African Journal of Biotechnology Vol. 8 (14), pp. 3244-3250, 20 July, 2009
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB09.587
ISSN 1684–5315 © 2009 Academic Journals

*Full Length Research Paper*

# Molecular analysis of genetic diversity in elite II synthetic hexaploid wheat screened against *Barley yellow dwarf virus*

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Accepted 3 June, 2009

**The presence of sufficient genetic diversity in the germplam is an important prerequisite for the improve-ment and efficient use of available material. Traditionally, the data on agronomic, morphological and physiological plants traits are used to estimate the genetic diversity. But now molecular markers are available for authenticated and reliable studies for genetic diversity. The present study was conducted to assess the genetic diversity of Elite-II synthetic hexaploid (SH) wheat by genome DNA fingerprinting as revealed by random amplified polymorphic DNA (RAPD) analysis. Ten decamer RAPD primers (OPG-1, OPG-2, OPG-3, OPG-4, OPG-5, OPA-3, OPA-4, OPA-5, OPA-8, and OPA-15) were used to evaluate the diversity profile of the selected SH entries. Primers OPG-2 and OPA-4 gave the highly polymorphic results. The pair wise similarity values shows that genotypes 1, 20 and 30 have most closest relationship with highest similarity values i.e., 100% while genotypes 9 and 13 show dictinct relationship with minimum similarity value that is, 54%. Therefore, the allelic variation of the SH resistance germplasm is a potent means to enrich and improve bread wheat cultivars where BYDV is a production threat and these can be used in future wheat breeding programme.**

**Key words:** Random amplified polymorphic DNA, primers, genotypes, synthetic hexaploid, wheat.

## INTRODUCTION

Wheat is one of the most important among cereal crops of the world as well as Pakistan. The history of cultivated wheat and human civilization are interwoven. Important species of wheat are common wheat or bread wheat (*Triticum aestivum* L.), club wheat (*T. compactum* L.) and durum wheat *(T. turgidum* L.). Most modern varieties belong to hexaploid wheat*, T. aestivum* var. *aestivum.* Wheat contributes 3.2% to GDP in Pakistan. Wheat being the staple grain food of Pakistan occupies the largest area i.e. 8330 thousand hectares with annual production of 21109 thousand tonnes (Anonymous, 2005).

It had been a general practice to lay more emphasis to increase production of wheat in the shape of increase of hectare under cultivation or increase per hectare yield. Diseases are one of constraints in this reference. There are a lot of diseases that attack wheat and include a number of biotic agents like fungi, bacteria, and viruses (Gair et al., 1987). Among viral diseases, Barley yellow dwarf virus (BYDV) disease is very common, serious, and destructive. It attacks all cereals including bread wheat (*T. aestivum*), durum wheat *(T. turgidum*), barley (*Hordeum vulgare*), oats (*Avena sativa* L.) and Triticale (*Tritico-Secale* Wittmack). The virus also infects at least 100 other grass species including maize and rice and these hosts can serve as reservoirs for virus and aphids (Jones and Catheral, 1970).

In order to exploit genetic resistance within the *Triticeae* family, various strategies allow the breeders to utilize genetic diversity. The most common of these is the conventional approach that forms the backbone of wheat breeding globally. However, when the genetic base gets narrow and new alleles are needed then

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diverse genetic resources come into consideration for which the family resources are a means to incorporate such novel diversity.

There are about 325 perennial and annual species within the *Triticeae* (Dewey, 1984) and these are distributed within three gene pools based upon their genetic distance and homology (Jiang et al., 1994). Thus development of diverse genetic stocks around this material resource forms a viable means of harnessing novel genes that have the potential to address production constraints for biotic and abiotic stresses. The choice of species provides the option to structure a research program and thus species within the primary gene pool are a good choice for rapid experimental returns (Mujeeb-Kazi and Rajaram, 2002). It is well known that *Ae. Tauschii* has a potential source for resistance to numerous production constraints of wheat (Mujeeb-Kazi et al., 2004) which include biotic, abiotic stresses, and pests (Mujeeb-Kazi, 2003, 2005, 2006; Raupp et al., 1993; Lutz et al., 1992, 1994). Due to the presence of extensive diversity in *Ae. Tauschii* (Roder et al., 1998) preference over the last twenty years has been given on exploiting this resource for wheat improvement.

Synthetic hexaploid wheats are created by artificially crossing between *Triticum turgidum* L. (AABB) and *Aegilops tauschii* Coss. (DD), the evolutionary progenytors of common bread wheat (*Triticum aestivum* L.; AABBDD). Different *Ae. tauschii* accesstions and va-rious form of *T. aestivum* can be used to create synthe-tic hexapolids, making synthetic hexaploids a unique germplasm resource for bread wheat breeding. Synthetic hexaploids can act as a vehicle for the introduction of specific characters or general genetic diversity from these progenitors into bread wheat backgrounds because the genetic diversity obtainable from synthetic hexaploid wheats may be novel alleles and genes for biotic and abiotic tolerances not currently represented within the *T. aestivum* gene pool (Reif et al., 2005). From the wide array of such synthetic hexaploids developed at the International Maize and Wheat Improvement Centre (CIMMYT) in Mexico (Mujeeb-Kazi, 2005), a subset of Elite-II was structured based upon multiple attributes and is currently available in Pakistan. A set of 33 entries has been screened against BYDV (Saffdar) and its DNA profiles have not been defined. Molecular markers showing levels of polymorphism among closely related genotypes include RAPDs (Willams et al., 1994; Diouf and Hilu, 2005; Zannou et al., 2008), microsatellites (Sonnante et al., 1994; Diouf and Hilu, 2005) and AFLPs (Vos et al., 1995; Gillaspie et al., 2005). RAPD markers have shown to be useful in assessing intraspecific or interspecific genetic variability in many crops (Diouf and Hilu, 2005; Zannou et al., 2008).

The objective of the present study was to investigate genetic variation among Elite II Synthetic Hexaploid wheats and to find out phylogenetic relationship among these entries for the best use of the genetic potential of the crop.

## MATERIALS AND METHODS

### Plant material

The germplasm consisted a set of 33 entries of synthetic hexaploid wheat, produced by crossing various *T. turgidum* (AB-genome) cultivars with *Ae. tauschii* (D-genome) accessions grouped into Elite II sub-set based upon multiple stress positive character (Table 1), and was obtained from NARC, cytogenetics and wide crosses laboratory. This germplasm was screened against BYDV (Saffdar, 2007) and out of these only 15 selected entries were analyzed for molecular analysis. Spikes were threshed manually and seeds of these selected entries were grown in controlled conditions (20-22°C) and sampling was done from 3 - 4 weeks old seedlings.

### DNA extraction

Leaf samples were used to isolate total genomic DNA following the protocol as described by Weining and Langridge (1991). In the growth room 5-7 cm long piece of fresh leaf material was cut from the plants and was placed in 1.5 ml microtubes. Liquified Nitrogen ($N_2$) was added and then plant material was crushed to a fine powder with a knitting needle while still inside the microtube. 500 µl DNA extraction buffer (1% SDS, 100 mM NaCl, 100 mM tris base, 100 mM $Na_2EDTA$, pH: 8.5 by HCl) was added to each microtube containing the crushed leaf material and was mixed well with the help of a knitting needle. 500 µl phenol : chloroform : isoamylalcohol (25:24:1) was added and tubes were well shaken until a homogenous mixture was made. Samples were then centrifuged at 5000 rpm for 5 min. The aqueous phase (supernatant) was transferred to another microtube and mixed with an equal volume of Isopropanol and 0.1 volume of 3 M sodium acetate (pH = 4.8). DNA was removed as a pellet by centrifugation and washed with 70% ethanol. Pellet was dried at room temperature for an hour and stored in 40 µl TE buffer (10 mM Tris, 1 mM EDTA and pH: 8.0) (Weining and Langridge, 1991). To remove RNA, DNA was treated with 40 µg RNAase-A (0.20 µl of commercially supplied RNAase-A purchased from Gene Link, USA) at 37°C for 1 h.

### Agarose gel electrophoresis

The quality of DNA was checked by running it on 1.2% agarose gel and stained in ethidium bromide (EtBr). The stained gel was photographed and quality of the DNA was assessed using documentation system (Kodak EDAS 290). The DNA was further quantified by spectrophotometer at 260 and 280 nm and a dilution of 1.5% was made in double distilled, deionized and autoclaved water for further RAPD analysis.

### DNA amplification

RAPD analysis was performed as described by Williams et al. (1990), with minor modifications. For polymerase chain reaction (PCR) 10 oligonucleotde (decamer) primers (Table 2) were used. The PCR reaction mixture (25 µl) contained genomic DNA of 50 ng concentration, 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 DNA Taq polymerase. The amplification conditions was as; an initial step of denaturizing for 1 min at 94°C followed by 45 cycles each consisting of a denaturizing step of 1 min at 94°C, an annealing step of 1 min at 34°C and an extension step of 2 min at 72°C.

Seven minutes was given after the last cycle to the extension

**Table 1.** List of elite-II synthetic hexaploid wheat along with their pedigree.

| Elite # | Pedigree |
|---|---|
| 1 | SORA / *Ae. tauschii* (192) |
| 2 | CROC 1 / *Ae. tauschii* (210) |
| 3 | DVERD 2 / *Ae. tauschii* (214) |
| 4 | DVERD 2 / *Ae. tauschii* (218) |
| 5 | TK SN1081 / *Ae. tauschii* (222) |
| 6 | CAN / *Ae. tauschii* (236) |
| 7 | SORA / *Ae. tauschii* (323) |
| 8 | D67.2 / P66.270 // *Ae. tauschii* (308) |
| 9 | STY-US / CELTA // PALS /3/ SRN 5 /4/ *Ae. tauschii* (431) |
| 10 | LCK59.61 / *Ae. tauschii* (693) |
| 11 | SKARV 2 / *Ae. tauschii* (304) |
| 12 | CETA / *Ae. tauschii* (1025) |
| 13 | DOY 1 / *Ae. tauschii* (1027) |
| 14 | CETA / *Ae. tauschii*(386) |
| 15 | CETA / *Ae. tauschii* (392) |
| 16 | CETA / *Ae. tauschii* (533) |
| 17 | CPI / GEDIZ /3/ GOO // JO / CRA /4/ *Ae. t*auschii (1018) |
| 18 | CETA / *Ae. tauschii* (1031) |
| 19 | CETA / *Ae. tauschii* (1038) |
| 20 | CETA / *Ae. tauschii* (1046) |
| 21 | CETA / *Ae. tauschii* (1053) |
| 22 | CROC 1 / *Ae. tauschii* (212) |
| 23 | CETA / *Ae. tauschii* (368) |
| 24 | ARLIN 1 / *Ae. tauschii* (430) |
| 25 | D67.2 / P66.270 // *Ae. tauschii* (497) |
| 26 | D67.2 / P66.270 // *Ae. tauschii* (1015) |
| 27 | GAN / *Ae. tauschii* (206) |
| 28 | ARLIN 1 / *Ae. tauschii* (335) |
| 29 | GAN / *Ae. tauschii* (335) |
| 30 | 68111 / RGB-U // WARD RESEL /3/ STIL /4/ *Ae. tauschii* (385) |
| 31 | CETA / *Ae. tauschii* (417) |
| 32 | 68.111 / RGB-U // WARD RESEL /3/ STIL /4/ *Ae. tauschii* (431) |
| 33 | DOY 1 / *Ae. tauschii* (534) |

**Table 2.** List and sequence of the 10-base nucleotide primers used for rapd analysis.

| Primer code | Oligo name | Nucleotide sequence (5'-3') |
|---|---|---|
| OPA-3 | DecamerA-03 | AGTCAGCCAC |
| OPA-4 | DecamerA-04 | AATCGGGCTG |
| OPA-5 | DecamerA-05 | AGGGGTCTTG |
| OPA-8 | DecamerA-08 | GTGACGTAGG |
| OPA-15 | DecamerA-15 | TTCCGAACCC |
| OPG-1 | DecamerG-1 | CTACGGAGGA |
| OPG-2 | DecamerG-2 | GGCACTGAGG |
| OPG-3 | DecamerG-3 | GAGCCCTCCA |
| OPG-4 | DecamerG-4 | AGCGTGTCTG |
| OPG-5 | DecamerG-5 | CAACTCACGA |

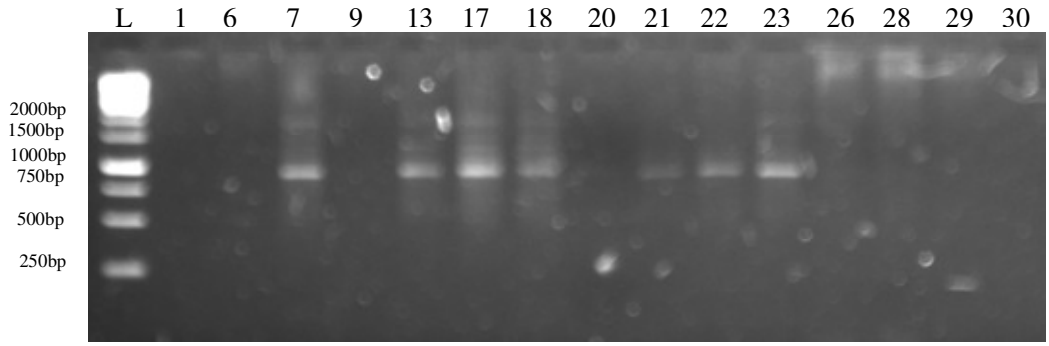


**Figure 1.** PCR amplification profile of Elite-II SH Wheat using the primer OPG-2.

step at 72°C to ensure the completion of the primer extension reaction. All amplification reactions were performed using Gene-Amp PCR system 2700. The amplification products were loaded on 1.5% agarose gel and visualized by staining ethidium bromide and viewed under the UV light chamber using the computer program UVIPhotoMW.

#### Data analysis

For genetic diversity analysis, every scorable band was considered as a single locus/allele and scored as present (1) or absent (0). The bivariate (1-0) data were used to estimate genetic distances (G.D) following the " Unweighted Pair Group of Arithmetic Means (UPGMA)" procedure as described by Nei and Li, (1979) and to construct a dendrogram using computer program "Popgene32" version 1.31 (http://www.ualberta.ca./~fyeh/fyeh).

## RESULTS AND DISCUSSION

In present study, ten randomly amplified polymorphic DNA (RAPD) primers were used to detect genetic polymorphism at DNA level in 15 selected Elite II synthetic hexaploid wheats, developed between various durums (AB-genome) with different accessions of *Ae. tauschii* (DD). Molecular sizes of amplified fragments ranged from 250-1000 bp. Diversity matrices were developed for the RAPD primers. The presence of genetic diversity ranging from 0-100% indicated that these synthetic genotypes were genetically diverse and possessed a high degree of polymorphism. It was necessary to know the genetic diversity of wheat germplasm for the identification of diverse parental combinations and for the creation of segregating progeny with high degree of genetic variability for promoting selection (Franco et al., 2001). To evaluate genetic diversity the present study depicted that RAPD markers is an excellent technique and Its technical simplicity has facilitated its use in the analysis of genetic variability, with results being successfully correlated with established relationships based on pedigree records (Kresovich et al., 1992; Yang and Quiros, 1993; Stiles et al., 1993; Liu et al., 1994).

Out of these tested ten RAPD primers, only five primers gave polymorphism with these genotypes whereas other five did not amplify any genotype. Out of the five primers that gave polymorphism, OPG-2 amplified 7 genotypes out of 15. All of the genotypes were monomorphic for the band at 1000 bp (Figure 1). The primer OPA-4 amplified 6 genotypes out of 15. Genotypes 26, 28 and 29 were monomorphic for the band at 750 bp. Genotype 22 was monomorphic for band between 1000 and 1500 bp. Genotype 6 was polymorphic for the bands at 750 and 1500 bp. Genotype 18 was polymorphic for the bands at 750 bp and in between 1000 and 1500 bp.

OPG-5 amplified 4 out of 15 genotypes All of them were monomorphic for the band at 750 bb. OPA-15 amplified 3 out of 15 genotypes. Genotypes 9 and 17 were monomorphic for the bands at 2000 bp. The genotype 29 was monomorphic for the band at 1500 bp. OPG-3 amplified one genotype out of 15, which was monomorphic for the band at 750 bp.

The similarity matrix (Table 3) was conducted on pair wise similarity values (which are calculated with similarity coefficient) to estimate genetic diversity and relatedness among 15 synthetic wheat genotypes. Similarity values ranged from 54-100%. It was observed that the genetic diversity was 46% among all genotypes (between 9 and 13) showing 54% similarity and genetic diversity was 0% among all those genotypes (1, 20, 30) showing 100% similarity. Mukhtar et al. (2002) studied genetic diversity among 20 wheat cultivars from a range of localities across Pakistan using RAPD analysis. Their results showed that similarity ranged from 75.60-92.74% that indicated the most of the cultivars have a narrow genetic back ground. Previous studies reported that level of polymorphisms in Pakistani wheat germplasm estimated through RAPD markers were 40.70% (Khan et al., 2005) but on the other hand a high level of polymorphism was observed among hexaploid wheat cultivars and breeding lines from two breeding centers in Groatia (Maric et al., 2004). Similarly the present study indicated that greater diversity in this sub set Elite II. De Freitas et al. (2000) also used RAPD in order to assess the variability of wheat (*T. aestivum*) genotypes. Their results also revealed extreme homogeneity

**Table 3.** Similarity matrix of pair wise distance constructed from RAPD banding among 15 Elite II genotypes.

| pop ID | 1 | 6 | 7 | 9 | 13 | 17 | 18 | 20 | 21 | 22 | 23 | 26 | 28 | 29 | 30 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | **** | 0.9167 | 0.9167 | 0.8750 | 0.6667 | 0.7917 | 0.7500 | 1.0000 | 0.8750 | 0.8333 | 0.7500 | 0.9167 | 0.9583 | 0.8750 | 1.0000 |
| 6 | | **** | 0.8333 | 0.7917 | 0.7500 | 0.7083 | 0.8333 | 0.9167 | 0.7917 | 0.8333 | 0.6667 | 1.0000 | 0.9583 | 0.8750 | 0.9167 |
| 7 | | | **** | 0.7917 | 0.7500 | 0.8750 | 0.8333 | 0.9167 | 0.8750 | 0.8333 | 0.8333 | 0.8333 | 0.8750 | 0.7917 | 0.9167 |
| 9 | | | | **** | 0.5417 | 0.8333 | 0.6250 | 0.8750 | 0.8333 | 0.7083 | 0.6250 | 0.7917 | 0.8333 | 0.9167 | 0.8750 |
| 13 | | | | | **** | 0.7083 | 0.9167 | 0.6667 | 0.7083 | 0.7500 | 0.7500 | 0.7500 | 0.7083 | 0.6250 | 0.6667 |
| 17 | | | | | | **** | 0.7917 | 0.7917 | 0.8333 | 0.7083 | 0.7083 | 0.7083 | 0.7500 | 0.8333 | 0.7917 |
| 18 | | | | | | | **** | 0.7500 | 0.7917 | 0.8333 | 0.7500 | 0.8333 | 0.7917 | 0.7083 | 0.7500 |
| 20 | | | | | | | | **** | 0.8750 | 0.8333 | 0.7500 | 0.9167 | 0.9583 | 0.8750 | 1.0000 |
| 21 | | | | | | | | | **** | 0.8750 | 0.7917 | 0.7917 | 0.8333 | 0.8333 | 0.8750 |
| 22 | | | | | | | | | | **** | 0.7500 | 0.8333 | 0.7917 | 0.7083 | 0.8333 |
| 23 | | | | | | | | | | | **** | 0.6667 | 0.7083 | 0.6250 | 0.7500 |
| 26 | | | | | | | | | | | | **** | 0.9583 | 0.8750 | 0.9167 |
| 28 | | | | | | | | | | | | | **** | 0.9167 | 0.9583 |
| 29 | | | | | | | | | | | | | | **** | 0.8750 |
| 30 | | | | | | | | | | | | | | | **** |

among Brazilian wheat genotypes with an average distance of 27 and 69% similarity.

The bivariate (1-0) data and similarity coefficients were used to construct a dendrogram using computer program “Popgene32” and genotypes grouped into two (2) major clusters that is A and B. Cluster A comprises on 11 genotypes and cluster B comprises only on 3 genotypes. However the genotypes 17 did not fall in any group.

Both clusters have been divided into subgroups and sub-clusters (Figure 2). Cluster A genotypes 1, 20 and 30 (genetically similar genotypes) together make a sub sub-group. Genotypes 6 and 26 are genetically similar and make another sub-group. The genotypes 28 did not make any sub group but it made a sub cluster comprising of genotypes 1, 6, 20, 30, 28 and 26. Genotypes 9 and 29 together made a group and genotypes 21 and 22 also made another group but genotype 7 did not fall into any group and sub group. Cluster B consists of 3 genotype. Genotypes 13 and 18 make a group. This group together with genotypes 23 forms a cluster (Figure 3). Asif et al. (2005) also employed RAPD analysis to estimate genetic diversity/genetic similarity among nine wheat (*T. aestivum*) genotypes and grouped these genotypes into two main clusters (A and B) and reported 61.01 to 78.81% genetically similarity among these genotypes but our present study showed 46% genetic diversity between 9 and 13 genotypes.

The genetic distance was observed minimum that is 0 between genotypes 1 and 20, 1 and 7, 7 and 30, 6 and 26. Maximum genetic distance was observed between genotypes 28 and 23 that is, 14.38 closely followed by 26 and 17 that is, 12.28 and 28 and 17 that is, 10.03 and genetic distances for rest of genotypes varied from “0-14.38”. Similar (though not as extreme) RAPD results were also obtained with British (Devos and Gale, 1992) or USA (He et al., 1992) varieties.

Attempts to relate these groups to characteristics of agronomic values, that is reaction to BYDV were unsuccessful. For instance genotypes 6 and 26 which are closely associated in the dendrogram, showed a completely different behavior in relation to the BYDV disease, genotype 6 being highly susceptible and 26 symptomless carrier or tolerant under natural conditions. Also the genotypes 20 being symptomless carrier or tolerant and 30 susceptible (Saffdar, 2007). Similar results were also observed by De Freitas et al. (2000) in relation to the stem rust disease. The dendrogram showed

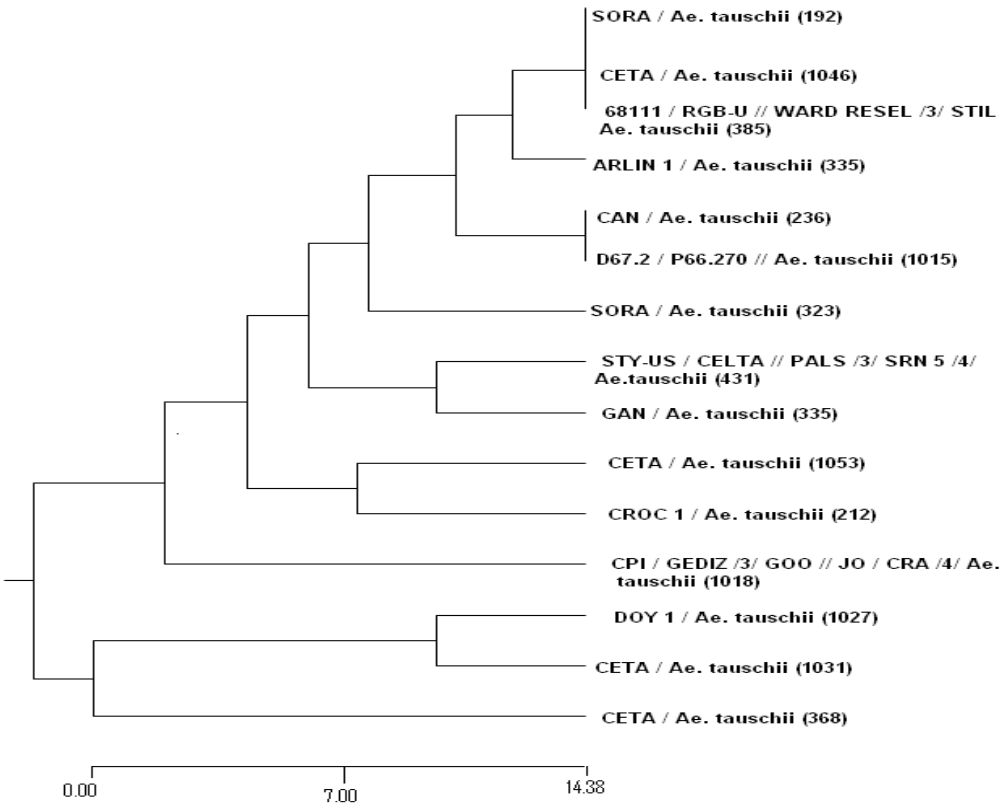


**Figure 2.** Dendrogram constructed for 15 Elite II synthetic hexaploid wheat genotypes based on genetic distance using 10 RAPD primers.

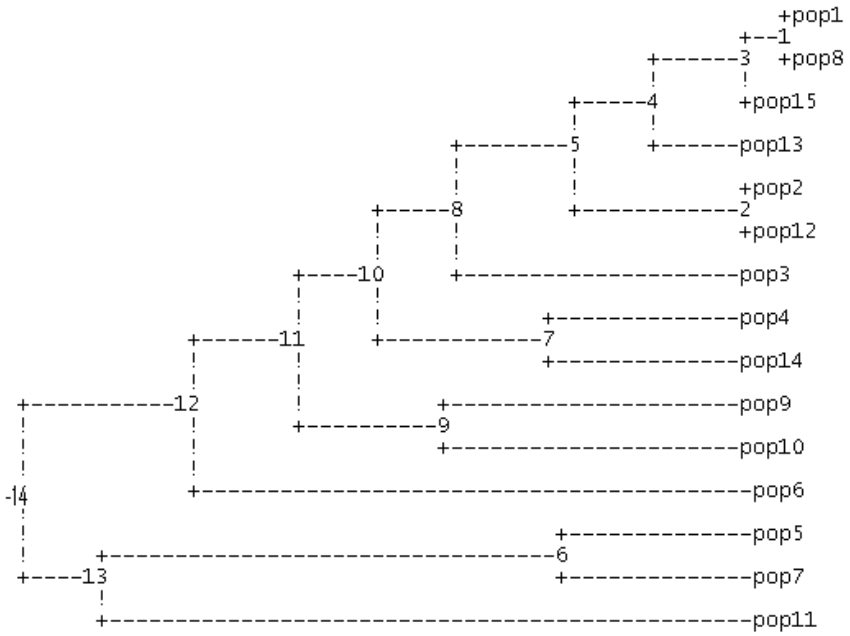


**Figure 3.** Dendrogram showing the pair wise similarities values among 15 Elite II synthetic hexaploid wheat genotypes: pop1, Elite # 1, pop2, Elite # 6, pop3, Elite # 7, pop4, Elite # 9, pop5, Elite # 13, pop6, Elite # 17, pop7, Elite # 18, pop8, Elite # 20, pop9, Elite # 21, pop10, Elite # 22, pop11, Elite # 23, pop12, Elite # 26, pop13, Elite # 28, pop14, Elite # 29, pop15, Elite # 30.

that genotypes 17 and 23 are most diverse. In screening experiment both of them are proved to be tolerant (Saffdar, 2007) hence are recommended for further breeding programs. In conclusion, RAPD analysis revealed high levels of genetic diversity in Elite II synthetic hexaploid wheat genotypes, even with use of limited set of primers. This study will also work as indi-cator for wheat breeders to evolve diverse genetic background to develop sustainable wheat in Pakistan.

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