ISSN 1682-296X (Print)
ISSN 1682-2978 (Online)

Bio Technology

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

Biotechnology 7 (4): 745-750, 2008
ISSN 1682-296X

Genetic Diversity of Wild and Cultivated Barley Genotypes Under Drought Stress Using RAPD Markers

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Abstract: The genetic diversity among cultivated barley with different responses to drought stress was investigated. Initial screens involved growing 16 cultivars under drought conditions in greenhouse. These tests involved 5 tolerant and 5 sensitive types. The results of RAPD analysis indicated its suitability for determination of polymorphism among the samples. Among 30 primers used with RAPD-PCR technique, primers No. 3, 26 and 28 did not assist amplification of any definite bands and primers No. 24, 25 did not produce any scoreable polymorphic bands. In all the 25 selected primers amplified a total of 275 amplicons from 16 barley genotypes were detected, among them, 65 fragments (23.6%) were monomorphic and the rest (76.4%) were polymorphic between one or more genotypes. The cultivar genotypes were clustered according to their simple matching coefficient and complete-link methods. The least similarity was observed between wild genotype (Plot 21) and Valfajr. In general, Rihane with the average of 50.6% and Kavir with the mean of 60.8% had the least and the most similarity with other genotypes, respectively. Primer No. 27 amplified a 600 base pair fragment characteristic of tolerant cultivar lines and absent in susceptible genotypes. Therefore, it is likely that this DNA band be associated with drought tolerance. Clustering on the basis of 51% similarity ranked genotypes into 5 groups. The resulting dendrogram indicated that the cross of tolerant wild genotype (Plot 21) and susceptible Sina genotype which have low genetic similarity, also grouped in distinct cluster. This may be suggested as the most suitable cross to analyze QTLs (Quantitative Trait Loci) involved in drought tolerance.

Key words: Drought tolerance, barley, RAPD markers, genetic diversity, genotypes

INTRODUCTION

Barley is a crop of major economic importance and also a model species for genetics and physiology (Koornneef *et al*., 1997). Wild barley, *Hordeum spontaneum* C. Koch, is the progenitor of the crop species *H. vulgare* L. (Harlan, 1995). Habitats of wild barley in the Fertile Crescent differ widely in water availability, temperature, soil type, altitude and vegetation. Morphological and physiological variation observed in these habitats has arisen by natural selection (Nevo, 1992). The genetic diversity of wild barley has been studied both within and between populations collected from the Fertile Crescent (Nevo, 1992; Forster, 1999).

Drought, like many other environmental stresses, has adverse effects on crop yield. Low water availability is one of the major causes for crop yield reductions affecting the majority of the farmed regions around the world. As water resources for agronomic uses become more limiting, the development of drought-tolerant lines becomes increasingly more important (Duvick, 1997).

To differentiate drought tolerance genotypes, several selection indices have been suggested on the basis of a mathematical relationship under favorable and stressed conditions (Huang, 2000). Tolerance index (TOL) and mean productivity (MP) (Rosielle and Hambling, 1981), Stress Susceptibility Index (SSI) (Clark *et al*., 1992), Geometric Mean Productivity (GMP) (Kristin *et al*., 1997; Fernandez, 1992), Stress Tolerance Index (STI) (Fernandez, 1992), Yield reduction ratio (Yr) (Golestani and Assad, 1998) have all been employed under various conditions.

The development of molecular techniques has allowed genetic diversity to be evaluated by means of molecular markers. These present some advantages over other methods, because, in addition to identifying great polymorphism, they do not show interaction with different environments and can also be evaluated at any developmental stage (Williams *et al*., 1990).

Randomly amplified polymorphic DNA sequences or RAPD markers are based on the amplification of unknown DNA sequences using single, short and random oligonucleotide primers (Ovesna *et al*., 2002). RAPD-PCR

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is currently used as a tool to assess genetic markers useful in breeding programs for assessment of genetic variability between genotypes (Hillel *et al*., 1992). RAPD markers have often been used for studying genetic diversity within plant germplasm collections (Campos *et al*., 1994; Daiya *et al*., 2002). They have been proposed by several groups as efficient tools for identification of DNA markers associated with agronomical important traits (Banerjee *et al*., 1999; Hittalmani *et al*., 1995). One practical application of molecular biological techniques in plants is the genetic identification or fingerprinting of molecular markers. The technique of analyzing molecular markers is based on the detection of the DNA sequences or combinations that are unique to the individual plant under study (Henry, 1997).

The objectives of this study were to use Random Amplified Polymorphic DNA (RAPD) markers to assess genetic diversity among barley genotypes with different responses to drought stress and to establish specific DNA markers associated with drought tolerance in barley genotypes using RAPD patterns.

MATERIALS AND METHODS

This study was conducted at College of Agriculture, Shiraz University, Iran, during 2005-2006. The plant material used in this study (Table 1), were chosen from Zarghan Research Center (Fars Province, Iran). These cultivars were planted in pots containing 5 kg pots using a completely randomized design with four replications under each of irrigated and water stressed conditions. Initiation of differential irrigation was started at 50% flowering stage and continued through crop maturity. Pots under drought stress were irrigated to FC (Field Capacity) when the weight of each pot reached to 50% of FC. Irrigation treatment was carried based on pot weight. Control was irrigated everyday. Fertilizers were applied at the rate of 120 mg pot^{-1}. Grain yield was determined under both moisture non-stress and moisture stress experiments and used as Yp and Ys, respectively. The stress tolerance attributes TOL, MP, SSI, GMP, STI and Yr were calculated by the related formulas to determine drought tolerance responses of the cultivar genotypes.

Genomic DNA extraction: For isolation of genomic DNA the seeds from barley germplasm (Table 1) were surface sterilized and sowed in pots containing 1 kg of soil maintained in a greenhouse. Fresh young leaves from 6 plants per cultivar type were collected after 10 day germination and stored at -70°C until further use.

Genomic DNA from fresh leaves was extracted by the modified CTAB (cetyltrimethylammonium bromide) method of Doyle and Doyle (1987). The DNA precipitate was spooled with Pasteur pipette using a flame-formed hook and transferred to 500 μL TE. Two microliter of RNase for RNA removal. After overnight RNase treatment, the pure DNA precipitate was collected by spooling, washed with ethanol, acetate sodium and ammonium sulphate and transferred to TE and stored at -20°C. The quality and quantity of DNA was tested by Nano-drop and agarose gel electrophoresis.

Table 1: Barley cultivar genotypes and their tolerance status

Cultivar	Tolerance	Cultivar	Tolerance
Wild (Vineyard)	Moderate	Arivat (Kavir)	Moderate
Wild (Plot 21)	Tolerant	Star	Sensitive
Wild (Plot 41)	Moderate	Aljerseres	Sensitive
Afzal	Moderate	Sina	Sensitive
Rihane	Tolerant	Valfajr	Tolerant
Victoria	Tolerant	Gohar	Moderate
Aras	Tolerant	Star/Jerusa/em/Rihane-03	Moderate
Karoon/Kavir	Sensitive	Himalya	Sensitive

RAPD-PCR of samples: For RAPD analysis DNA extracted from young leaves from each barley cultivar genotype was used as template in the PCR mix. Other components of the reaction mixtures (20 μL total volume) were: 10xPCR buffer, $MgCl_2$ (0.9 μL), dNTPs (0.4 μL), primer (0.4 μL), template DNA (1.5 μL), Taq DNA polymerase (1 units). DNA amplification was carried out according to the method of Williams *et al*. (1990). Amplifications were performed in a Techno gene thermolcycler. The PCR mix samples were preheated at 94°C for 4 min. This was followed by amplification reactions were run for 35 cycles consisting of 1 min at 94°C, 1 min at 36°C, 2 min at 72°C with a final extension time of 15 min at 72°C. The amplification products were analyzed by agarose gel electrophoresis in 1% TBE, stained with ethidium bromide, visualized by ultraviolet illumination and pictured with Gel Documentation. The molecular weight of all bands was calculated by comparison with the 100 base pair marker. All reactions were repeated at least twice and only reproducible bands were scored for statistical analysis. Other visible bands were not considered because of their ambiguous nature.

Data analysis: RAPD marker bands obtained were scored as present (1) or absent (0). Similarity among genotypes was computed as simple matching coefficients (SMC), Sij = (a+d)/(a+b+c+d), between each pair of genotypes, where, a is number of fragments in common between lines; d is number of fragments absent in both lines and b and c is number of fragments not in common between two lines (Sokal and Michener, 1958). Cluster analysis was performed according to SMC method assisted with NTsys 2.02 software.

RESULTS

RAPD analysis was used to determine the genetic variation and molecular markers among the selected cultivar genotypes. Thirty primers (Table 2) were used to differentiate between the sixteen genotypes of barley. Primers No. 3, 26 and 28 did not produce any detectable band and primers No. 24 and 25 did not detect any polymorphic band. Therefore, clustering was based on polymorphic bands produced by the rest primers. A total of 275 DNA bands, ranging from 400 to 2000 bp, were generated for the 16 tested genotypes. Out of these amplified DNA bands, 65 were conserved and fixed (monomorphic) among all genotypes, while 210 (76.4 %) were polymorphic. The number of RAPD amplicons produced by each primer varied from 4 (primer No. 4) to 17 (primer No. 27) with an average of 11/primer. The highest numbers of polymorphic DNA fragments were obtained with primer No. 27 and 1.

The patterns obtained with primer P27 for genotypes suggested that this primer has the ability to produce fingerprints specific to drought tolerance. A 600 bp DNA fragment was present exclusively in tolerant genotypes (Fig. 1).

Table 2: Primer nucleotide sequence used to amplify DNA

Name	Sequence 5'→3'	Name	Sequence 5'→3'
P1	ACACAGAGGG	P16	CCTGGGCTTC
P2	CCTCTCGACA	P17	CCTGGGCTTG
P3	TCTCAGCTGG	P18	CCTGGGCCTA
P4	GTGTGCCCCA	P19	CCTGGGCCTC
P5	CCACGGGAAG	P20	TGCCCCGAGC
P6	TCGGCGGTTC	P21	TTCCCCGACC
P7	CTGCATCGTG	P22	GAGGGCGGGA
P8	TGAGCCTCAC	P23	AGGGGCGGGA
P9	TCGGCACGCA	P24	GAGGTCCAGA
P10	CTGCGCTGGA	P25	GGGGGTTAGG
P11	CCATTCCCCA	P26	ATCGGGTCCG
P12	GGTGAACGCT	P27	CCGTGCAGTA
P13	CTCCCTGAGC	P28	TAGCCGTGGC
P14	TTCCGGGTGA	P29	GGCTAGGGGG
P15	GAGCTCGCGA	P30	TACGTGCCCG

Bands generated from 25 RAPD primers, were utilized to calculate the genetic similarity index (RAPD-GS) among the 16 barley genotypes (Table 3). Based on the 210 polymorphic bands obtained, a genetic distance matrix was constructed using the complement of simple matching similarity coefficient. Genetic Distances (GD) among cultivar genotypes ranged from 0.12 to 0.65, with an average of 0.45 (Table 3). The smallest genetic distance obtained was observed between wild (Vineyard) and (Plot 41), while the largest distance was between wild (Plot 21) and Valfajr barley types. Rihane and Star/Jerusa/em/ Rihane-30 had low similarity (37%) in spite of existence of Rihane in pedigree of this line. Wild genotypes had the highest similarity with each other. The highest similarity between wild genotypes and cultivated genotypes belonged to Rihane. In general, among studied genotypes, Rihane and Kavir (with average of 0.506 and 0.608) had the lowest and the highest similarity with other genotypes, respectively. The similarity between wild genotypes was higher than that between cultivated genotypes.

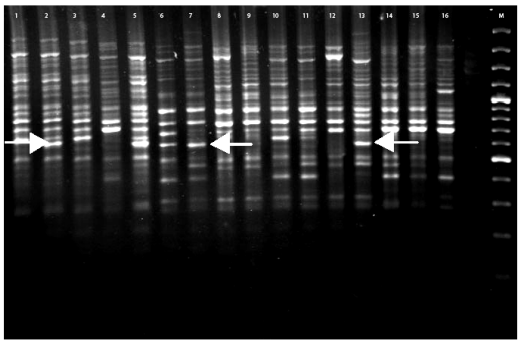

Fig. 1: Results of RAPD amplification based on the use of primer No. 27 in tolerant and non-tolerant barley genotypes. A 600 bp fragment which is present in tolerant and absent in non-tolerant genotypes, is indicated with an arrow

Table 3: Similarity (%) between 16 barley genotypes based on simple matching coefficient (SMC)

Genotypes	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	1.00															
2	0.81	1.00														
3	0.88	0.84	1.00													
4	0.48	0.54	0.46	1.00												
5	0.79	0.75	0.81	0.37	1.00											
6	0.48	0.39	0.41	0.46	0.43	1.00										
7	0.50	0.46	0.49	0.49	0.45	0.64	1.00									
8	0.44	0.45	0.48	0.50	0.44	0.73	0.55	1.00								
9	0.46	0.53	0.48	0.53	0.53	0.67	0.52	0.78	1.00							
10	0.54	0.55	0.58	0.50	0.54	0.65	0.53	0.67	0.68	1.00						
11	0.44	0.48	0.45	0.50	0.41	0.73	0.65	0.62	0.55	0.64	1.00					
12	0.44	0.41	0.51	0.55	0.39	0.58	0.58	0.70	0.62	0.62	0.57	1.00				
13	0.44	0.35	0.45	0.48	0.46	0.49	0.55	0.53	0.58	0.50	0.48	0.65	1.00			
14	0.41	0.40	0.43	0.60	0.44	0.60	0.55	0.69	0.69	0.56	0.54	0.62	0.63	1.00		
15	0.42	0.41	0.38	0.62	0.37	0.62	0.59	0.68	0.67	0.62	0.63	0.61	0.60	0.76	1.00	
16	0.49	0.48	0.43	0.65	0.41	0.53	0.48	0.49	0.53	0.51	0.59	0.50	0.54	0.49	0.57	1.00

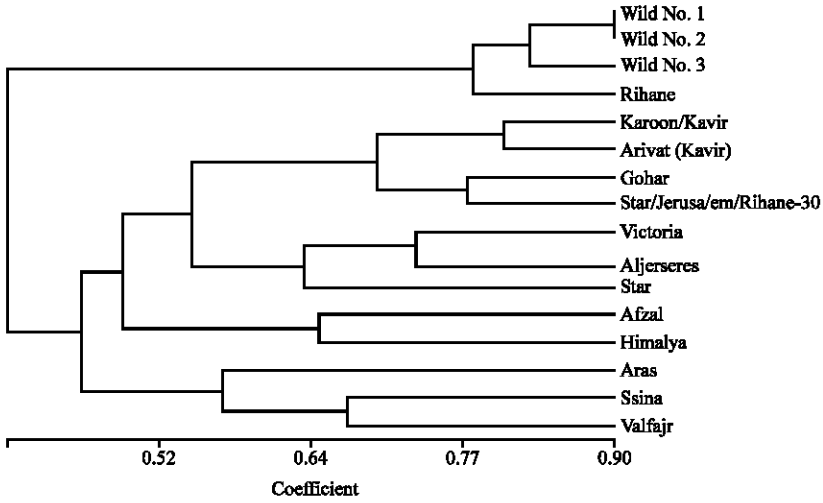

Fig. 2: Dendrogram for 16 barley genotypes using 30 primers based on SMC cluster analysis of RAPD data

Totally, evaluated genotypes did not exhibit enough genetic variation and this can result in the loss of such genotypes from the populations owing to lack of tolerance trait and subsequently broken genotype resistance. Therefore, we suggest using new cultivar genotypes in breeding to improve such genotypes.

Figure 2 presents the dendrogram for barley genotypes, according to the SMC clustering method. The 16 genotypes were separated into five distinct groups. Considering the genetic distances detected by the RAPD markers, the first group (a) is comprised by Afzal and Himalya; the second group (b) is comprised of Valfajr and Sina; the third group (c) comprises of Star/Jerusa/em/Rihane-30, Gohar, Kavir and Karoon/Kavir; the fourth group (d) is formed by genotypes, Aljerseres Victoria and Star and the fifth and last group (e) is comprised of wild genotypes and Rihane.

DISCUSSION

The drought resistance differences are in partial agreement with the relationships determined from the distance matrix. Drought tolerant and non-tolerant genotypes were placed in the same group. Present results suggest that one characteristic is not a good predictor of genetic similarity or dissimilarity. For example Gohar, Kavir, Karoon/Kavir and Star/Jerusa/em/Rihane-03 are tolerant, while star is a susceptible genotype, yet they are grouped together in Fig. 2 because this is probably a function of RAPD markers used in analyzing genetic distances. The 25 markers selected amplified portions of genomic DNA sequence of unknown function and are based only on the randomly produced sequence of each oligonucleotide. The marker sequences thus may originate from any part of the genome, including genes, promoters, repeat stretches of DNA and non-coding regions. Additional RAPD markers linked to the genes controlling drought resistance are needed to enable better discrimination of this phenotypic character. Also genotypes Aras and Victoria are tolerant, while Aljerseres is susceptible; still, they are placed in the same group. Aras and Victoria have the same origin (France), so we expected this result because they have similar background. Although Rihane was involved in pedigree of Star/Jerusa/em/Rihane-03, they are grouped in different clusters. Therefore, the use of molecular markers in investigating genome directly is a useful tools to determine relationship between genotypes.

If there is possibility of several crosses, two parents should be crossed in order they have the farthest genetic distance in order to determine QTLs involved in drought tolerance. On this basis, the cross of tolerant wild genotype (Plot 21) and susceptible genotype (Sina) is suggested as the most suitable cross for drought tolerance analysis studies as they have low genetic similarity and also grouped in distinct cluster

Through the technique of RAPD, which is relatively cheap and requires small quantities of DNA, it was possible to identify one primer (P27) that generated polymorphic band in tolerant and non-tolerant genotypes. This band can be considered as a potential marker to identify drought resistant cultivar genotypes or may even be more useful when converted into a simple-sequence PCR-based marker that can be used for large-scale water-stress tolerance screening of genotypes, or converted to SCAR individual primers in order to distinguish drought resistant genes. Also each RAPD marker is the equivalent

of a Sequence Tagged Site (Olson *et al*., 1989), which can greatly simplify information transfer in collaborative research programs.

Pakniyat *et al*. (2004) introduced markers linked to salt tolerance in cultivated and wild barley using these primers. Also Pakniyat and Tavakol (2007) found markers related to drought tolerance in bread wheat genotypes using these markers.

The use of RAPD has been demonstrated to have several advantages over other techniques of DNA fingerprinting (Keil and Griffin, 1994). In contrast with other molecular techniques such as, SNPs (Simple Nucleotide Polymorphisms), SSRs (Simple Sequence Repeats), RFLP, DNA sequencing and allozymes, the technique of RAPD is very simple to carry out and it does not require previous knowledge of the genome in study. This is a rapid method that is technically and financially less demanding compared to the AFLP method and thus could be more useful for routine germplasm screening. It allows examination of a large number of loci and primers are made on aleatory sequences. Despite of its advantages this technique has some practical problems, for example reproducibility (Van Oppen *et al*., 1996; Jones *et al*., 1997). However, if it is possible to standardize the DNA extraction procedure and find the optimal PCR conditions, the reproducibility of the patterns should not be a problem. Nearly all RAPD markers are dominant, as DNA segments of the same length are amplified from one individual but not from another. It is not possible to distinguish whether a DNA segment is amplified from a locus that is heterozygous (1 copy) or homozygous (2 copies) with a dominant RAPD marker. Co-dominant RAPD markers, observed as different-sized DNA segments amplified from the same locus, are detected only rarely. Bands of identical size amplified with the same primer are considered to be at the same locus consisting of two alleles. This interpretation could be justified because barley exhibits very high rate of selfing (99%) (Brown *et al*., 1978). As they are selfers, the widely recorded problem of RAPDs being dominant markers is not of relevance in this study.

It is possible that products of different loci can have similar molecular weights and for this reason, establishing the identity of the marker bands can be difficult. Rieseberg (1996) analyzed the homology of 220 co-migrating fragments of RAPD in populations of wild sunflowers and found that 91% of them turned out to be homologous. Results of Rieseberg (1996) indicated that fragments of similar size are good indicator of homology. In present study, a distinct band characteristic to tolerance trait was noticed. In addition similar amplicons were obtained using primers P24 and P25.

REFERENCES

Banerjee, N.S., P. Manoj and M.R. Das, 1999. Male sex associated RAPD markers in *Piper longum* L. Curr. Sci., 77: 693-695.

Brown, A.H.D., E. Nevo, D. Zohari and O. Dagan, 1978. Genetic variation in natural populations of wild barley (*Hordeum spontaneum*). Genetica, 49: 97-108.

Campos, L.P., J.V. Raelson and W.F. Grant, 1994. Genome relationships among Lolus species based on random amplified polymorphic DNA (RAPD). Theor. Applied Genet., 88: 417-422.

Clark, J.M., R.M. DePauw and T.F. Townley Smith, 1992. Evaluation of methods for quantification of drought tolerance in wheat. Crop Sci., 32: 723-728.

Daiya, K.K., S. Archak and J.L. Karihaloo, 2002. DNA fingerprinting of guava (*Psidium guajava* L.) cultivars using RAPD markers. Indian J. Genet. Resour., 15: 112-115.

Doyle, J.J. and J.L. Doyle, 1987. A rapid DNA isolation procedure for small quantities of fresh leaf tissue. Phytochem. Bull., 19: 11-15.

Duvick, D.N., 1997. What is yield. In: Developing Drought and Low N-tolerant Maize, Edmeads, G., M. Banzinger, H.R. Mickelson and C.B. Pena-Valdivia (Eds.). CIMMYT, El Batan, Mexico, ISBN: 968-6923-93-4 pp: 332-335.

Fernandez, G.C.J., 1992. Effective selection criteria for assessing plant stress tolerance. Proceedings of The International Symposium on Adaptation of Vegetables and other Food Crops in Temperature and Water Stress, August 13, Asian Vegetable Research and Development Center, Shanhua, Taiwan, pp: 257-270.

Forster, B.P., 1999. Studies on Wild Barley, *Hordeum spontaneum* C. Koch at the Scottish Crop Research Institute. In:. Evolutionary Theory and Processes: Modern Perspectives. Papers in honour of Eviatar Nevo, Wasser, S.P. (Ed.). Kluwer Academic Publishers, Kluwer, ISBN: 0792355180 pp: 325 344.

Golestani, S.A. and M.T. Assad, 1998. Evaluation of four screening techniques for drought resistance and their relationship to yield reduction ratio in wheat. Euphytica, 103: 293-299.

Harlan, J.R., 1995. Barley. In: Evolution of Crop Plants, Smartt, J. and N.W. Simmonds (Eds.). Longman, London, ISBN: 13978-0470233726, pp: 140-147.

Henry, R.J., 1997. Practical Applications of Plant Molecular Biology. 1st Edn., Published Chapman and Hall, Chapman, ISBN: 0-41-273220-3.

Hillel, J., E.A. Dunnington and P.B. Siegel, 1992. DNA markers in poultry breeding and genetic analysis. Poult. Sci. Rev., 4: 169-186.

Hittalmani, S., M.R. Foolad, T.V. Mew, R.L. Rodriguez and N. Huang, 1995. Development of PCR-based marker to identify rice blast resistance gene in a segregating population. Theor. Applied Genet., 91: 9-10.

Huang, B., 2000. Role of Root Morphological and Physiological Characteristics in Drought Resistance of Plants. In: Plant-Environment Interactions, Wilkinson, R.F. (Ed.). Marcel Inc., New York, ISBN: 13978-0824703776, pp: 39-64.

Jones, C.J., K.J. Edwards, S. Castaglione, M.O. Winfield and F. Sala *et al*., 1997. Reproducibility testing of RAPD, AFLP and SSR markers in plants by a network of European laboratories. Mol. Breed., 3: 381-390.

Keil, M. and R.A. Griffin, 1994. Use of random amplified polymorphic DNA (RAPD) markers in the discrimination and verification of genotypes in *Eucalyptus*. Theore. Applied Gene., 89: 442-450.

Koornneef, M., C. Alonso-Blanco and A.J.M. Peeters, 1997. Genetic approaches in plant physiology. New Phytologist, 137: 1-8.

Kristin, A.S., R.R. Serna, F.I. Perez, B.C. Enriquez and J.A.A. Gallegos *et al*., 1997. Improving common bean performance under drought stress. Crop Sci., 37: 51-60.

Nevo, E., 1992. Origin, Evolution, Population Genetics and Resources for Breeding of Wild Barley, *Hordeum spontaneum*, in the Fertile Crescent. In: Genetics, Biochemistry, Molecular Biology and Biotechnology, Shewry, P. (Ed.). CAB International, Wallingford, UK., pp: 19–43.

Olson, M., L. Hood, C. Cantor and D. Botstein, 1989. A common language for physical mapping of human genome. Science, 245: 1434-1435.

Ovesna, J., K. Polakova and L. Leisova, 2002. DNA analysis and their application in plant breeding. Czech. J. Genet. Plant Breed, 38: 29-40.

Pakniyat, H., A. Namayandeh and B.P. Forster, 2004. Identification of RAPD markers linked to salt tolerance in cultivated and wild barley. Proceedings of the 9th International Barley Genetics Symposium, June 20-26, Brono. Czech Republic, pp: 191-194.

Pakniyat, H. and E. Tavakol, 2007. RAPD markers associated with drought tolerance in bread wheat (*Triticum aestivum* L.). Pak. J. Biol. Sci., 10: 3237-3239.

Rieseberg, L.H., 1996. Homology among RAPD fragments in inter-specific comparisons. Mol. Ecol., 5: 99-105.

Rosielle, A.A. and J. Hamblin, 1981. Theoretical aspects of selection for yield in stress and non-stress environments. Crop Sci., 21: 943-945.

Sokal, R.R. and C.D. Michener, 1958. A statistical method for evaluating systematic relationship. Kans. Univ. Sci. Bull., 38: 1409-1438.

Van Oppen, M.J, H.H. Klerk, M. Graaf, W.T. Stam and J.L. Olsen, 1996. Assessing the limits of random amplified polymorphic DNAs (RAPDs) in seaweed biogeography. J. Phycol., 32: 433-444.

Williams, 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. Nucleic Acids Res., 18: 6531-6535.