Pak. J. Bot., 42(4): 2443-2452, 2010.

MOLECULAR MARKER ASSISTED SELECTION FOR DROUGHT TOLERANT WHEAT GENOTYPES

SAJIDA BIBI[1], MOHMUMMAD UMAR DAHOT[2], GHULAM SHAH NIZAMANI, IMTIAZ AHMAD KHAN[1], ABDULLAH KHATRI[1], MAZHER HUSSAIN NAQVI[1] FATEH CHAND OAD[3] AND UMEED ALI BURIO[3]

[1]*Plant Breeding and Genetics Division, Nuclear Institute of Agriculture, Tando Jam, Pakistan*
[2]*Institute of Biotechnology and Genetic Engineering, University of Sindh, Jamshoro, Pakistan*
[3] *Sindh Agriculture University, Tando Jam, Pakistan*

Abstract

A diverse population was generated through induced mutation with an objective to develop drought tolerant wheat genotypes. Population was first accesses for genetic diversity through random amplified polymorphic DNA and then investigated for drought tolerant through sequence tagged sites technique. A total of 100 alleles were amplified with 15 random primers out of which 78 % were polymorphic and 22 % were monomorphic. Fragments size ranged from 198bp-1.842kbp and fragments produced by various primers ranged from 2-13 with an average of 7.4 fragments per primer. Genetically most similar genotypes were SMP1 and KMP4 (95.2%) followed by TMP3 and TMP4 (94.9%) while most dissimilar genotypes were SMP5 and KMP2 (55.8%). On the basis of results achieved, the varieties could be divided into four clusters. Of eighteen, eleven genotypes amplify the DREB F1R1 fragment. Fourteen genotypes tagging the specific sites in the wheat genome with F2R2 and thirteen genotypes were amplified with F3R3 (DREB sequence). Wheat DNA amplification with DREB gene yields 190-220bp bands with F2R2 and F3R3 whereas F1R1 yields 1.9-3.0 kb.

Introduction

Wheat (*Triticum aestivum* L.) is a major cereal, which is cultivated under irrigated rain-fed conditions on 19% and the area near the tail end of canals where shortage of water is often experienced. The rain-fed area alone covers about 1.50 millions hectares, which is one fifth of the total area of cultivation in Pakistan. National yield average in Pakistan is 2.5 t/ha (Anon., 2006-07). During 2007-08, wheat was cultivated on an area of 8414 thousand hectares showing 1.9% decrease over last year area of 8578 thousand hectares. During the Rabi season the canal head withdrawals decreased by 10.5%, as it remained at 27.93MAF compared to 31.18MAF during the same period of last year. The average yield of wheat was decreased by 6.6% as compare to 2006-07 and 9.4% less than the target for this year. This was because of 23.3% shortage of irrigation water. This creates the gap between production and demand thus, enforcing wheat import to fulfill the country requirement (Anon., 2007-08). The imbroglio necessitates addressing the drought problem as a front line task for the wheat improvement. The best answer to this problem is drought tolerant wheat genotype.

Drought is the stress that has adverse effect on the growth of the plants and crop yields. The physiological response to this stress arises from the changes in the cellular gene expression profile, and a number of genes are induced by exposure to such conditions (Shinozaki & Yamaguchi, 2000). The constraints with the conventional breeding approaches are complexity of drought traits (Zhang, 2004) with low genetic variance of yield component under stress conditions, which make it, very difficult due to lack of the proper screening procedure (Alan, 2007) and absence of suitable genetic model systems. Hence breeders are extremely interested in new technologies that could make this procedure more efficient.

Selection of wheat varieties is always challenging and understanding of the genotyping characteristics and relationships of the germplasm is still very limited, which stuck their effective exploitation. Traditionally, the varietals selection is based only on morphological features hence, polygenic characters were very difficult to analyze thus, such constraints can be overcome by using molecular marker assisted selection for trait of interest (William *et al.,* 2007). Techniques which are particularly promising in assisting selection for desirable characters involves the use of molecular markers such as random amplified polymorphic DNA (RAPD) and sequence tagged sites (STS) (Yang-Dong *et al.,* 2003; William *et al.,* 2007). The random amplified polymorphic DNA (RAPD) technology provides a powerful tool for the selection of genetic variation of population/organisms (Williams *et al.*, 1990) while sequence tagged sites (STS) is a short stretch of genomic sequence that are used as landmarks in genome mapping (Saiki *et al.*, 1985). The specific objective of this study was to screen the wheat genotypes mutants for drought tolerance, developed through induced mutation.

Materials and Methods

Plant material: Fifteen mutants of wheat were selected from different doses of gamma rays i.e. 50Gy, 100Gy, 150Gy, 200Gy and 250Gy along with their parents Sarsabz, Kiran-95 and TD1. Seeds of 18 wheat mutants/varieties (Table 1) were grown in bowls at control condition and seedlings were harvested after 15 days.

DNA extraction through MATAB Method: DNA was extracted from fresh leaves of wheat genotypes using MATAB method. Fresh leaves (3g) were grounded in liquid nitrogen; MATAB buffer (20ml) was placed in a 50ml falcon tube, grounded material was transferred in MATAB solution (Tris-HCl pH 8.1, ethelenediaminetetra acetic acid (500mM) Sodium Chloride (5M), MATAB (10g), PEG-6000 (5g) and sodium sulphite). Incubate the mixture at 74^{o}C for 30 minutes and then cooled at room temperature. Equal amount of chloroform isoamyl alcohol (CIAA) (24:1) was added in the cool mixture and mixed by inversion 100 times. The mixture was then centrifuged at 4000 rpm for 30 minutes. The supernatant was poured into a new clean falcon tube which is already containing 20ml CIAA, mixed the sample and then again centrifuge at 4000 rpm for 30 minutes. Supernatant containing DNA was collected in the separate 50ml falcon tube containing 20ml of absolute isopropanol. Collected the DNA with pasture pipette. Transfer the DNA in 2.0 ml Eppendrof tube. Ethanol 70% was used to wash the pellet and the DNA samples were then hydrated with 1000µl of TE buffer.

The concentration of the DNA was measured with spectrophotometer at absorbance 260/280 nm. The quality of DNA was checked on 0.8% agarose gel.

PCR with random primers: For RAPD analysis, the PCR reaction was carried in 25µl reaction mixture containing 1µM of primer (Gene link) in 1x reaction buffer, 0.33mM of each dNTPs, 2.5mM $MgCl_2$, 0.1u/µl of Taq polymerase and 2.6ng/µl of template (Genomic DNA) were used for the amplification of DNA. The amplification reaction was performed in the Eppendorf Master Cycler with an initial denaturation for 5 minute at 94^{o}C, then 33 cycles: 1 minute denaturation at 94^{o}C; 1 minute annealing at 40^{o}C; 2 minute extension at 72^{o}C. Final extension was carried out at 72^{o}C for 10 minute. Amplified products were analyzed through electrophoresis on 1.5% agarose gels containing 0.5 x TBE (Tris Borate EDTA) and 0.5µg/ml Ethidium bromide to stain the DNA. Gel electrophoresis at 72 volts for 2 hours and photograph was taken under UV light using gel documentation system.

Table 1. Mutants/parent varieties released from different doses of gamma rays employed in the study.

S. No.	Mutant/verities	Dose (Gy)	S. No.	Mutant/verities	Dose (Gy)
1.	Sarsabz (parent)	Control	10	KMP3	150
2.	SMP1	50	11	KMP4	200
3.	SMP2	100	12	KMP5	250
4.	SMP3	150	13	TD1 (parent)	Control
5.	SMP4	200	14	TMP1	50
6.	SMP5	250	15	TMP2	100
7.	Kiran-95 (parent)	Control	16	TMP3	150
8.	KMP1	50	17	TMP4	200
9.	KMP2	100	18	TMP5	250

PCR with specific (DREB) primers: National Center for Biotechnology Information (NCBI) website was used to obtain the EST nucleotide sequences of model plant *Arabidopsis thaliana* for drought tolerance (DREB2) expression and three sets of primers of 18-nucleotide base pair was designed using a primer design software 'Primer3' for the selection of drought tolerant genotypes through STS (Olson *et al*., 1989). PCR reaction was carried out in 25µl reaction mixture containing 2.6ng of template (Genomic DNA), 2.5mM MgCl, 0.33mM of each dNTPs, 2.5U of Taq polymerase and 0.25µM of each primer in a 1X PCR reaction buffer. The amplification reaction was performed in the Eppendorf Master Cycler with an initial denaturation for 4 minutes at 94^{o}C, then 30 cycles: 1 minute denaturation at 94^{o}C; 2 minute annealing at 55^{o}C; 3 minutes extension at 72^{o}C. Final extension was carried out at 72^{o}C for 7 minutes. Amplified products were analyzed through electrophoresis on 1.5% agarose gels containing 0.5X TBE (Tris Borate EDTA) and 0.5µg/ml ethidium bromide to stain the DNA. Gel electrophoresis at 72 volts for 2 hours and photograph was taken under UV light using gel documentation system.

Data analysis: Data was scored as presence of band as (1) and absence of bands as (0) from RAPD and STS of amplification profile. Coefficient of similarity among cultivars was calculated according to Nei & Li (1979). Similarity coefficient was utilized to generate a dendrogram by means of Unweighted Pair Group Method of Arithmetic means (UPGMA).

Results and Discussion

STS analysis: Specific primers corresponding to DREB2 sequences were used to screen the 15 mutants and their parents for drought tolerance. Primer F1R1 (DREB sequence) (Fig. 4) amplified the eight mutants with parents viz. Sarsabz, SMP1, SMP2, SMP3, SMP4, Kiran-95, KMP1, KMP2, KMP3, KMP4 and TD1, DNA amplification with DREB sequence yields 2.1 to 1.6 kbp bands whereas Sarsabz, SMP1, SMP3, SMP4, SMP5, Kiran-95, KMP1, KMP4, KMP5, TMP1, TMP2, TMP3, TMP4, TMP5 amplified with the primer F2R2 (DREB sequence) (Fig. 5) which yields 190-200bp bands. Of 18, thirteen mutants/varieties viz. Sarsabz, SMP2, SMP3, SMP4, Kiran-95, KMP1, KMP2, KMP4, KMP5, TMP1, TMP3, TMP4 and TMP5 tagging the specific gene (F3R3) responsible for drought tolerance in the genome. DNA amplification with DREB sequence yields 214bp bands (Fig. 6). The presence and absence of bands indicates the variation in sequence of wheat genome due to mutation. A number of the polygenic loci for candidate gene have been identified in a numbers of the plants. The DREB2 genes are

involved in drought-responsive gene expression (Liu *et al.*, 1998; Nakashima & Yamaguchi, 2005) and a great number of cDNA encoding DREB transcription factors have been identified in various plants, such as AtDREB in *Arabidopsis*, OsDREB in rice, TaDREB1 in wheat and GmDREB in soybean (Liu *et al.*, 1998; Dubouzet *et al.,* 2003; Shen *et al.*, 2003; Li *et al.,* 2005). Tagging of useful genes like the ones responsible for conferring the resistance to plant, synthesis of plant hormone and drought tolerant genes, is a major target for enhancing crop productivity (Lopez *et al.*, 2003). Gene tagging is very useful for detecting the presence of desire gene in the new ideotypes for effective and meaningful selection (Semagn *et al.,* 2006). The very first report on gene tagging were from tomato (Paterson *et al.,* 1988; Williamson *et al.,* 1994), availing the means for identification of markers linked to gene involved in several traits like water use efficiency (Martin *et al.,* 1994), leaf rust resistance genes Lr9 and 24 (Schachermayr *et al.,* 1995). Xiao *et al.*, (1997) reported the utility of RFLP markers in identifying the trait improving QTL alleles from wild rice relative *O. rufipogon*. Involvement of diverse genome in breeding programme is therefore, important not only to widen the crop genetic base but also to introgress useful gene (s) for yields, quality and stability (Svetlana *et al.,* 2007).

Plant improvement, either by natural selection or through efforts of breeders has always relied upon creating, evaluating and selecting the right combination of alleles. With the use of molecular marker it is now easy to trace valuable allele in a segregating population (Svetlana *et al.,* 2007). Drought tolerant alleles (DREB2) developed in this study produced clear, strong, reproducible signals, which is easy to score in a segregating population.

RAPD analysis: Genomic DNAs of 18 wheat mutants/varieties (Table 1) produced multiple fragments with ten base arbitrary primers. Of 40 primers 15 were able to amplify the genomic DNA. The total number of 100 alleles were amplified, out of which 78 (78 %) were polymorphic and 22 (22 %) were monomorphic (Table 2). Size of the alleles ranged from 198bp to 1.842kbp (Figs. 1, 2, 3). Maximum 13 alleles were amplified with primer A-17 and minimum two were amplified with primer A-20. Moreover, the amplification of monomorphic loci is depicting sharing of common blood among the genotypes (Asif *et al.*, 2005). RAPD method has the potential for fingerprinting at molecular level and more efficient selection of genotypes for crossing and enhancing wheat breeding strategies (Li *et al.,* 2005).

Primer-A-17 (Fig. 2) amplified thirteen alleles were polymorphic, which ranged between 222 bp-1.4 kb. All mutants with their parents contain alleles of 1.211kb, 818bp and 406bp except mutant SMP5. Only mutant KMP3 contain a specific allele of 930bp whereas SMP3 mutant containing another specific allele of 560bp. Sarsabz, Kiran-95, TD1 parents did not contain 300bp allele. However SMP1, SMP3 and SMP4 contain allele of 222bp.

Primer B-10 (Fig. 3) amplified 7 alleles in which 4 were polymorphic and three were monomorphic (1.37kb, 645bp & 252bp), with the range of 252bp-1.4kb. All mutants and parents contain alleles of 832bp and 500bp except mutant KMP2. Some specific RAPD markers have been also observed. Mutants SMP3 and KMP2 contain a specific allele of 436bp. KMP2, KMP3, TD1, TMP3 and TMP4 contains a different allele of 395bp.

There were 06 segments amplified using primer A-03 (Fig. 1) in 15 mutants with parents in which four were polymorphic and 2 monomorphic (968 bp and 726bp). All mutants and parents contain alleles of 1.018kb and 998bp except mutant SMP5.Band 456 disappeared in Sarsabz, SMP2, SMP3, SMP5 KMP3 and KMP5, whereas band 396bp disappeared in Sarsabz, KMP2, KMP3, KMP5 indicating the specificity of genotype.

Table 2. Sequence of the primers for wheat RAPD study.

Primer	Primer sequence	Polymorphic loci	Monomorphic loci	Total no. of loci
A-02	TGCCGAGCTG	07	----------	07
A-03	AGGGGTCTTG	04	02	06
A-04	AATCGGGCTG	02	01	03
A-08	GTGACGTAGG	04	01	05
A-10	GTGATCGCAG	04	02	06
A-13	CAGCACCCAC	06	03	09
A-15	TTCCGAACCC	10	----------	10
A-16	AGCCAGCGAA	05	02	07
A-17	GACCGCTTGT	13	----------	13
A-20	GTTGCGATCC	01	01	02
B-05	TGCGCCCTTC	03	01	04
B-07	GGTGACGCAG	03	01	04
B-10	CTGCTGGGAC	05	03	08
B-17	AGGGAACGAG	06	02	08
B-19	ACCCCCGAAG	05	03	08
		78 (78 %)	22 (21 %)	100

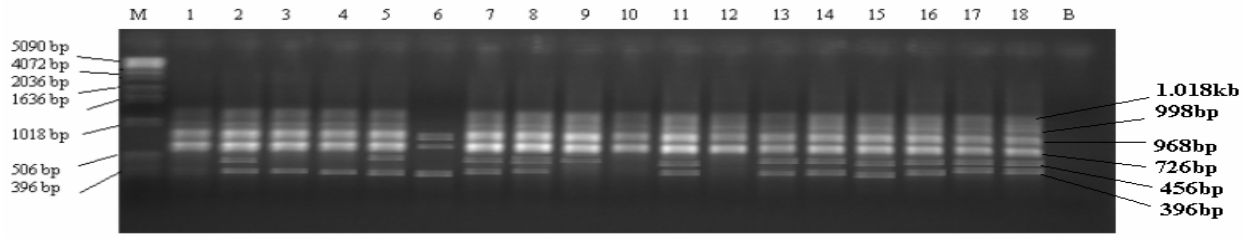

Fig. 1. Amplification profile of eighteen wheat genotypes with primer A-03 by RAPD-PCR. M=1kb (DNA marker), 1=Sarsabz 2=SMP1, 3=SMP2, 4=SMP3, 5=SMP4, 6=SMP5, 7=Kiran-95, 8=KMP1, 9=KMP2, 10=KMP3, 11=KMP4, 12=KMP5, 13=TD1, 14=TMP1, 15=TMP2, 16=TMP3, 17=TMP4, 18=TMP5, B=Blank

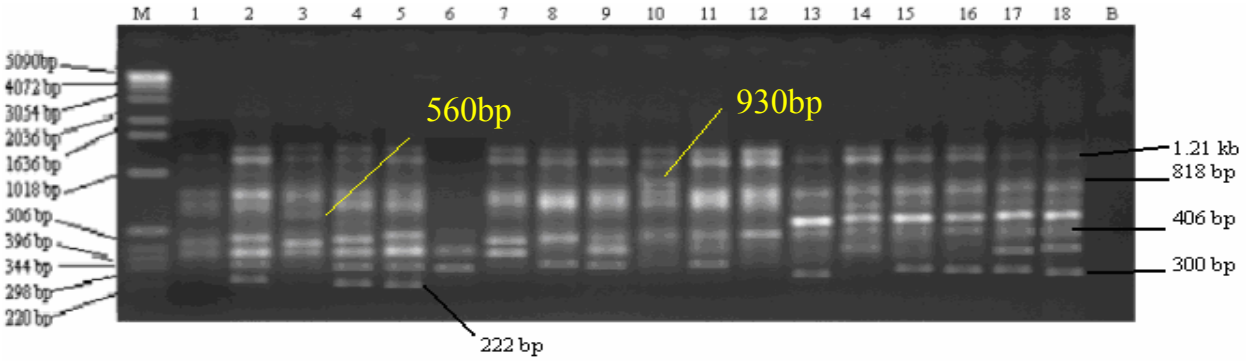

Fig. 2. Amplification profile of eighteen wheat genotypes with primer A-17 by RAPD-PCR. M=1kb (DNA marker), 1=Sarsabz 2=SMP1, 3=SMP2, 4=SMP3, 5=SMP4, 6=SMP5, 7=Kiran-95, 8=KMP1, 9=KMP2, 10=KMP3, 11=KMP4, 12=KMP5, 13=TD1, 14=TMP1, 15=TMP2, 16=TMP3, 17=TMP4, 18=TMP5, B=Blank

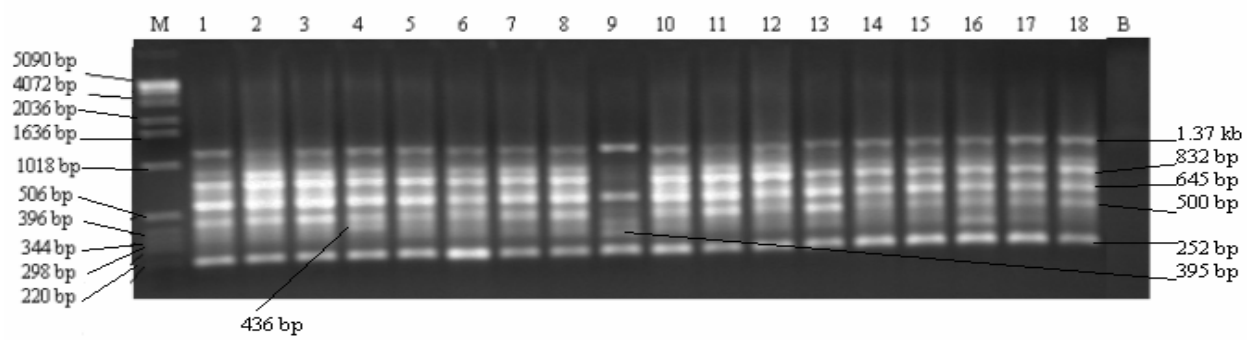

Fig. 3. Amplification profile of eighteen wheat genotypes with primer B-10 by RAPD-PCR. M=1kb (DNA marker), 1=Sarsabz 2=SMP1, 3=SMP2, 4=SMP3, 5=SMP4, 6=SMP5, 7=Kiran-95, 8=KMP1, 9=KMP2, 10=KMP3, 11=KMP4, 12=KMP5, 13=TD1, 14=TMP1, 15=TMP2, 16=TMP3, 17=TMP4, 18=TMP5, B=Blank.

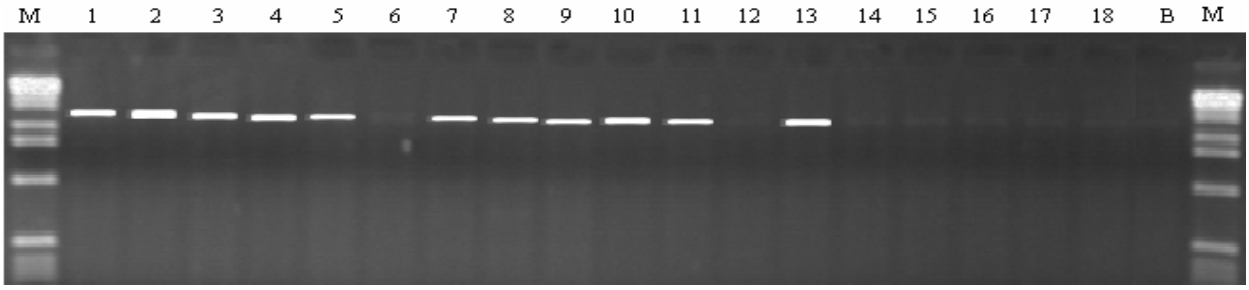

Fig. 4. Result of STS-PCR with DREB sequence (F1R1) M=DNA marker (1kb), 1=Sarsabz 2=SMP1, 3=SMP2, 4=SMP3, 5=SMP4, 6=SMP5, 7=Kiran-95, 8=KMP1, 9=KMP2, 10=KMP3, 11=KMP4, 12=KMP5, 13=TD1, 14=TMP1, 15=TMP2, 16=TMP3, 17=TMP4, 18=TMP5, B=Blank.

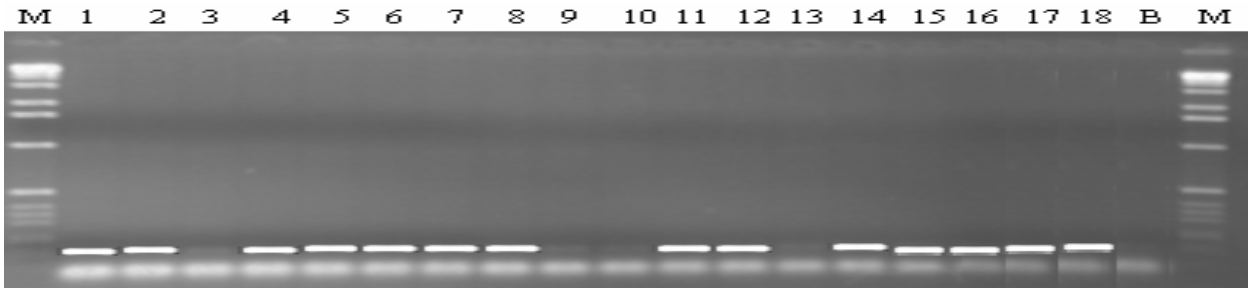

Fig. 5. Result of STS-PCR with DREB sequence (F2R2) M=DNA marker (1kb), 1=Sarsabz 2=SMP1, 3=SMP2, 4=SMP3, 5=SMP4, 6=SMP5, 7=Kiran-95, 8=KMP1, 9=KMP2, 10=KMP3, 11=KMP4, 12=KMP5, 13=TD1, 14=TMP1, 15=TMP2, 16=TMP3, 17=TMP4, 18=TMP5, B=Blank.

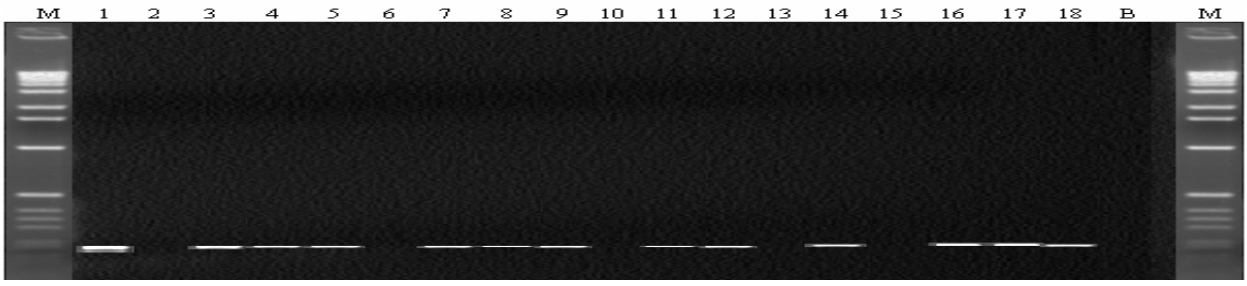

Fig. 6. Result of STS-PCR with DREB sequence (F3R3) M=DNA marker (1kb), 1=Sarsabz 2=SMP1, 3=SMP2, 4=SMP3, 5=SMP4, 6=SMP5, 7=Kiran-95, 8=KMP1, 9=KMP2, 10=KMP3, 11=KMP4, 12=KMP5, 13=TD1, 14=TMP1, 15=TMP2, 16=TMP3, 17=TMP4, 18=TMP5, B=Blank.

Seven bands were amplified by primer A-02 which was polymorphic, ranged between 258 bp-1.1 kb while 10 bands were amplified with primer A-15. Primer A-04, A-10 and A-13 produced 66.67% polymorphic alleles and 33.33% monomorphic alleles, with the range of 400bp-2.5kb, 617bp-3.9 and 220bp-1.78kb. Primer B-19, 8 bands were amplified, in which five were polymorphic and three monomorphic, size ranged between 275bp-1.936bp. With the primer B-05, primer B-07 and primer B-17 produced 75% polymorphic alleles. Primer A-08 amplified five bands, four were polymorphic and one was monomorphic, size ranged between 328bp-2.0kb. Primer A-20 amplified two bands in which one was monomorphic (1.96kb). Primer A-16 amplified seven bands, five were polymorphic and two were monomorphic, size ranged between 345bp-2.0kb (Table 2).

The RAPD data revealed that genetically most similar mutants were SMP1 and KMP4 (95.2%) followed by TMP3 and TMP4 (94.9%) while most dissimilar mutants were SMP5 and KMP2 (55.8%) (Table 3) The close genetic relationships are quite alarming and may impede further plant improvement. It has been very well documented that plant improvement is based on the information about the genetic relationships among accessions within and between species (Thormann *et al.,* 1994).

On the basis of dendrogram, the varieties could be divided into four clusters, designated A through D (Fig. 7). Cluster 'A' comprising of TMP3, TMP4 and KMP1. Cluster B contained predominantly Kiran-95 and TMP1 showing more genetic similarity among each other and cluster C contained SMP3, SMP4, Sarsabz and TD1. Cluster D consisted of SMP1, KMP4, SMP2, KMP5 and KMP2. Cluster A, B and C with distinct mutants TMP2 and KMP3 forming one group which are designated as group 'One'. Whereas, cluster D forming second group. TMP5 and SMP5 were found genetically distinct to all other mutants/varieties (dissimilarity i.e. 27.3% and 37% respectively). Mutant KMP3, TMP2, TMP5 and SMP5 did show some peculiarity and it will be examine in the field closely for there agronomical and morphological distinctness. The distinct mutants was out come of the mutation breeding Kiran-95 mutant (KMP3) was out come of 150Gy radiation doses and TMP2 and TMP5 (100 & 250Gy) was irradiated with gamma rays. However Sarsabz mutant (SP5) was result of 250Gy. While, 50 and 200Gy irradiated doses did not produce any mutant among three selected varieties. This showed that mutation breeding have the potential to create new ideotypes which can be useful for enhancing the crop productivity.

The results revealed that all selected mutants of 50Gy showed more than 75-96% similarity. Population of 250Gy exhibited 20-44.2% dissimilarity, which is a highest polymorphism in the mutated population of Sarsabz, Kiran-95 and TD1. Irradiation dose of 200Gy gave 20% genetic variability while 7% genetic variants were observed in 100 and 150Gy. Two mutants of 250Gy occupying a distinct place in dendrogram. This may be due to enhancing effect of high doses (Alikamanoglu, 2002). In the present study it was observed that 250Gy generate more variation than 100 and 150Gy.

It is extremely important to study the genetic composition of the germplasm of existing modern-day cultivars in comparison with their ancestors and related species. This will not only provide information on their phylogenetic relationship but will also indicate a chance of findings new and useful genes, as the accessions with most distinct DNA profiles are likely to contain a greater number of novel alleles (Messmer *et al.,* 1992).

The results provide valuable information for fingerprinting at molecular level and more efficient selection of genotypes for crossing and enhancing wheat breeding strategies that will maintain the steady genetic improvement.

Table 3. Similarity co-efficient among the wheat mutants with parents calculated according to Nei & Li's coefficient.

	L1	L2	L3	L4	L5	L6	L7	L8	L9	L10	L11	L12	L13	L14	L15	L16	L17	L18
L1	1																	
L2	0.811	1																
L3	0.877	0.897	1															
L4	0.915	0.844	0.882	1														
L5	0.93	0.822	0.859	0.936	1													
L6	0.759	0.648	0.688	0.765	0.752	1												
L7	0.844	0.883	0.883	0.874	0.914	0.711	1											
L8	0.877	0.855	0.865	0.87	0.875	0.746	0.892	1										
L9	0.755	0.847	0.835	0.784	0.821	0.558	0.861	0.802	1									
L10	0.831	0.835	0.813	0.879	0.854	0.696	0.816	0.877	0.783	1								
L11	0.817	0.952	0.901	0.851	0.831	0.647	0.878	0.866	0.859	0.857	1							
L12	0.799	0.875	0.862	0.825	0.82	0.645	0.846	0.82	0.832	0.806	0.882	1						
L13	0.895	0.8	0.824	0.912	0.904	0.815	0.85	0.895	0.736	0.869	0.816	0.773	1					
L14	0.858	0.836	0.864	0.878	0.865	0.663	0.891	0.862	0.796	0.812	0.875	0.881	0.875	1				
L15	0.817	0.775	0.808	0.833	0.825	0.715	0.827	0.885	0.72	0.787	0.804	0.791	0.883	0.878	1			
L16	0.821	0.863	0.854	0.87	0.847	0.71	0.898	0.898	0.814	0.855	0.864	0.823	0.883	0.9	0.869	1		
L17	0.82	0.83	0.836	0.861	0.853	0.735	0.865	0.911	0.791	0.846	0.842	0.809	0.889	0.893	0.891	0.949	1	
L18	0.798	0.677	0.731	0.791	0.827	0.601	0.738	0.754	0.689	0.751	0.69	0.7	0.779	0.792	0.75	0.787	0.817	1

L1=Sarsabz L2=SMP1, L 3=SMP2, L4=SMP3, L5=SMP4, L6=SMP5, L7=Kiran-95, L8=KMP1, L9=KMP2, L10=KMP3, L11=KMP4, L12=KMP5, L13=TD1, L14=TMP1, L15=TMP2, L16= TMP3, L17= TMP4, L18= TMP5.

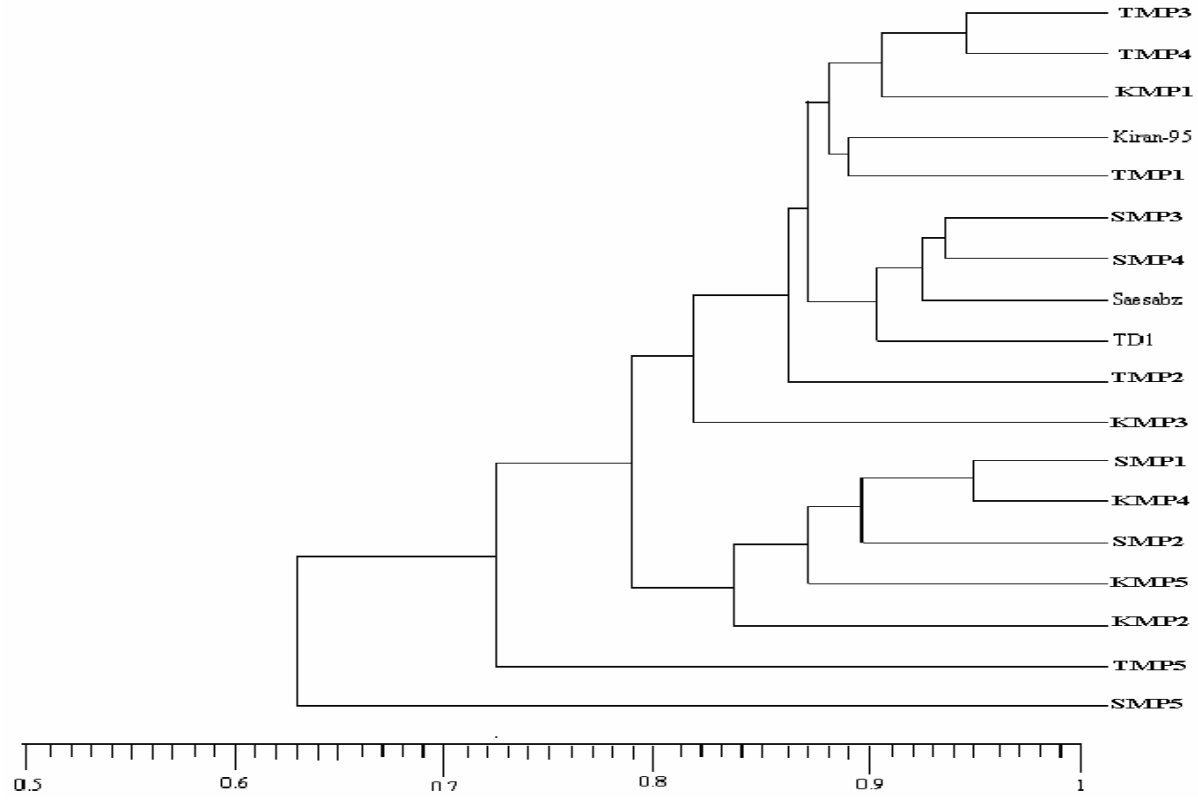

Fig. 7. Dendrogram of fifteen wheat mutants and three parents developed from RAPD data using un-weight pair group method of arithmetic means (UPGMA).

References

Alan, H.S. 2007. Molecular markers to assess genetic diversity. *Euphytica.,* 158: 313–321.

Alikamanoglu, S. 2002. Efficiency of the gamma irradiation in the induction of in vitro somatic mutations*. J. Cell and Molecular Biol.* 1: 19-24.

Anonymous, (a) 2006-07. *Agriculture Statistics of Pakistan*. 2006-07. MINFAL. Islamabad. Pakistan. pp: 4, 110.

Anonymous, (b) 2007-08. *Economic Survey of Pakistan*. 2007-08. MINFAL. Islamabad. Pakistan. pp: 19, 22, 28.

Asif, M., M. Rahman and Y. Zafer. 2005. DNA fingerprinting studies of some wheat (*Triticum aestivum* L.) genotypes using random amplified polymorphism DNA (RAPD) analysis. *Pak. J. Bot*., 37(2): 271-277.

Dubouzet, J.G., Y. Sakuma, Y. Ito, M. Kasuga, E.D. Dubouzet, S. Miura, M. Seki, K. Shinozaki and K.S. Yamaguchi. 2003. OsDREB genes in rice, *Oryza sativa* L., encoded transcription activators that function in drought-, high salt- and cold- responsive gene expression. *Plant J*., 33: 751-63.

Li, X.P., A.G. Tian, G.Z. Luo, Z.Z. Gong, J.S. Zhang and S.Y. Chen. 2005. Soybean DRE-binding transcription factors that is responsive to abiotic stresses. *Theor. Appl. Genet*., 110: 1355-62.

Liu, Q., M. Kasuga, Y. Sakuma, H. Abe, S. Miura, K. Yamaguchi-Shinozaki and K. Shinozaki. 1998. Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought-and low-temperature-responsive gene expression, respectively, in Arabidopsis. *Pl. Cell*. 10: 1391–1406.

Lopez, C.G., G.M. Banowetz, C.J. Peterson and W.E. Kronstad. 2003. Dehydrin expression and drought tolerance in seven wheat cultivars. *Crop Sci.* 43: 577-582.

Martin, G.B., A. Frary, T. Wu, S. Brommonschenkel, J. Chunwongse, E.D. Earle and S.D. Tanksley. 1994. A member of the tomato Pto gene family confers sensitivity to fenthion resulting in rapid cell death. *Pl. Cell.* 6: 1543-1552.

Messmer, M.M., A.E. Melchinger, J. Boppenmaier, R.G. Herrmann and E. Brunklaus-Jung. 1992. RFLP analysis of early-maturing Eurpeon maiz germplsm. *Theor. Appl. Genet.* 83: 1003-1112.

Nakashima. K. and K.S. Yamaguchi. 2005. Molecular studies on stress-responsive gene expression in *Arabidopsis* and improvement of stress tolerance in crop plants by regulon biotechnology. *JARQ.* 39: 4.

Nei. M. and W.H. Li. 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. *Proc. Natl. Acad. Sci. USA.* 76: 5269–5273.

Paterson, A.H., E.S. Lander, J.D. Hewitt, S.E. Peterson, S.E. Lincholn and S.D. Tanksley. 1988. Resolution of quantitative traits into Mendelian factors by using a complete linkage map of restriction fragment length polymorphism. *Nature.* 335: 721-726.

Saiki, R.K., S. Scharf, F. Faloona, K.B. Mullis, G.T. Horn, H.A. Erlich and N. Arnheim. 1985. Enzymatic amplification of β-globin genomic sequence and restriction sites analysis for diagnosis of sickle-cell anemia. *Sci.* 230: 1350-1354.

Schachermayr, G.M., M.M. Messmer, C. Feuillet, H. Winzeler, M. Winzeler and B. Keller. 1995. Identification of molecular markers linked to the *Agropyron elongatum*- derived leaf rust gene Lr24 in wheat. *Theor. Appl. Genet.* 90: 982-990.

Semagn, K., A. Bjornstad and M.N. Ndjiondjop. 2006. An overview of molecular marker methods for plants. *African J. Biotech.* 5 (25): 2540-2568.

Shen, Y.G., W.K. Zhang, S.J. He, Z.S. Zhang, Q. Liu and S.Y. Chen. 2003. An EREBP/AP2-type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration, and ABA stress. *Theor. Appl. Genet.* 106: 923–30.

Shinozaki, K. and K.S. Yamaguchi. 2000. Molecular responses to dehydration and low temperature: differences and cross-talk between two stress signaling pathways. *Curr. Opin. Plant Biol.* 3: 217–23.

Svetlana, L., K. Viktor and B. Andreas. 2007. Molecular markers: actual and potential contributions to wheat genome characterization and breeding. *Euphytica.* 156: 271–296.

Thormann, C.E., M.E. Ferreira, L.E.A. Camargo, J.G. Tivang and T.C. Osborn. 1994. Comparison of RFLP and RAPD markers to estimating genetic relationship within and among cruciferous species. *Theor. Appl. Genet.* 88: 973-980.

William, H.M., R. Trethowan and E.M. Crosby-Galvan. 2007. Wheat breeding assisted by markers: CIMMYT's. Experience. *Euphytica.* 157: 307–319.

William, J.G.K., A.R. Kubelik, K.J. Livak, J.A. Rafalski and S.V. Tingey. 1990. DNA polymorphisms amplified by arbitrary primers are useful as genetic markers. *Nucl. Acids. Res.* 18(22): 6531-6535.

Williamson, V.N., J.Y. Ho, F.F. Wo, N. Miller and I. Kaloshian. 1994. A PCR based marker tightly linked to nematodes resistance genes, Mi, in tomato. *Theor. Appl. Genet.* 87: 757-763.

Xiao, J., J. Li, S. Grandillo, S.N. Ahn, L. Yuan, S.D. Tanksley and S.R. McCouch. 1997. Identification of hetrosis enhancing QTL alleles from a rice wild relative, *Oryza rufipogon*. *Plant and Animal Genome* V., abstracts, San Diego, USA, 88.

Yang-dong, G., T. Yli-Mattila and S. Pulli. 2003. Assessment of genetic variation in timothy (*Phleum pratense* L.) using RAPD and UP-PCR. *Hereditas.* 138: 101–113.

Zhang, J.Z. 2004. From laboratory to field. Using information from Arabidopsis to engineer salt, cold, and drought tolerance in crops. *Plant Physiol.* 135: 615–621.

(Received for publication 8 January 2010)