8th International Conference on Water Resources and Arid Environments (ICWRAE 8): 150-156
22-24 January 2019*, Riyadh, Saudi Arabia*

Theme: Water Conservation Molecular Characterization of Multifarious Corn Genotypes under Divergent Moisture Regimes

[1]Ijaz Rasool Noorka, [1]Taufiqullah, [2]Azeem Iqbal Khan and [3]J.S. (Pat) Heslop-Harrison

[1]Department of Plant Breeding and Genetics, College of Agriculture,
University of Sargodha, Sargodha, Pakistan
[2]Centre of Agricultural Biochemistry and Biotechnology, University of Agriculture, Faisalabad, Pakistan
[3]Department of Biology, University of Leicester, Leicester LE1 7RH, United Kingdom (UK)

Abstract: Variability in soil moisture response was assessed in twelve maize genotypes under contrasting water regimes using 32 RAPD primers. RAPD markers are considered very useful to study the genetic variability of different plant species including maize. In present study, RAPD markers produced 115 polymorphic bands; however maximum number of polymorphic bands wereamplified by the primer GLK-02 followed by the primer GLI-16 by amplifiing seven bands. Narrow genetic base was observed among twelve genotypes with the range of genetic similarity from 84.04% to 57.32%. Genotype DR-185 was found to be most dissimilar among all the genotypes studied. The present study confirmed that the selection of promising maize genotype should be based on morphological and molecular characterization maize to combat water shortage prevailing throughout the globe.

Key words: DNA marker · Genetic difference · Polymorphism · RAPD · Drought Stress · Maize

INTRODUCTION

All biological plants face several distinct types of biotic as well as abiotic stresses. These occur persistently or rarely at different growth stages of plant in their life [1, 2] Among abiotic stresses plant faced water shortage, salinity, extreme weather conditions and temperature fluctuations [3, 4]. Abiotic stresses affect the crop productivity [5]. Drought affects plants at different levels, from slight to complete wilting even death. Drought tends to reduce crop yields and is considered as a serious threat of food security [6].The destructive effect of drought may occur seriously in the future years due to changing climatic conditions.World food security is depending on crop improvement process to introduce and new blood varieties with increased tolerance to stresses particularly drought and salinity stresses [7, 8]. Improved genetics enclosed in seed, adaptation to improved agricultural management, availability of proper inputs, infrastructure, skill in crop breeding and soil management practices are the way to ensure food provision [9, 10].

Maize grain is one of the versatile and most important cereal used both as fresh and processed form in human diet having value added concept of economic in corn markets [11]. In many regions of the world, maize is one of the staple foods and ranked prime source of diet like in Latin America, Africa and Asia. Without the required increase in production area of maize, its annual growth rate will be 1.5% to meet its requirement annually. Worldwide, maize growth rate was 1.2% but it is only 1% in developed countries. It ranks third in cereals after wheat and rice. Under irrigated condition, it is grown on 0.98 million ha. Pakistan maize production is 3.70 million ton with an average 3.8 ton/ha. It's very useful to have superior lines best adapted to Pakistani conditions. Genetic variation for drought tolerance gives an insight to the plant breeders to develop maize tolerant lines [12, 13, 14]. With the availability of genetic variation the maize plant may combat against drought tolerance. Hybrid maize is highly productivity more than land laces [15].

Genetics principles have been used for the improvement of drought tolerance and yield components [16, 17]. Conventional breeding methodologies are still valid but to access complex genetic nature of character, the advanced methods of microbiology are needed [18, 19]. Biotechnological applications in plant breeding, agronomy, physiology etc., made through the identification of quantitative trait loci that are responsible

Corresponding Author: Ijaz Rasool Noorka, Department of Plant Breeding and Genetics, College of Agriculture, University of Sargodha, Sargodha, Pakistan. Tel: 00923006600301, E-mail: ijazphd@ yahoo.com.

for yield traits. Application of molecular markers have opened a new vistas of development to identify genetic difference for the last decade and it has been found that technique of molecular marker has been advanced as an alternate approach [20]. RAPD marker has been used to estimate genetic difference in maize [21, 22]. Due to its simplicity and power of detection differences, random amplified polymorphism (RAPD) has widely been used to estimate the genetic variation, even in closely related individuals. The present study focused on the estimation of genetic difference among the screened maize germplasm to validate the conventional results and its onward use in maize germplasm in line × testers mating design [23].

MATERIALS AND METHODS

Plant Material: Total twelve maize inbred lines (DR-187, DR-177, DR-198, DR-194, DR-158, DR-185, DR-189, DR-159, Pak Afgoee, Sadaaf, EV-1098 and EV-6098) were selected on the basis of their physiological responses to different moisture levels by the initial experiment conducted at College of Agriculture, University of Sargodha. Eight inbred lines were found to have best tolerance level and four inbred lines were taken from susceptible inbred lines. Seeds of the concerned genotypes were planted in pots in a growth chamber (C-606, C.E.Ltd. Willipeg Manitoba, Canada) at the Center of Biochemistry and Biotechnology (CABB), University of Agriculture, Faisalabad and sample were taken at 3-4 leaves stage of each seedling at the age of ten days for genomic DNA extraction. Principal component analysis was performed using PAST software. Analysis of Molecular Variance (AMOVA) was calculated using Arequin version 3.5.

Extraction of Genomic DNA: Total genomic DNA was extracted by the modified CTAB method. The concentration of the DNA samples was determined by Nano Drop ND-1000 spectrophotometer (Nano Drop Technologies, Wilmington, Delaware). DNA quality was checked by running it on 0.8% agarose gel with 0.5x TBE buffer. DNA samples with good quality were selected and DNA giving smears in the gel were rejected and re-extraction was done. Concentration of $MgCl_2$, Taq DNA polymerase and DNA template were optimized for best amplification.

RAPD Analysis: All polymerase chain reactions were used to run in a thermal cycler (Peq lab, AG No. 533300839; Germany) by using 15 ng DNA as template. The conditions were optimized with the concentration of genomic DNA, 10X PCR buffers, $MgCl_2$, dNTPs, Decamer primer, GL Decamer I-01, GL Decamer I-03, GL Decamer I-04, GL Decamer I- 06, GL Decamer I-16, GL Decamer I-19, GL Decamer I-20, GL Decamer J-01, GL Decamer J-04, GL Decamer J-05, GL Decamer J-08, GL Decamer J-09, GL Decamer J-10, GL Decamer J-12, GL Decamer J-14, GL Decamer J-15, GL Decamer J-16, GL Decamer J-17, GL Decamer J-18, GL Decamer J-20, GL Decamer K-01, GL Decamer K-02, GL Decamer K-03, GL Decamer K-04, GL Decamer K-08, GL Decamer K-11, GL Decamer K-12, GL Decamer K-15, GL Decamer K-16, GL Decamer K-17, GL Decamer K-19, GL Decamer K-20 (Gene Link Company, Hawthorne, NY, USA, USA) and Taq DNA polymerase (MBI, Ferments, Vinius, Lithuania). The temperature for DNA amplification was fixed at 94°C for the period of 5 min as initial denaturation, then 40 cycles with same temperature for 1 minute followed by 36°C for 1 min, 72°C for 2 min and 72°C for 10 min as extension temperature. The PCR product was run on 1.2% (w/v) agarose gel stained with Ethidium bromide at 100 V for 2 hrs, checked under UV trans- illuminator at 300nm and photographed in a gel doc system (SynGen, SynopticsLtd, UK) [24].

Scoring of Bands: The finger prints of DNA fragments amplified by RAPD Polymerase chain reaction (PCR) counted for each primer scored as present (1) and the absent as (0). Coefficients of similarity matrix among inbred lines were observed by the method delineated [25]. Dendrogram was constructed based on these similarity indices by Pop gene software (version 1.44) using Un-weighted Pair Group of Arithmetic Means (UPGMA) to check the genetic distance among twelve maize inbred lines.

RESULTS AND DISCUSSION

The genetic difference was checked by 32 random primers on twelve different maize inbred lines resulted in 29 RAPD Decamer as polymorphic (Table 2). Total of 157 loci were amplified, with an average of 5.41 loci per primer and 115 loci showed polymorphism with an average 3.96 loci per primer whereas the other 42 loci were found monomorphic in all twelve maize inbred lines Maximum number of bands was amplified by the primer GLK-02 which amplified nine bands followed by the primer GLI-16 which amplified seven bands. Two primers GLK-04 and GLJ-04 amplified six polymorphic bands each, while the primers GLI-01 and GLI-10 amplified single polymorphic bands. RAPD technology has been efficiently used to observe genetic difference and variations among the

maize genotypes [26]. The findings of this research are in agreement with the results of [27, 28]. However, the range of polymorphic bands are comparatively low as revealed by the findings [26] who reported number of polymorphic bands varied from 3-18, while [29] reported the number of loci amplified by RAPD primers for genetic analysis of six maize hybrids ranged from 4-13. RAPD data for the genetic similarity of twelve maize inbred lines was constructed by the method of [25] coefficient of similarity matrix as shown in the Table 2. Narrow genetic base was found among twelve maize inbred lines with a range from 84.04% to 57.32%. Inbred lines DR-189 and DR-194 have the greatest similarity which is 84.04%. These two lines differed from each only in 11 bands with 29 different primers followed by the genotypes EV-6098 and DR-159 which has the genetic similarity of 83.44%. Genotypes, Pak Afgoee and DR-194 were more genetically dissimilar than other genotypes with the genetic similarity of 57.32%. These two inbred lines showed difference among each other by 51 bands with 29 different primers followed by inbred lines DR-177, DR-187, DR-185 and DR-177 which showed 64.97% similarity. Genetic relationship among 12 maize inbred lines was estimated by analyzing the dendrogram. Cluster analysis revealed that the first group comprising 9 inbred lines and inbred lines DR-194 and DR-189 are closely linked followed by the inbred lines DR-158 and DR-198. The genotypes DR-187 showed some dissimilarity from the rest of the members of this group. Other closely linked inbred lines of this group are DR-159, EV-6098, Sadaaf and EV-1098 respectively. The inbred lines DR-185 was unclustered and showed its dissimilarity from rest of the inbred lines whereas two other inbred lines DR-177 and Pak Afgoee form a separate cluster (Fig. 2). It is evident from the cluster analysis that these

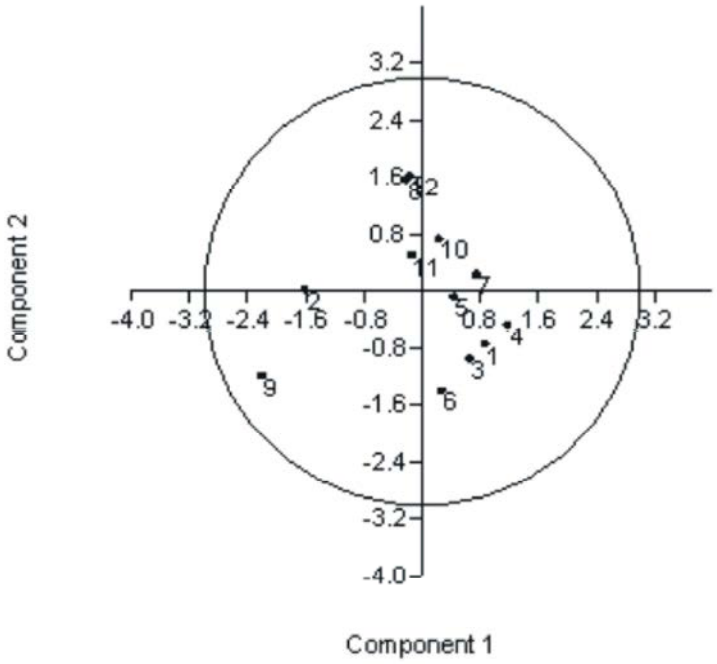

Fig. 1: Principal Component analysis of 12 Maize accessions using RAPD data

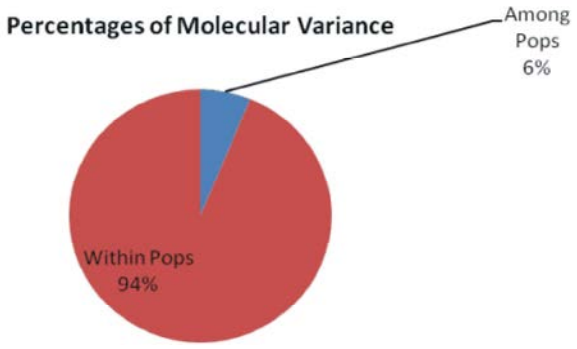

Table 1: Summary AMOVA Table

Source of variation	df	SS	MS	Est. Var.	%
Among Pops	1	26.917	26.917	1.311	6%
Within Pops	10	199.250	19.925	19.925	94%
Total	11	226.167		21.236	100%

Table 2: List of RAPD primers whom amplified loci was found

Sr.No	Primer Name	Sequence	TNB	NPB	PIC Value		MBF
1	GL I-01	ACCTGGACAC	2	1	0.49	0.71	
2	GLI-03	CAGAAGCCCA	6	4	0.13	0.93	
3	GL I-04	CCGCCTAGTC	6	3	0.34	0.81	
4	GL I-16	TCTCCGCCCT	6	4	0.39	0.78	
5	GL I-19	AATGCGGGAG	5	4	0.36	0.80	
6	GL I-20	AAAGTGCGGG	9	6	0.40	0.77	
7	GL J-01	CCCGGCATAA	6	4	0.31	0.83	
8	GL J-04	CCGAACACGG	7	6	0.51	0.70	
9	GL J-05	CTCCATGGGG	5	5	0.24	0.87	
10	GL J-09	TGAGCCTCAC	5	3	0.43	0.75	
11	GL J-10	AAGCCCGAGG	5	1	0.05	0.97	
12	GL J-12	GTCCCGTGGT	7	5	0.59	0.64	
13	GL J-14	CACCCGGATG	4	3	0.43	0.75	
14	GL J-15	TGTAGCAGGG	5	3	0.48	0.72	
15	GL J-16	CTGCTTAGGG	4	4	0.19	0.90	
16	GL J-17	ACGCCAGTTC	3	2	0.31	0.83	
17	GL J-20	AAGCGGCCTC	5	2	0.31	0.83	
18	GL K-01	CATTCGAGCC	5	4	0.36	0.80	
19	GL K-02	GTCTCCGCAA	9	9	0.55	0.67	
20	GL K-03	CCAGCTTAGG	5	5	0.66	0.58	
21	GL K-04	CCGCCCAAAC	7	6	0.40	0.77	
22	GLK-08	GAACACTGGG	5	2	0.09	0.95	
23	GL K-11	AATGCCCCAG	3	2	0.55	0.67	
24	GL K-12	TGGCCCTCAC	6	6	0.73	0.51	
25	GL K-15	CTCCTGCCAA	6	6	0.49	0.71	
26	GL K-16	GAGCGTCGAA	7	7	0.64	0.60	
27	GL K-17	CCCAGCTGTG	3	2	0.48	0.72	
28	GL K-19	CACAGGCGGA	8	4	0.17	0.91	
29	GL K-20	GTGTCGCGAG	3	2	0.31	0.83	
		Total	157	115	11.39	22.31	
		Mean	5.41	3.96	0.39	0.76	

Table 3: Nei's unbiased measurement constructed on twelve maize genotypes genetic distance

Pop ID	1	2	3	4	5	6	7	8	9	10	11	12
1	****	0.6497	0.7516	0.8217	0.7707	0.7197	0.7771	0.7389	0.5987	0.7516	0.7261	0.7006
2		****	0.6688	0.6752	0.7261	0.6497	0.6688	0.7452	0.7452	0.6815	0.7070	0.7197
3			****	0.8280	0.8153	0.7516	0.7962	0.7070	0.6433	0.7834	0.7707	0.7452
4				****	0.8217	0.7707	0.8408	0.7643	0.5732	0.7771	0.7516	0.7516
5					****	0.7325	0.7898	0.7771	0.6369	0.7771	0.7516	0.7771
6						****	0.7771	0.7134	0.6497	0.7134	0.7261	0.7134
7							****	0.7834	0.6306	0.8217	0.8217	0.7834
8								****	0.6561	0.7834	0.7707	0.8344
9									****	0.6688	0.6943	0.6561
10										****	0.8089	0.7962
11											****	0.7834
12												****

whereas; 1=DR187, 2= DR-177, 3=DR 198, 4=DR 194, 5=DR 158, 6=DR 185, 7=DR 189, 8= DR 159, 9=Pak Afgoee, 10= Sadaf, 11=EV 1098, 12=EV6098

inbred lines in the same group and also in the same sub group were similar with respect to their physical and morphological traits because the characters are under genetic control. Similar clustering was reported [30] which showed the association of flint, semi flint, dent and semi-dent germplasm by placing them in different groups through RAPD methodology. The polymorphic information content gives an idea to estimate the efficiency of markers for genetic difference evaluation. The present study revealed the PIC estimated for all 29 RAPD primers varied from 0.06 (GL J-10) to 0.73 (GL K-12) with the mean of 0.39 (Table 1). The PIC values are quite

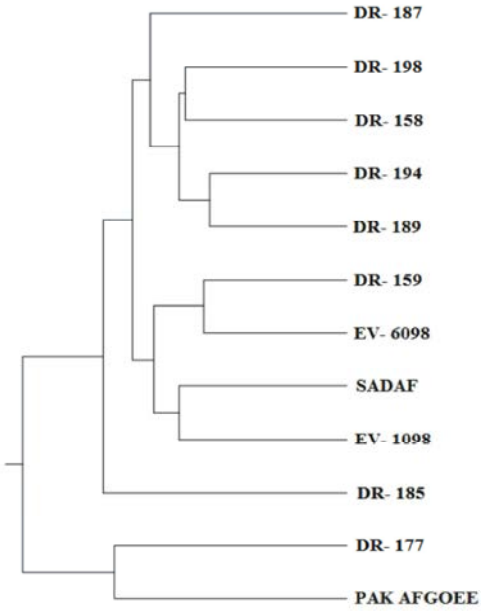

Fig. 2: Genetic distance of 12 maize genotypes developed from RAPD data using unweighted pair group of arithmetic means (UPGMA)

comparable to the early studies on maize [31, 32, 33]. Principal component analysis was performed to investigate the distribution of genetic variability among eight inbred lines and four cultivars and it was found that close association was found among most of the inbred lines except DR-177 and DR-159 whereas the cultivar Pak Afgoee exhibited a distinct behavior as compared to Sadaaf, EV-1098 and EV-6098. The results are in line with the earlier researchers research [34, 35].

Analysis of Molecular Variance: An analysis of molecular variance (AMOVA-1) was performed which helps to partition the total variance among groups and among populations of within groups. It was found that total of 6% variation was detected among the population where as 94% variability was found within populations.

CONCLUSIONS

It was concluded that besides the relatively low level of genetic variation among the maize inbred lines RAPD can be used as a reliable technique for identification of genotypes along with interspecific variation. Being simple and easy, RAPD method of analysis for genetic difference among maize genotypes, the polymorphism among the genotypes can be further used in plant breeding research programs for maximum exploitation of genetic resources.

ACKNOWLEDGMENTS

The authors acknowledge the management of Maize and Millet Research Institute (MMRI) Sahiwal for providing the seeds of maize genotypes used in this study. We are highly grateful to the Centre of Agricultural Biochemistry and Biotechnology (CABB), University of Agriculture Faisalabad, for providing the facilities to conduct this part of research. The manuscript is a part of Ph. D, Dissertation of Mr. TaufiqUllah.

REFERENCES

1. Khaliq, I., I.R. Noorka and R. Khaliq 2009. Estimation of heritability and genetic advance for some quantitative characters in spring wheat. Int. J. Agric. Appl. Sci., 1(2): 100-103
2. Rasool, I., 2008. Physio-genetic Basis of Water Stress Tolerance in Spring Wheat (*Triticum aestivum* L.) Doctoral dissertation, University of Agriculture, Faisalabad.
3. Tester, M. and A. Bacic, 2005. Abiotic stress tolerance in grasses. From model plants to crop plants. Plant Physiol., 137: 791-793.
4. Noorka, I.R., S. Tabasum and M. Afzal, 2013. Detection of genotypic variation in response to water stress at seedling stage in escalating selection intensity for rapid evaluation of drought tolerance in wheat breeding. Pak. J. Bot., 45(1): 99-104.
5. Araus, J.L., G.A. Slafer, M.R. Reynolds and C. Royo, 2002. Plant breeding and drought in C3 cereals: what should we breed for? Ann. Bot., 89: 925-940.
6. Noorka, I.R, I. Khaliq, Z.Akram and S. Iqbal, 2009. Inheritance studies of physio-genetic traits in spring wheat under normal and moisture stress environments. Int. J. Agric. Appl. Sci., 1(1): 29-34.
7. Katherine, D. and C. Gehring, 2005. Engineering drought and salinity tolerance in plants: lessons from genome-wide expression profiling in *Arabidopsis*. Trends Biotech., 23: 547-552.
8. Noorka, I.R. and S.A. Shahid, 2013. Use of conservation tillage system in semiarid region to ensure wheat food security in Pakistan. Development in Soil Salinity Assessment and Reclamation ISBN 978-94-007-5683-0.in Springer Book, http//www.springer.com/environmrnt/soil+science/book/978978-94-007-5683-0.
9. Campos, H., M. Cooper, J.E. Habben, G.O. Edmeades and J.R. Schussler, 2004 Improving drought tolerance in maize: a view from industry. Field Crops Res., 90: 19-34.

10. Noorka, I.R. and J.S. Heslop- Harrisan, 2014. Water and Crops Molecular Biologists', Physiologists' and Plant Breeders' Approach in the Context of Evergreen Revolution. Handbook of Plant and Crop Physiology, pp: 967-978.CRC Press, Taylor and Francis, USA.
11. Hallauer, A.R. and J.B. Miranda, 2000. Quantitative genetics in Maize Breeding. 3rdedn. Iowa State University Press, USA.
12. Froya, C., G. Krajewski, M. di Fonzo, N. Villa and M. Sari-Gorla, 1999. Genetic analysis of drought tolerance in maize by molecular markers. I. Yield components. Theor. Appl. Genet., 99: 280-288.
13. Hassan, S.M.F., M.S. Iqbal, G. Rabbani, Naeem-ud-Din, G. Shabbir, M. Riaz and I.R. Noorka, 2013. Correlation and path analysis for yield and yield components in sunflower (*Helianthus annus*. L) Afr. J. Biotech., 12(16): 1968-1971.
14. Rauf, S., M.S. Haider, S.A. Tariq, M. Ejaz, E. Ashraf and I.R. Noorka. 2013. Prospects of Inducing Resistance in Fodder Species against Toxic Ions and Metals. Bioremediation Journal, 17(4): 212-230.
15. Tabassum, S., I.R. Noorka, M. Afzal and A. Ali, 2012. Screening best adopted wheat lines against aphid (*Schizaphisgraminum rondani*) population. Pak. Entomol., 34(1): 51-53.
16. Batool, A., I.R. Noorka, M. Afzal and A.H. Syed, 2013. Estimation of heterosis, heterobeltiosis and potence ratio over environments among pre and post green revolution spring wheat. J. Basic Appl. Sciences, 9: 36-43.
17. Noorka, I.R., 2014. Effect of Drought/Water Stress and Adaptation to Unintended Consequences of Wheat Growth and Development in Pakistan. Handbook of Plant and Crop Physiology, pp: 441-452. CRC Press, Taylor and Francis, USA.
18. Noorka, I.R. and J.A. Teixeira Da Silva, 2012. Mechanistic Insight of Water Stress Induced Aggregation in Wheat (*Triticumaestivum* L.) Quality: The Protein Paradigm Shift. Notulae Scientia Biologicae, 4(4): 32-38.
19. Ahmad, I., F. Mohammad, A. Zeb, I.R. Noorka, Farhatullah and S.A. Jadoon, 2013. Determination and inheritance of phytic acid as marker in diverse genetic group of bread wheat. Amer. J. Mol. Biol., 3(7): 158-164 doi:10.4236/ajmb.2013.33021.
20. Joyce, T.A., T.M. Abberton, T.P.T. Michaelson-Yeates and J.W. Forster, 1999. Relationships between genetic distance measured by RAPD-PCR and Heterosis in inbred lines of white clover (*Trifoliumrepens* L.) Euphytica, 107: 159-165.
21. Noorka, I.R., S.U. Rehman, J.R. Haidry, I. Khaliq, S. Tabassam and M. Din, 2009. Effect of water stress on physico-chemical properties of wheat (*Triticumaestivum* L.)" Pak. J. Bot., 41(6): 2917-2924
22. Smith, O.S., J.S.C. Smith, S.L. Bowen, R.A. Tenborg and S.J. Wail, 1990. Similarities among a group of elite maize inbreeds as measured by pedigree, F1 grain yield, grain yield, Heterosis and RFLDs. Theor. Appl. Genet., 91: 1148-1152
23. Kempthorne, O., 1957. An introduction to genetical statistics. Jhon Wiley and Sons, Inc., New York.
24. Aslam, M., F.S. Awan, I.A. Khan and A.I. Khan, 2009. Estimation of genetic distance between 10 maize genotypes with varying response to different levels of soil moisture. Gen. Mol. Res., 8(4): 1459-1465.
25. Nei, M. and W.H. Li, 1979. Mathematical model for studying genetic variation in terms of restriction endonucleases. Proc. Nalt. Acad. Sci., 76: 5269-5273.
26. Souza, S.G.H.D., V. Carpentieri-Pipolo, C.D.F. Raus, V.D.P. Carvalhoa, P.M. Ruas and A.C. Gerage, 2008. Comparative analysis of genetic diversity among the maize inbred lines (*Zea mays* L.). Obtained by RAPD and SSR markers. Inter. J. Biol. Technol., 51(1): 183-192.
27. Carvalho, V.P., P.M. Ruas, C.F. Ruas, J. M. Ferreira and M.P. Moreira, 2004. Genetic diversity among maize (*Zea mays* L.) landraces assessed by RAPD markers .Genetics Mol. Biol., 27: 228-236.
28. Bauer, I., S.M. Drinic, M. Filipovic and K. Konstanti, 2005. Genetic characterization of early maturing maize hybrids (*Zea mays* L.) obtained by protein and RAPD markers. Genetika, 37(3): 235-243
29. Asif, M., M. Rahman and Y. Zafar, 2006. Genotypic analysis of six maize (*Zea mays* L.) hybrids using DNA fingerprinting technology. Pak. J. Bot., 38(5): 1425-1430.
30. Parentoni, S.N., J.V. Magalhaes, C.A.P. Pacheco, M.X. Santos, T. Abadie, E.F.G. Gama, P.E.O. Guimaraes, W.F. Meirelles, M.A. Lopes, M.J.V. Vasconcelos and E. Paiva. 2001. Heterotic groups based on yield specific combining ability data and Phylogenetic relationship determined by RAPD markers for tropical maize open pollinated varieties. Euphytica., 121: 197-208.
31. Amorim, E.P., C.C. De Souza, M.J.C. Almeida, F.B. MeloSereno and J.F.B. Neto, 2003. Genetic variability in sweet corn using molecular markers. Maydica, 48: 177-181.
32. Ceccarelli, S., S. Grando and J.A.G. Van Leur, 1987. Genetic diversity in barely landraces from Syria and Jordan. Euphytica., 36: 389-405.

33. Saadia, A., A.I. Khan, F.S. Awan, A. Riaz and S. Bahadur, 2012. Genetic diversity of rose Germplasm in Pakistan characterized by random amplified polymorphic DNA (RAPD) markers. Afr. J. Biotech., 11(47): 10650-10654.
34. Troyer, A.F., S.J. Openshaw and K.H. Knittle, 1988. Measurement of genetic diversity among popular commercial corn hybrids. Crop Sci., 28: 481-485.
35. Khan, I.A., F.S. Awan, A. Ahmad, F. Yong-Bi and A. Iqbal, 2005. Genetic diversity of Pakistan wheat Germplasm as revealed by RAPD markers. Genetic Resources and Crop Evolution, 52: 239-244.