*INT. J. BIOL. BIOTECH.,* **10 (2): 221-228, 2013.**

# EVALUATION OF DIFFERENT WHEAT GENOTYPES FOR QUALITY TRAITS THROUGH MOLECULAR CHARACTERIZATION

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## ABSTRACT

The present study was conducted in the Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University, Rawalpindi and National Agricultural Research Center (NARC) Islamabad during the years 2010-2011. The experimental material consisted of thirty promising wheat genotypes obtained from various research Institutes of Pakistan. The research material was planted at NARC during *Rabi* 2010-11 and was then subjected to molecular characterization for estimation of genetic diversity. DNA extraction was done from wheat seeds of all genotypes and PCR reaction using SSR primers was carried out. The SSR primers generated 21 alleles with number of alleles per locus varying from 1 to 4. The highest number of scorable bands (4) was obtained with the primer XGBSS-4A while the lowest number (1) was produced by the primers XGDM33- 1D, XGLU-D1 and XPINa-1D. Maximum genotypes (25) were amplified by the primer XPINb (wild)-1D and minimum (8) by the primers XGBSS-4A and XGDM33-1D. Dendrogram was developed on the basis of the scorable banding patterns of the thirty wheat varieties using ten SSR primers by grouping the genotypes on the basis of similarities and differences. During the present investigation, genetic similarity ranged from 0.6-0.9%. This showed the presence of wide range of genetic diversity for quality traits among all genotypes studied.

**Key Words:** Wheat, Gluten, PCR, Simple Sequence Repeats (SSR).

## INTRODUCTION

Wheat (*Triticum aestivum* L.) is a direct source of food for human beings cultivated worldwide. Some wheat species are diploid, with two sets of chromosomes, but many are stable polyploids, with four (tetraploid) or six (hexaploid) sets of chromosomes. Cultivated wheat belongs to the tribe Triticeae of the family *Graminae* and is hexaploid (6x), which regularly form 21 pairs of chromosomes (2n = 42) during meiosis (Briggle and Curtis, 1987). Cultivated wheat emerged about 10,000 years ago in an area belonging now to north-western Iran or north-eastern Turkey (McFadden and Sears, 1946). Protein content is a key quality factor that determines the suitability of wheat for a particular type of product as it affects other factors including mixing tolerance, loaf volume and water absorption capacity (Shah *et al*., 2008). To understand the meaning of protein quality for bread making, the protein of endosperm has been characterized by Gel electrophoresis and their controlling genes have been subjected to genetic analysis (Payne *et al.,* 1980). Previously research attempts were focused mainly to improve the quantity of the wheat because of increasing population demand, whereas little attention was paid to the quality.DNA marker technology is an important area of biotechnology which can enhance the efficiency of plant breeding practices and crop improvement programs (Allard, 1996; Hoisington *et al.,* 1999). Microsatellite markers are used for different applications in wheat breeding due to their high level of polymorphism and easy handling. Genetic diversity of hexaploid wheat (*Triticum aestivum* L.) landraces in relation to their geographic origin has been evaluated via microsatellite technology (Roder *et al*., 1995). SSR markers are excellent markers for genetic diversity analyses and genotype identification in self-pollinated species such as wheat (Domini *et al.,* 2000). The main objective of this study was molecular characterization of promising wheat genotypes for yield and quality parameters.

## MATERIALS AND METHODS

The present study was conducted in the Department of Plant Breeding and Genetics, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi and at National Agricultural Research Center (NARC) Islamabad during the years 2010-2011. The research material comprised of 30 new promising wheat genotypes obtained from various Research Institutes of Pakistan. Different genotypes were subjected to molecular characterization for estimation of genetic diversity. For this purpose SSR primers were used. Seeds of the different wheat genotypes were grown in growth chamber. Young leaf tissues were taken from all genotypes for DNA extraction. In the growth room, 5 to 7 cm long piece of fresh leaf was cut from three weeks old seedlings and was placed in 1.5 ml eppendorf tubes. The plant material was then crushed to a fine powder with a micro pestle while still inside the tube. Five hundred micro-litre DNA extraction buffer (1% SDS, 100Mm NaCl, 100 Mm Tris base, 100 Mm EDTA, pH: 8.5) was added to

each eppendorf tube containing the crushed leaf material and mixed well with the help of a micro pestle. 500 µl phenol: chloroform: isoamyalcohol (in the ratio of 25:24:1) was added and shaken well to get homogenous mixture. Samples were then centrifuged at 5000 rpm for 5 minutes. The aqueous phase (supernatant) was then transferred to a fresh tube. To precipitate the DNA, 50 µl 3M sodium acetate (pH = 4.8) and 500 µl isopropanol was added to the tube and mixed gently. To make the DNA pellet, samples were centrifuged at 5000 rpm for 5 minutes. After discarding the supernatant, pellet was washed with 70% ethyl alcohol. Pellet was dried at room temperature for an hour and resuspended in 40 µl TE buffer (10 Mm Tris, 1 MmEDTA and pH: 8.0) (Weining and Langridge, 1991). To remove RNA, DNA was treated with 40 µg RNAse-A (0.2µl) at 37$^0$C for 1 hour. After RNAse treatment, DNA samples were run on 1% gel to check the quality of DNA and then stored at 4$^0$C. For further 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 reaction was carried out in 25 µl reaction containing : 50-100 ng total genomic DNA template, 0.25 µM of each primer, 200 µM of each dATP, dGTP, dCTP, dTTP, 50 mM KCl, 10 mM Tris, 1.5 mM $MgCl_2$ and 2.5 units of Taq DNA polymerase (Dweikat *et al.* , 1993).

**AMPLIFICATION CONDITIONS**

With SSR primers the amplification conditions were: denaturation, an initial step for 1 minute at 93$^0$C followed by 40 cycles each consisting of a denaturation step of 30 seconds at 93$^0$C. An annealing step of 1 minute at 60$^0$C. An extension step of 1 minute at 72$^0$C. Five minutes were given after the last cycle to the extension step at 72$^0$C to ensure the completion of the primer extension reaction. Gene Amp PCR system 2700 was used for all amplification reaction.

**GEL ELECTROPHORESIS**

For electrophoresis of the amplification products using SSR, 3 % agarose /TBE gel (Roder *et. al.,* 1998) was used. Gels were visualized by Ethidium Bromide under the UV light chamber and observed using the computer program UVI PhotoMW.

**DNA LADDER**

The genetic ruler 50bp DNA ladder (Catalouge # SMO313, Lot: 00018968, concentration: 0.1 µg/ml) for SSRs by Fermentas was used for sizing and approximate quantification of wide range double stranded DNA fragments on agarose gel. The ladder was pre-mixed with 6 X Loading Dye solutions for direct loading on gel.

**STATISTICAL ANALYSIS**

For statistical analysis of molecular studies, all the scorable bands were considered as a single locus/allele. Genetic distance was calculated using Unweighted Pair Group of Arithmetic Means (UPGMA) procedure. The presence of bands was shown by score 1 while absence of bands was shown by score 0. The 1-0 bivariate data matrix for each set of wheat lines based on the data SSR primer sets was used to construct dendrogram using computer program "Popgene32" version 1.31 (http://www.ualberta.ca./~fyeh/fyeh) (Yeh *et al*., 1999).

**RESULTS AND DISCUSSION**

The selected wheat genotypes were subjected to molecular characterization using single sequence repeats (SSR). Ten wheat microsatellite markers for ten loci representing at least one microsatellite marker from chromosomes (1B, 2B, 1D, 4A and 7A) were selected for genotyping (Table 1). These microsatellites were selected on the basis of their known genetic diversity among locations to give a uniform coverage for all three wheat genomes (A, B and D) and a total of 21 polymorphic alleles were detected at 10 loci with a mean of 2.1 alleles per locus (Fig 11). More alleles were observed in B and D genome as compared to A genome. The maximum number of alleles (4) was observed at XGBSS-4A and XWMC41-2B and their size ranged from 50-200 bp and 150-200 bp respectively. An average of 2.1 alleles per locus was detected for all the thirty wheat genotypes. This level of polymorphism is lower than the average of 10 alleles per locus as reported by Fahima *et al*. (1998) using different wheat genotypes. Furthermore, the detected genetic diversity for the thirty wheat genotypes is also lower than that reported by Plaschke *et al*. (1995) studying closely related European wheat cultivars having an average of 6.2 alleles per locus. The highest number of scorable bands (4) was obtained with the primer XGBSS-4A and XWMC41-2B while the lowest number (1) was obtained with the primers XGDM33- 1D, XGLU-D1 and XPINa-1D. Maximum genotypes (25) were amplified by the primer XPINb (wild)-1D and minimum (8) by the primers XGBSS-4A and

XGDM33-1D. Different primers showed variation in their ability to detect polymorphism. Primer XPINb (wild)-1D and XPINa-1D showed higher polymorphism. Primer XGBSS-4Aband XGDM33-1D showed lowest polymorphism. Gel electrophoresis patterns of XBARC108-7A, XBXMARF-1B, XGDM33-1D, XGBSS-4A, XPINa-1D, XPINb(wild)-1D, XPINb (mutant)-1D, XUHW89bf-1B and XWMC41-2B, SSR primers are depicted in figure 4.1 to 4.10.

**SSR amplification pattern using the primer *XBARC* 108-7A:**

The primer Xbarc 108-7A showed the amplification profile of 30 samples in which 21 samples gave visible bands. Sizes of the scorable bands ranged from 75 to 300 bp (Fig 1). Maximum scorable bands (3) were detected for the genotypes 7, 8, 9, 25 and 26. The genotypes that showed band size of 75 bp were 1, 2, , 6, 7, 8, 9, 12, 13, 14, 15, 16, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27and 28 and were monomorphic with respect to each other but polymorphic to rest of the genotypes. Similarly the genotypes 7, 26 and 28 showed the band size of 300 bp and hence were polymorphic to the rest of genotypes.

**SSR amplification pattern using the primer *XBXMARF*-1B:**

The primer Xbxmarf-1B showed the amplification profile of 30 samples in which 15 samples gave visible bands (Fig 2). Sizes of the scorable bands ranged from 50 to 350bp. The genotypes with common band size 50 bp were 1, 2, 3, 4, 5, 6, 7, 8, 11, 12, 13, 14, 15, 27, and 28 and were monomorphic to each other and polymorphic to rest of the genotypes. Similarly genotypes with band size of 350 bp were 13, 27 and 28 and scored maximum bands (2).

**SSR amplification pattern using the primer XGDM33-1D:**

The primer Xgdm 33-1D manifested the amplification profile of 30 samples in which 8 samples gave visible bands. Sizes of the scorable bands were 120 bp (Fig 3). The genotypes 3, 4, 7, 12, 14, 16, 19, 26, 27 that showed scorable bands were monomorphic for the band size of 120bp**.** No unique band was present. Total genomic DNA from remaining 22 samples did not amplify using Xgdm33-1D and hence were not included in the analysis.

**SSR amplification pattern using the primer XGLU-1D:**

The primer XGlu-1D manifested the amplification profile of 30 samples in which 19 samples gave visible bands (Fig 4). Sizes of the scorable bands ranged from 50 to 75bp. A common band of 50bp was only present in genotypes 2, 21, 22, and 24. These genotypes were monomorphic to each other but polymorphic to rest of the genotypes. Similarly the genotypes 1, 3, 5, 10, 12, 13 14, 15, 16, 17, 18, 19, 26, 27 and 28 showed band size of 75bp and were monomorphic for each other and polymorphic for rest of the genotypes.

**SSR amplification pattern using the primer XGBSS-4A:**

The primer Xgbss-4A manifested the amplification profile of 30 samples in which only 8 samples gave visible bands (Fig 5). Sizes of the scorable bands ranged from 50 to 200 bp. Maximum scorable bands (4) were given by only genotype 5. A unique band of 200 bp was present in genotype 5. The other genotypes 1, 9, 11, 12, 13, 14 and 22 that showed scorable bands and were monomorphic for the band size of 50 bp. Genotype having unique band were polymorphic to rest of genotypes. Total genomic DNA from remaining 22 samples did not amplify using Xgbss-4A and hence were not included in the analysis.

**SSR amplification pattern using the primer XPINa-1D:**

The primer Xpina-1D manifested the amplification profile of 30 samples in which 24 samples gave visible bands (Fig 6). The size of the scorable bands was 50 bp. All the genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 20, 21, 23, 24, 27 and 28 that showed scorable bands were monomorphic for the band size of 50 bp. No unique band was present. Total genomic DNA from remaining 7 samples did not amplify using Xpina-1D and hence were not included in the analysis.

**SSR amplification pattern using the primer XPINb-D1 (wild):**

The primer Xpinb-D1 manifested the amplification profile of 30 samples in which 25 samples gave visible bands (Fig 7). Sizes of the scorable bands were 50 to 200 bp. A common band of 50 bp was present in the genotypes 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 20, 21, 22, 24, 25, 26, 27 and 28 and these genotypes were monomorphic to each other. Band size of 200bp was present in only genotypes 5 and 12. Total genomic DNA from remaining 7 samples did not amplify using Xpinb-1D and hence were not included in the analysis.

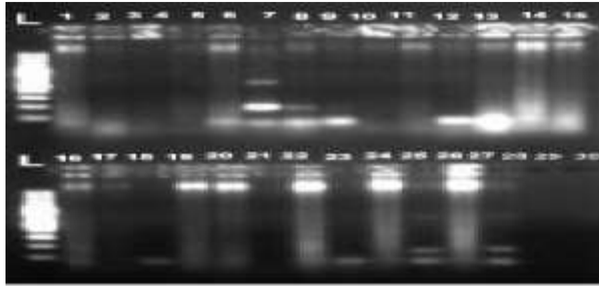

Fig 1: SSR amplification pattern using *xbarc* 108-7A

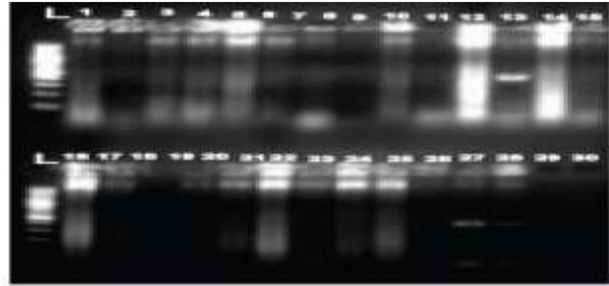

Fig 2: SSR amplification pattern using the primer *xbxmarf* - 1B

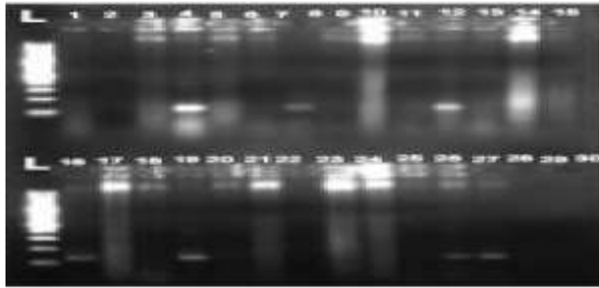

Fig 3: SSR amplification pattern using the primer the primer *xgdm33*-1D

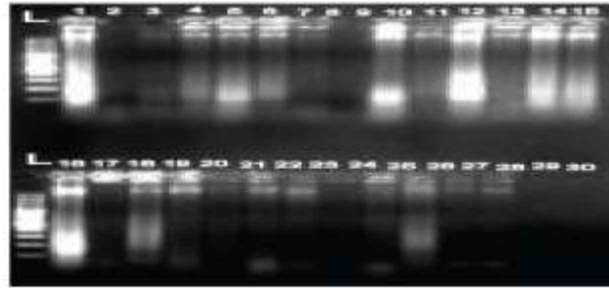

Fig 4: SSR amplification pattern using*xglu* 1D

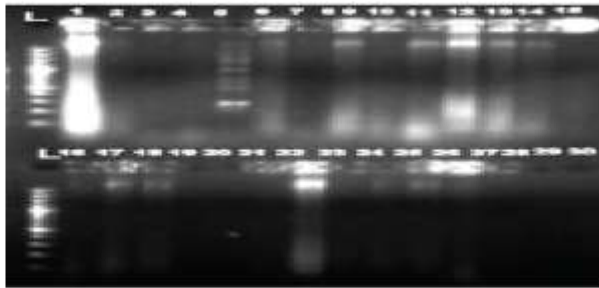

Fig 5: SSR amplification pattern using the primer *xgbss*-4A

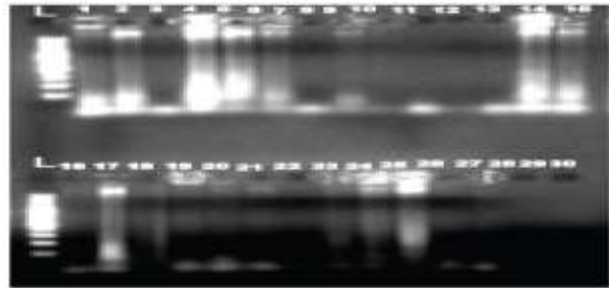

Fig 6: SSR amplification pattern using the primer the primer *xpin*a-1D

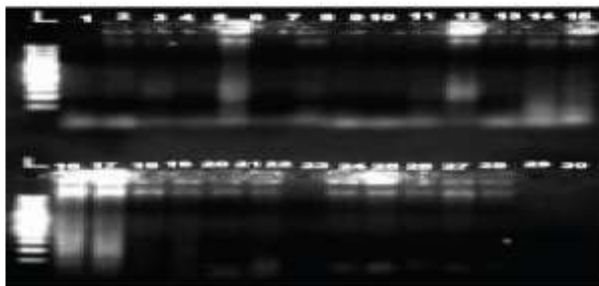

Fig 7. SSR amplification pattern using primer *xuhw89-bf*-1B

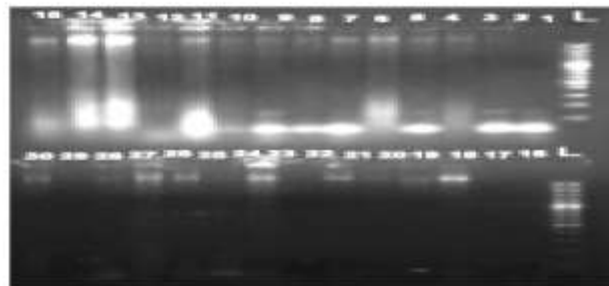

Fig 8. SSR amplification pattern using primer *xpin*b-1D (wild)

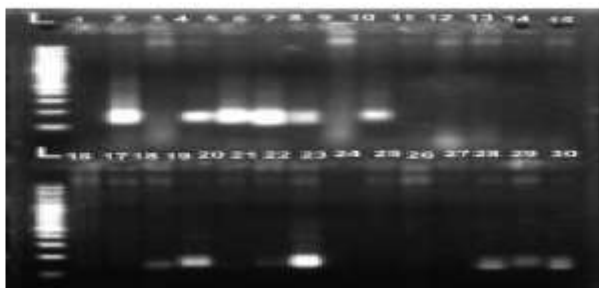

Fig 9. SSR amplification pattern using primer *xpin*b-1D (mutant)

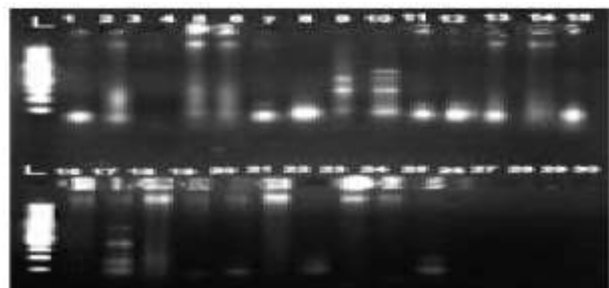

Fig 10. SSR amplification pattern using primer *xwmc-41*-2B

**SSR amplification pattern using the primer XPINb-D1(mutant):**

The primer Xpinb-D1 showed the amplification profile of 30 samples in which 16 samples gave visible bands (Fig 8). Sizes of the scorable bands ranged from 50 to 150 bp. Maximum scorable bands (2) were detected for the genotype 1, 2, 4, 7 and 8. A common band of 50bp was present in genotypes 1, 2, 3, 5, 7, 8, 9, 10, 11, 12, 19, 25 and 28 and these genotypes were monomorphic to each other and polymorphic to the rest of the genotypes. Similarly genotypes that showed band size of 150 bp were 13, 14 and 15 and are monomorphic to each other.

Fig 11. List of SSR Primers used for Molecular Characterization.

| Oligo Name | Primer Sequence (`5-3`) | Location | Annealing Tm.($^{O}C$) |
|---|---|---|---|
| XBARC 108 | FW: GCGGGTCGTTTCCTGGAAATTCATCTAA | 7A | 50 |
| | RVS: GCGAAATGATTGGCGTTACACCTGTTG | | |
| XBX7MARF | FW: TTT TACCCAAACCCCAAC TG | 1B | 50 |
| | RVS: CACGCCATCACTAATATTCCA | | |
| XGDM 33 | FW: GGCTCAATTCAACCGTTCTT | 1D | 55.4 |
| | RVS: TACGTTCTGGTGGCTGCTC | | |
| XGLU | FW: GCCTAGCA CCTTC CAATC | 1D | 50 |
| | RVS: GAAACCTGC TGC GGACAAG | | |
| XGBSS | FW: TGGCCTGCTACCTCAAGA | 4A | 49.9 |
| | RVS: CCCTCCACCGGC TTGT | | |
| XPINA | FW: CCCTGTAGAGACAAAGCTAA | 1D | 40.7 |
| | RVS: TCACCA TAATAGCCAATACTG | | |
| XPINB(a) Wild | FW: ATGAAGACCTTATTCCTCCTA | 1D | 40.1 |
| | RVS: CTCATGCTCACAGCC GCC | | |
| XPINB (b) mutant | FW: ATGAAG ACTTATTCCTCCTA | 1D | 49.9 |
| | RVS: CTCATGCTCACAGCCGCT | | |
| XUHW89BF | FW: TCTCCAAGAGGGGAGAGACA | 1B | 56.1 |
| | RVS: TTCCTCTACCCATGAATCTAGCA | | |
| XWMC41 | FW: TCCCTCTTCCAAGCGCGGATAG | 2B | 56.1 |
| | RVS: GGAGGAAGATCTCCCGGAGCAG | | |

Fig 12. SSR Markers, their names, chromosomal location, no. of alleles, Range of allele size (bp) and their genetic diversity for the microsatellite Markers used in this study.

| *Oligo Name* | *Chromosomal Location* | *No. of Alleles* | *Range of Allele Size (bp)* | *Genetic Diversity* |
|---|---|---|---|---|
| *Xbarc* 108 | 7A | 2 | 75-300 | 0.48 |
| xbx7marf | 1B | 2 | 50-350 | 0.54 |
| *Xgdm* 33 | 1D | 1 | 120 | 0.60 |
| *Xglu* | 1D | 1 | 50-75 | 0.66 |
| *xgbss* | 4A | 4 | 50-200 | 0.71 |
| *Xpin*a | 1D | 1 | 50 | 0.77 |
| *Xpin*b(a) wild | 1D | 2 | 50-200 | 0.83 |
| *Xpin*b(b) mutant | 1D | 2 | 50-150 | 0.89 |
| *Xuhw89bf* | 1B | 2 | 150-350 | 0.94 |
| *Xwmc*41 | 2B | 4 | 150-400 | 1.00 |
| **Total** | | 21 | | 7.42 |
| **Mean** | | 2.1 | | 0.742 |

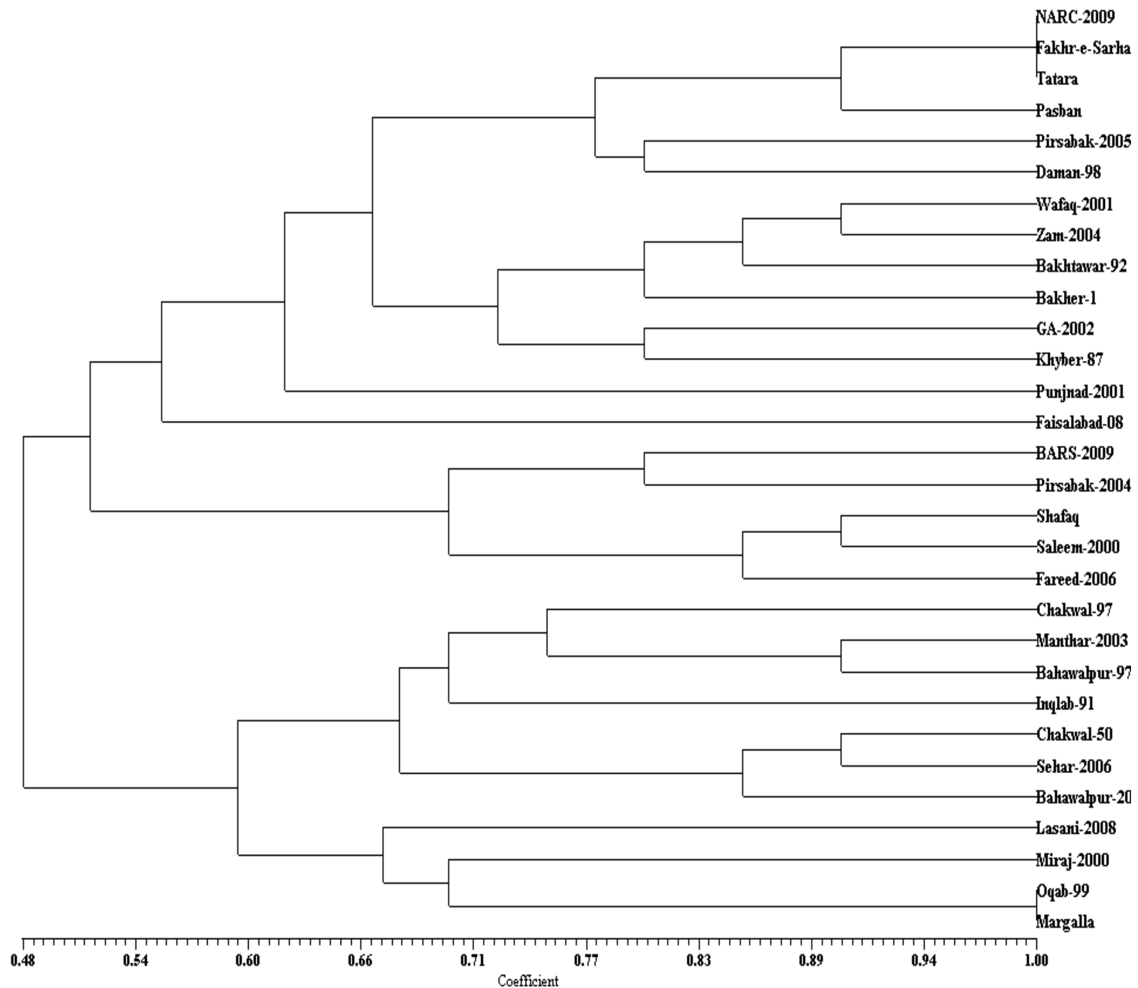


Fig. 13. Dendrogram of 30 wheat genotypes based on genetic distances.

**SSR amplification pattern using the primer XUHW89-BF-1B:**

The primer Xuhw89-BF showed the amplification profile of 30 samples in which 13 samples gave visible bands (Fig. 9). The size of the scorable bands ranged from 150 to 350 bp. Maximum scorable bands (2) were detected for the genotype 23. A unique band of 350 bp was present only in 23 and was absent in all other genotypes. This band is making this genotype diversified from the rest of the genotypes. Other genotypes 2, 4, 5, 6, 7, 8, 10, 19, 20, 22, 23, 28, 29 and 30 showed band size of 150 and were monomorphic to each other.

**SSR amplification pattern using the primer XWMC41-2B:**

The primer Xwmc-41 manifested the amplification profile of 30 samples in which 17 samples gave visible bands (Fig. 10). Sizes of the scorable bands ranged from 150 to 400 bp. Maximum scorable bands (4) were detected for the genotype 9, 10, 17. A unique band of 400 bp was present in 10, 17 and was absent in all other genotypes. A common band size of 150bp was present in 1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13, 15, 17, 19, 20, 23 and 25.

**Dendrogram Interpretation:**

The dendrogram was developed on the basis of the scorable banding patterns of the thirty wheat genotypes using ten SSR primers by grouping the genotypes on the basis of similarities and differences. This dendrogram demonstrated the ability of microsatellites to detect large amount of genetic diversity in genotypes with expected narrow genetic pool (Fig. 13). The dendrogram of SSR based genetic diversity evaluation categorized the genotypes into five main clusters A, B, C, D and E. Genetic distances estimated for these genotypes are mentioned in Fig. 13.

**CLUSTER A:**

This is the large cluster and sub-divided into two sub-clusters. First sub-cluster included NARC-2009, Fakhr-e-Sarhad, Tatara, Pasban, Pirsabak 2005 and Daman-98 while second sub cluster comprised of genotypes i.e. Wafaq-2001, ZAM-2004, Bakhtawar-92, Bhakar-1, GA-2002 and Khyber-87. The similarity indices showed that genotypes Wafaq-2001, GA-2002, Khyber-87, Pasban, Fakhr-e-Sarhad, Tatara, ZAM-2004, Chakwal-50, and Bakhtawar-92 were closely related cultivars with genetic distance of 0.9 (90%). While genotypes Chakwal-50 and BARS-2009 were most distantly genotypes showing genetic distance of 0.4 (40%).

**CLUSTER B:**

This cluster included only one genotype i.e. Punjnad-2001 and was genetically diverse showing genetic distance of 0.7 (70%) with rest of the remaining genotypes.

**CLUSTER C:**

This cluster also included only one genotype i.e. Faisalabad-2008. This genotype showed genetic diversity having genetic distance of 0.6 (60%) with rest of the other remaining genotypes.

**CLUSTER D:**

This cluster further included one sub-cluster comprised of total five genotypes i.e. BARS-2009, Pirsabak-2004, Shafaq, Saleem-2000 and Fareed-2006. The similarity indices showed that two most closely similar genotypes were Saleem-2000 and Fareed-2006 with highest similarity index 0.8 (80%). On the other hand the most distantly related cultivars were BARS-2009 and Pirsabak-2004 with lowest similarity index 0.4 (40%).

**CLUSTER E:**

This cluster consists of one sub cluster. This cluster comprised of eleven genotypes; Chakwal-97, Manthar-2003, Bahawalpur-97, Inqlab-91, Chakwal-50, Sehar-2006, Bahawalpur-2000, Lasani-2008, Miraj-2000, Uqab-99 and Margalla-99. The similarity indices showed that the genotypes Bahawalpur-97 and Manthar-2003 were closely related with similarity index 0.9 (90%). While the most distantly related genotypes were Manthar -2003 and Miraj-2000 with similarity index 0.6 (60%).During the present studies, a wide range of genetic diversity among all genotypes was observed. In previous studies, a higher number of wheat genotypes from the same origin have been analyzed using different DNA marker systems that produced the genetic diversity or similarity levels within a specific group of genotypes (Paull, *et al*., 1998; Szucs, *et al*., 2000; Eujay *et al.,* 2001). Findings of these studies clearly demonstrated the reliability, usefulness and efficiency of SSRs in analyzing genomic diversity. Thus, it should be possible to establish a collection of highly polymorphic SSRs for genetic diversity studies cultivar identification and quality in wheat (Rongwen, *et al.,* 1995). Knowledge of genetic diversity among different genotypes has a significant impact on the improvement of crop plants and this information has been successfully used for efficient genotype selection for different breeding purposes. Molecular markers have the potential to detect genetic diversity and to aid in the management of plant genetic resources (Ford-Lloyd *et al.,* 1997; Virk *et al.,* 1995; Song *et al*., 2003). The genetic diversity levels observed in this study would be useful indicators if such an approach is planned for the wheat genome. This makes genomic diversity estimates a potentially valuable predicting source for selecting diverse parent genotypes in a wheat improvement program that aims to broaden the genetic basis and progeny performance for complex traits such as yield and quality (Rolda´n-Ruiz, *et al*., 2001). Zhang *et al.,* (2005) also reported that hexaploid wheats are a valuable source for broadening the genetic base of wheat breeding germplasm. Our data showed significant variation in microsatellite DNA polymorphism among wheat varieties. The present study, using wheat microsatellite markers, revealed considerable amount of genetic diversity among wheat genotypes i.e. Wafaq-2001, GA-2002, Khyber-87, Pasban, Fakhr-e-Sarhad, Tatara, ZAM-2004, Chakwal-50, Bakhtawar-92, Bahawalpur-97 and Manthar-2003. The genetic diversity observed among different wheat genotypes can be utilized in future breeding program for wheat improvement.

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(*Accepted for publication March 2013*)