d_{xy},$$

Where:

GD_{xy} = Genetic distance between two genotypes, x (1) and y (2)

d_{xy} = total number of common loci (bands) in two genotypes

d_x = Total number of loci (bands) in genotype 1 and

d_y = Total number of loci (bands) in genotype 2.

The 1-0 bivariate data matrix for each set of wheat lines based on the data of RAPD primers were used to construct dendrogram using computer program “Popgene32” version 1.31(The 1-0 bivariate data matrix for each set of wheat lines based on the data of RAPD primers were used to construct dendrogram using computer program “Popgene32” version 1.31 (http://www.ualberta.ca./~fyeh/fyeh).

Results

Genetic diversity evaluation

In this study PCR based random decamer primers were used. Randomly Amplified Polymorphic DNAs (RAPD) were used to detect genetic polymorphism at DNA level in 53 genotypes of Richard’s selection.Only the scorable bands were included in the analyses. Every single band was considered as a single locus/allele for all the genetic analyses. The loci were scored as present / absent. Unweighted Pair Group of Arithamatic Means (UPGMA) function (Nei and Li, 1979) was used to estimate genetic distances between the genotypes.

Genetic diversity evaluation of Richard’s selection

Out of total 46 available primers initially we used 10 primers and out of which 2 primers had shown relatively better amplification in terms of band number and sharpness. The total numbers of loci traced by these primers were 33 and out of these 21 were polymorphic. The percentage of polymorphism among these genotypes was 69.70%. Figure 1 shows the amplification profile of 53 genotypes selected by Richard by using RAPD primer OpG-2(GGCACTGAGG). This primer has amplified highest number of genotypes. Maximum number of bands detected was three and minimum was one. Out of 53 genotypes the genomic DNA of 33 genotypes had been amplified by this primer. The size of scorable bands that were detected in amplification profile by using this primer ranged from 250 to 2500bp.

According to Fig.1 three scorable bands were detected in genotypes number 1, 4, 3, 5, 6, 7, 10, 11,14,15,16, 17 and 19. These genotypes were monomorphic for the band length of 750-2000bp. Similarly three bands were scored in genotypes 30 and 31 having size of 500 to 1500 bp. Two scorable bands were detected in genotype number, 20, 21, 23,25,27,33 and 37, and the size of these scorable bands was from 250-1500bp. One scorable band was detected in genotype number 30, 32,34,35,36, and 38, and the size of this scorable band was from 500-750bp. Two bands were scored in genotypes 40, 41, 44 and 47 having a band size of 750 to 2000bp. According Fig.1 the band having a length of 1500bp was found in genotypes 1, 2,3,4,5,6,10,11,15,16 and 19 and this band was not found in remaining genotypes by using primer OpG-2. So these genotypes are monomorphic with respect to this band but polymorphic as compared with remaining genotypes that were lacking this band. A band of size 750 to 1000bp is present only in the genotypes 40, 41, 44 and 47 these genotypes are monomorphic with respect to this band but polymorphic as compared with other genotypes. No amplification was shown by this primer in remaining genotypes, hence these were not included

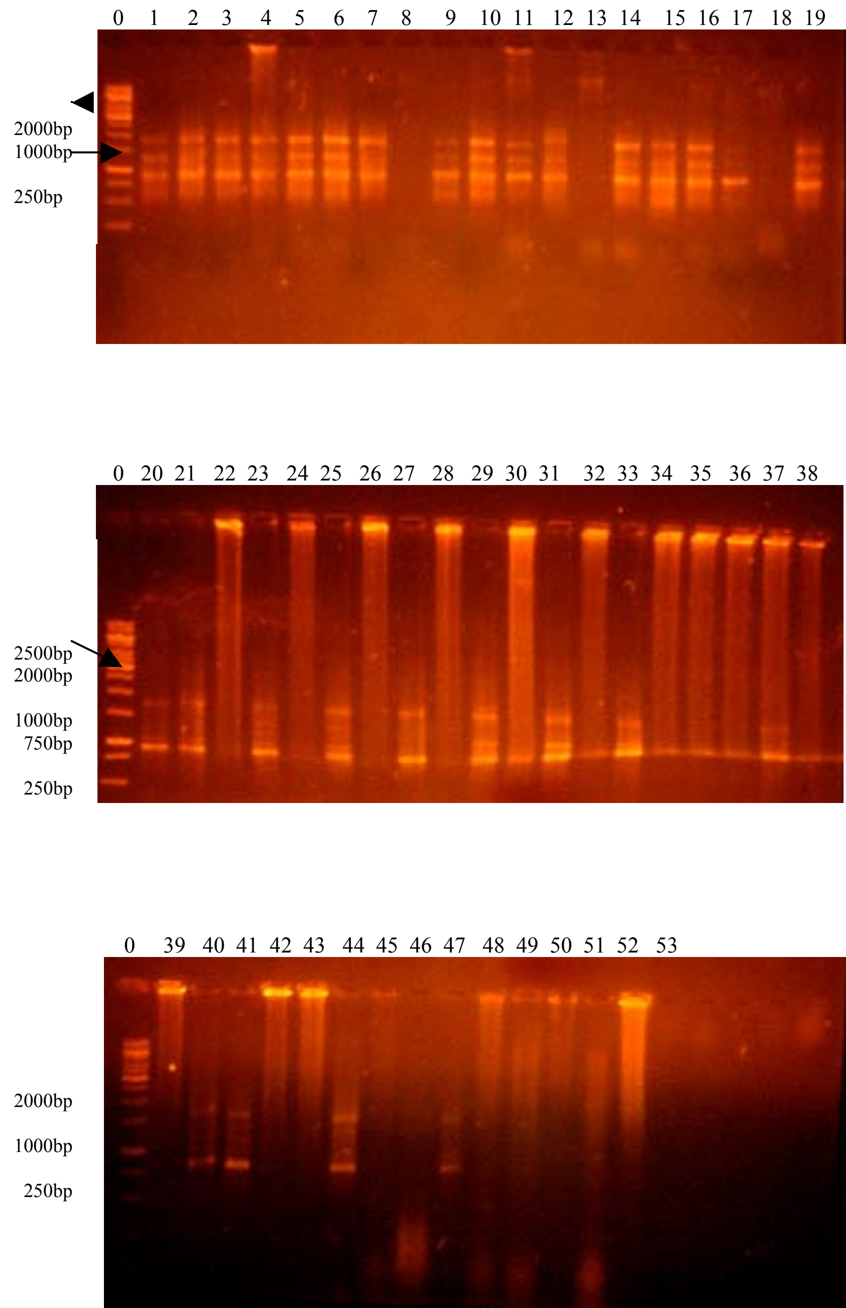

Figure 1. Agarose (1.7%) gel showing PCR amplification products of decamer primer OpG-2 (GGCACTGAGG) from DNA of various wheat genotypes of Richard's selections. Lane 0 is molecular weight marker (GeneRulerTM 1kb DNA Ladder, Cat # SM0313, Fermentas) and lanes 1-53 are representing the genotypes in the same order as indicated in Table 1.

in analysis. Other markers did not show amplifications in these genotypes. The value of similarity coefficient range from 0.6364(63.64%)-1.00(100%). Genotype 1and 8 are showing least similarity of 63.64%.

Dendogram interpretation of richard's genotypes

For proper cluster analysis we devide it into four clusters A, B, C and D (Fig 2). Dendogram as shown in Fig. 3 has been formulated on the basis of genetic distances. CLUSTER A: This cluster include total twelve genotypes pop-1,pop-2,pop-5,pop-7,pop-9,pop-3,pop-11,pop-17,pop-25,pop-29,pop-6 and pop-10(Sequence is in accordance to dendogram). In this cluster minimum genetic distance zero is present between pop-2, pop-5, pop-7 and pop-9. So we can say these genotypes are genetically identical and are not genetically diversified. In this cluster at point 43, pop-1 is showing maximum genetic diversity of 4.78 percent in comparison to the all members which are included in subcluster 40.

The genetic distance of remaining genotypes of this cluster lies between the ranges of 0.00 to 4.78 percent.

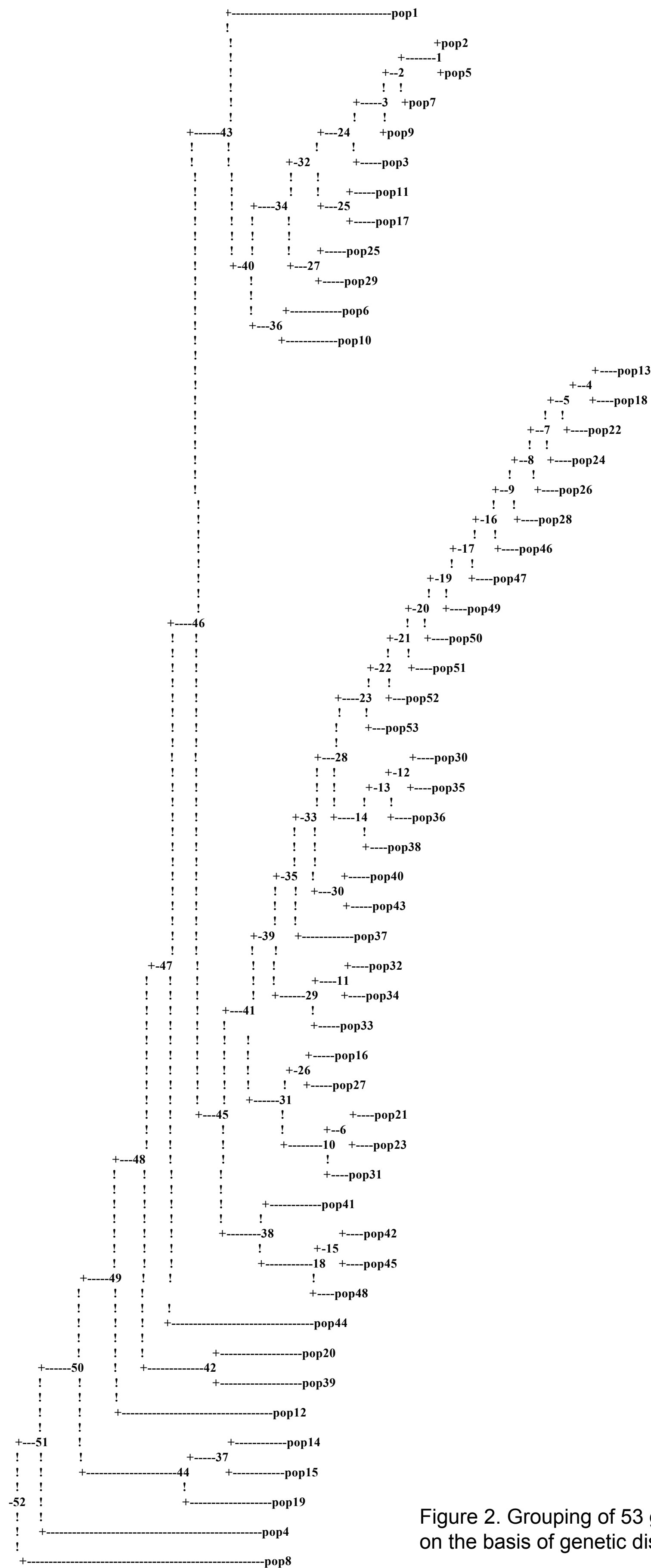

Figure 2. Grouping of 53 genotypes of Richard's selection on the basis of genetic distances.

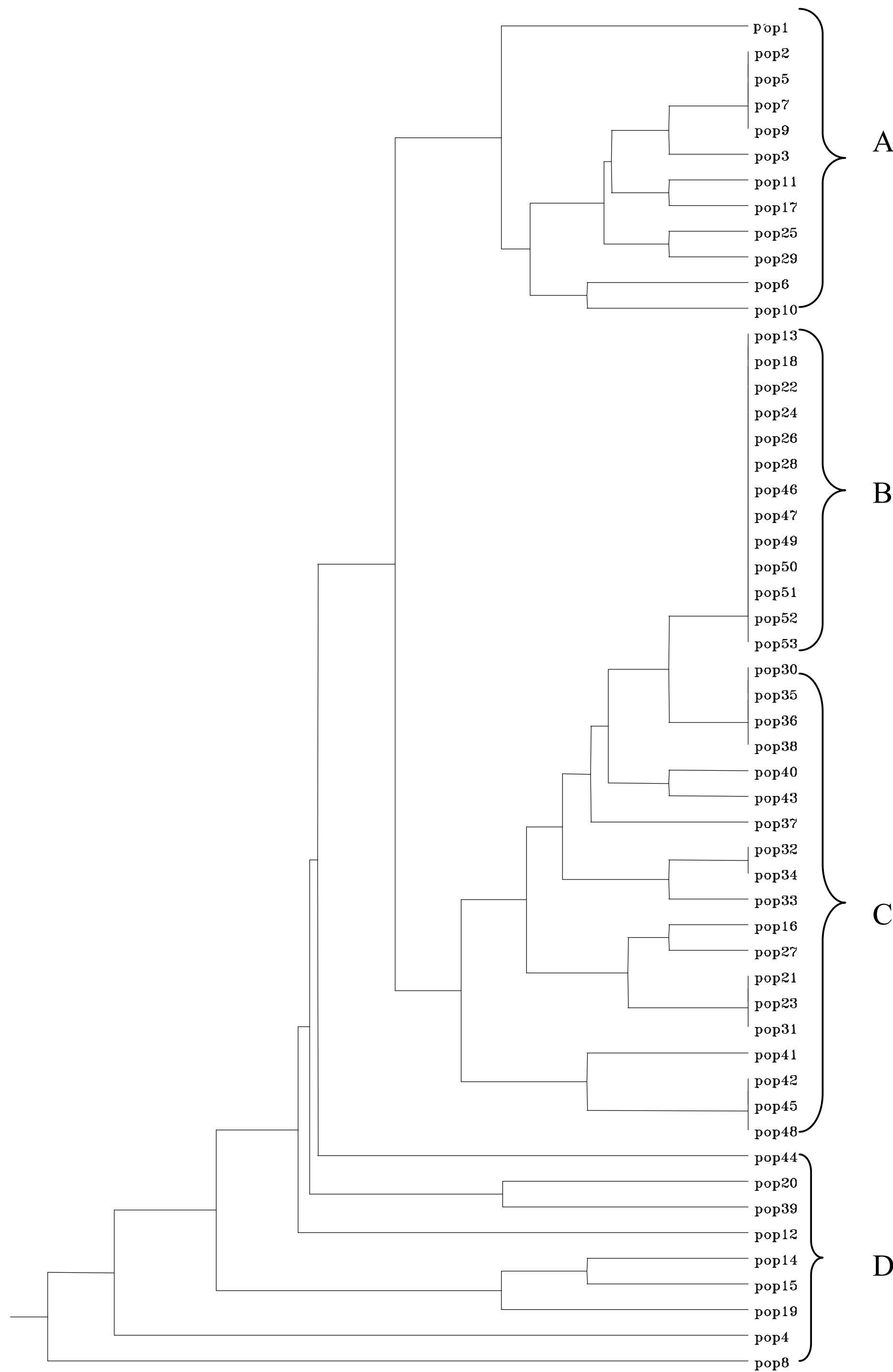

Figure 3. Dendogram formulated on the basis of genetic distances. See text for details.

CLUSTER B: This cluster include total twelve genotypes pop-13,pop-18,pop-22,pop-24,pop-26,pop-28,pop-46,pop-47,pop-49,pop-50,pop-51,pop52 and pop-53. These genotypes show zero percent genetic diversity among them. So we can say these genotypes are identical. CLUSTER C: this cluster include total nineteen genotypes pop-30,pop-35,pop-36,pop-38,pop-40,pop-43,pop-37,pop-32,pop-34,pop-33,pop-16,pop-27,pop-21,pop-23,pop-31,pop-41,pop-42,pop-45 and pop-48. In this cluster zero percent genetic distance is present among genotypes,

pop-30,pop-35,pop-36 and pop-38

pop-32 and pop-34

pop-21,pop-23 and pop-31

pop-42, pop-45 and pop-48

So genotypes which are showing zero percent genetic distance with each other are identical.

Within this cluster maximum genetic distance is shown by genotype pop-41. This genotype is showing a genetic

distance of 3.13 percent with the genotypes pop-48, pop-45 and pop-42 which are included within group 38 of dendogram. So genotype pop-41 is showing more diversity within this cluster as compare to remaining genotypes. While the genetic distance of remaining genotypes lie within the range of 0.00 to 3.13%. CLUSTER D: This cluster includes total nine genotypes i.e pop-44, pop-20, pop-39, pop-12, pop-14, pop-15, pop-19, pop-4 and pop-10. This cluster includes more genetically diversified genotypes i.e pop-8 and pop-4. These two genotypes are genetically more distinct as compare to the genotypes of all clusters. Pop-8 is showing a maximum genetic distance of 13.66 percent as compare to the all genotypes grouped at point 52 in dendogram. Similarly pop-4 is showing a genetic distance of 12.3 percent as compared to the all genotypes grouped at point 51 in dendogram. So pop-4 and pop-8 have shown high degree of polymorphism in molecular diversity analysis in comparison to the genotypes of all other clusters. From this cluster analysis it is revealed that pop-1, pop-4 and pop-8 are highly diversified genotypes. Genotypes of cluster A are showing a genetic distance of 2.07 percent with cluster B and cluster C. While cluster B is showing a genetic distance of 1.54 percent with cluster C. similarly Cluster D is showing a genetic distance of 1.5 percent as compare to cluster B, cluster C and cluster D.

Discussion

Over the past century, the development and successful application of plant breeding methods has produced high-yielding crop varieties upon which modern agriculture is based. New varieties are usually bred by crossing a set of genetically related modern varieties, followed by an intensive selection in succeeding generations (Ceccarelli *et al*. 1987). Introduction of valuable genes from exotic donors via wide crosses has been proposed to broaden the genetic base of many crop plants with known and closely related wild relatives (Zohary *et al*.1969). Forms in the primary gene pool have genetic proximity to the genomes A, B, and D of bread wheat thus all conventional wheat breeding utilizes its cultivars that reside in this gene pool (Coghlan 2006). Several types of diversity can be measured in the context of breeding programs (Smale *et al*. 1996). Apparent and latent genetic diversity are directly related to the performance of crops. Measures of apparent diversity are manifested in phenotypic differences of populations or cultivars in the field (Souza *et al.* 1994). Latent diversity refers to parentage analysis and molecular measurements that are not necessarily expressed in crop performance or phenotypes. Consequently, the genetic variation of crop plants is continued to be reduced by plant breeding (Tanksley and McCouch, 1997). It is the plant breeding process itself that threatens the genetic base on which breeding depends (Russell et al. 2000). In the light of these developments, the main objective of our research was to examine the genetic diversity in modern wheat breeding materials and genetic resources provided by and stored at CIMMYT. There has been a large transformation in the productivity of wheat due to the application of Green Revolution technology (Byerlee and Moya, 1993). The development of DNA markers in wheat is somewhat problematic due to three features. First, the size of the wheat genome (16×10^9 bp, compared to barley or maize with 5×10^9 bp), which makes the application of several marker techniques difficult. Second, the hexaploid nature of wheat adds complexity to many marker assays (Chao *et al.* 1989). Three sets of bands usually appear (often in the same size range), which are difficult to manage and interpret. Third, there is a generally low level of polymorphism in wheat relative to other cereal crops. This implies that a larger number of markers must be screened than in the case of rice, barley or maize (Chao *et al.* 1989; Lui *et al*. 1990). Furthermore, the level of polymorphism is not consistent across genomes and crosses. Commonly, the D genome tends to have the poorest marker coverage (Chalmers *et al*. 2001). Lack of genetic polymorphism in crops such as wheat and soybeans, and the consequent problems to identify molecular markers, has been a major limitation to the impact of marker assisted selection (MAS) in wheat breeding (Sorrells and Wilson 1997).

Criteria for the estimation of genetic diversity can be different pedigree records, morphological traits or molecular markers (Heckenberger et al. 2002). Molecular markers detect variation of the DNA sequences among cultivars and therefore directly bypass problems connected with environmental effects (Cox *et al*. 1985 and Maric *et al*. 1998). In 1991 Welsh and McClelland (Welsh *et al*.1999) developed a new PCR-based genetic assay namely randomly amplified polymorphic DNA (RAPD). This procedure detects nucleotide sequence polymorphisms in DNA by using a single primer of arbitrary nucleotide sequence. On an average, each primer directs amplification of several discrete loci in the genome, making the assay useful for efficient screening of nucleotide sequence polymorphism between individuals (Tingey *et al*. 1993). During the past decade the focus shifted to surveys at the level of DNA. Using DNA markers, diversity is measured as the average allelic divergence between any two individuals for given loci (Huang *et al*. 2002). Sufficiently large numbers of samples allow robust analyses of open questions in population genetics (Hamrick and Godt 1990). The random amplified polymorphic DNA (RAPD) technique, regardless of its sensitivity to reaction conditions and problems with repeatability and amplifying of non-homologous sequences has been successfully used for the assessment of genetic diversity in diploid, tetraploid and hexaploid wheat (Sivolap *et al*. 1999). In hexaploid wheat, large genome size, a high proportion of repetitive DNAs, continuous inbreeding caused by self-pollination and a narrow genetic base represent the di.culties for use of molecular markers (Joshi and Nguyen, 1993). Knowledge of genetic diversity among adopted cultivars or elite breeding materials has a significant impact on the improvement of crop plants (Franco *et al.* 2001). It can be obtained from pedigree analysis, morphological traits or using molecular markers (Pejic *et al*. 1998). Molecular markers can support a more detailed characterization of genetic resources (Paull *et al.* 1998). A vast potential lies in their ability to identify the structure of genetic diversity within and among accessions, which can be of great importance for the optimization of collections, the planning of seed regeneration, and the successful implementation of prebreeding approaches (Borner *et al*. 2000).

SSRs show potential for large-scale DNA fingerprinting of wheat genotypes due to the high level of polymorphisms detected their ability to be analyzed using automated systems and their high accuracy and repeatability (Christiansen et al. 2002). The T1BL1RS translocation in wheat also has a demonstrated advantage in dry land wheat areas, and

search for other diverse sources to exploit continues. One such unique gene pool resides in the primary Triticeae diploid *Ae. tauschii* (Dvorak *et al.* 1998). We have combined this diploid grass with elite durum cultivars to produce synthetic hexaploids. Field testing under reduced irrigation over the past several years have led to the identification of some synthetics classified as drought tolerant (Mujeeb-Kazi, 2003) evaluated that one successful method for introducing variation from the progenitors of BW into the elite breeding germplasm is the creation of synthetic hexaploid wheats (SHWs) (Mujeeb-Kazi et al. 1996). SSRs were applied to characterize the genetic relationships between hexaploid wheat landraces with different geographical origins and compare the results with a previous study carried out using AFLP and RAPD markers (Strelchenko *et al*. 2003). Simple sequence repeats (SSRs) represent the most suitable marker system in wheat (Li *et al*. 2000 and Hammer, 2000). They allow an even coverage of the genome, are abundant, genome specific, co-dominant in nature, and have been successfully used to characterize genetic diversity in advanced wheat breeding materials (Dreisigacker *et al*. (2004). The 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).

Drought is a major wheat production constraint, synthetic hexaploid yielded enormous desirable diversity when screened under reduced irrigation (Mujeeb Kazi, 1998). This whole study is basically a comparative study of elite Pakistani drought tolerant bread wheat varieties and SHs based derived lines. By using RAPD markers diversity is to be exploited at the molecular level. The annual increase in genetic potential in drought environments is only about half of that obtained in irrigated, optimum conditions (Trethowan *et al*. 2000). Genotypic analysis by using RAPD primer shows that SHs derived genotypes of Richards's selection are highly diversified as compare to Pakistani drought tolerant and rainfed genotypes. The polymorphism percentage among Richards's selection material was 69.70%. This reveals that the SHs derived genotypes of Richards's selection are highly diversified, and can be used for the improvement of local Pakistani cultivars.

Among SHs based material, pop-1, pop-4 and pop-8 are showing maximum genetic distance of 4.78%, 12.3% and 13.66% respectively with remaining genotypes. The genetic enhancement of germplasm pools in breeding programes has largely resulted from the wider utilization of plant genetic resources (Rajaram and Van Ginkel 1996). Bread wheat contains perhaps 30% of the diversity levels found in its diploid relatives. With the development of SHWs, which repeat the natural hybridization event, genetic diversity of *T. durum* and *T. tauschii* can be incorporated into modern breeding lines (Galili *et al*. 2000) (Diaby and Casler 2003). However, due to the stoichastic nature of DNA amplification with random sequence primers, it is important to optimize and maintain consistent reaction conditions for reproducible DNA amplification. They are dominant markers and hence have limitations in their use as markers for mapping, which can be overcome to some extent by selecting those markers that are linked in coupling. RAPD assay has been used by several groups as efficient tools for identification of markers linked to agronomically important traits, which are introgressed during the development of near isogenic lines. The application of RAPDs and their related modified markers in variability analysis and individual-specific genotyping has largely been carried out, but is less popular due to problems such as poor reproducibility faint or fuzzy products, and difficulty in scoring bands, which lead to inappropriate inferences. The identification of a high number of polymorphisms in single sequence repeats (SSR) therefore should greatly enhance the potential to find molecular markers in wheat (Snape 1998). SSRs are relatively uncomplicated in their use because low amounts of DNA are required and the assay can be automated (Roder *et al*. 2002).

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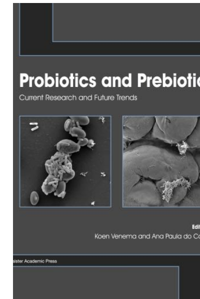

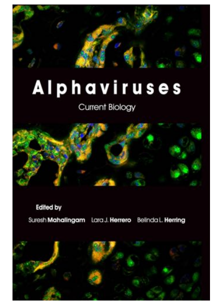

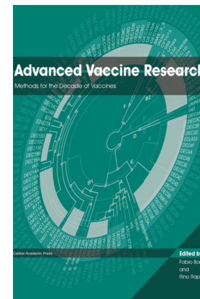

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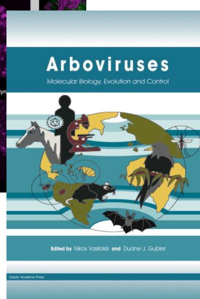

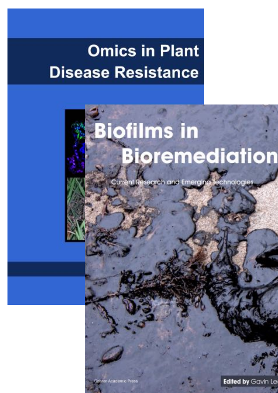

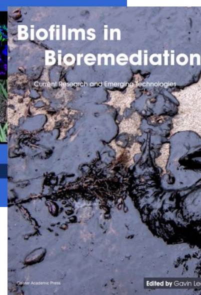

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