Responses of wheat seedling to varying moisture conditions and relationship between morphological and molecular characterization

Fareeha Arooj[1], Abdul Qayyum[2], Seema Mahmood[3]

[1,3]Institute of Pure and Applied Biology Bahauddin Zakariya University Multan Pakistan
[2]Department of plant breeding and genetics BZU Multan Pakistan

Abstract— *The following study was conducted to estimate the genotypic differences among 30 wheat (Triticum aestivum L.) genotypes under different moisture regimes and relationship between morphological and molecular characterization. Eight seedling parameters root length (RL), shoot length (SL), root fresh weight (RFW), shoot fresh weight (SFW), root dry weight (RDW), shoot dry weight (SDW), chlorophyll rate (CR) and survival rate (SR) were studied at four different soil moisture conditions ($T_1$40%,$T_2$60%,$T_3$80%,$T_4$100%) using two factor factorial complete randomized design (CRD). Significant differences among genotypes were observed by analysis of variance. For heritability estimates, survival rate showed lowest heritability under all the treatments. Principal components analysis accounted 81.4% variation in T_1, 81.9% in T2, 87.7% in T3 and 84.7% in T4 conditions in first PC. Selected diverse genotypes were further fingerprinted with 10 ISSR markers. A total of 74 DNA fragments were detected and 72.7% of was polymorphic. The amplified DNA fragments were ranged from 4 (UBC-809) to 11 (UBC-808). PIC values were ranged from 0.32 to 0.81. Cluster analysis grouped the genotypes into 4 clusters on the basis of molecular and phenotypic characterization under T4 normal conditions whereas under T1 (moisture stress) conditions genotypes were grouped into 5 clusters explaining genotypic differences under different moisture conditions. The present results showed that phenotypic difference in wheat seedling expression under different water regimes is accompanied with molecular basis, which offer a prospective to enhance wheat adaptation under moisture stress conditions.*

Keywords— Principal component, Dendogram, Genotypes, Polymorphism.

I. INTRODUCTION

Different types of biotic and abiotic stresses are affecting the efforts of researchers working to evanesce the increasing demands of wheat. Drought may cause 10% to 90% yield losses depending upon the intensity of drought and the stage of plant development (Dhanda et al. 2004; Reynolds et al. 2004). The decreasing water resources demands immediate actions for the genetic improvement of crops which requires plant evaluation under stress conditions and their genetic exploration. Drought stress retards plant growth, reduces performance, and has negative impact on development (Shao et al. 2009). Moisture stress not only affects the morphology but also badly affects the metabolism of plant.

The genetic basis for drought tolerance can be predicted by evaluating genotypes under stress condition (Ceccarelli and Grando 1997). Genetic improvement involves selection of genotypes with favorable alleles. Furthermore, screening techniques should be precise to evaluate plant performance at suitable developmental stage. Seedling survivability is a simple and well documented method used to screen wheat germplasm (Singh et al. 1999; Tomar and Kumar 2014). It discriminates between drought susceptible and tolerant genotypes under artificial moisture conditions. Uniform and rapid germination and good seedling emergence are necessary components of crop establishment. Root system helps plants to maintain their growth under moisture stress conditions. Limited water conditions can reduce seedling germination and growth which leads to less plant population per unit area. Khan et al. (2004) analyzed that drought adapted plants are often characterized by deep and vigorous root systems. Therefore, genetic basis of these seedling traits should be exploited to know the inheritance of these traits.

Development of molecular markers have provided new possibilities to evaluate genetic diversity, inter and intra species genetic relationship and to locate QTLs responsible for specific trait development (Sofalian et al. 2003). Inter simple sequence repeats (ISSR) are the DNA based markers which are being used for molecular characterization of different crops. Najaphy et al. (2012) showed that ISSR markers provide adequate polymorphism and reproducible fingerprinting profile for genetic characterization of wheat.

To analyze the genetic diversity various biometric tools are being used by plant breeders. Multivariate techniques which are commonly used to explore genetic diversity include cluster analysis, principal coordinate analysis (PCoA) and principal component analysis (PCA) (Brown-Guedira et al. 2000; Melchinger, 1993; Thompson and Nelson, 1998). The following study was conducted to gain a better understanding of different seedling traits under different moisture conditions and to measure the extent of genetic diversity contributing to drought tolerance at seedling stage.

II. MATERIALS AND METHODS

2.1 Phenotypic characterization:

Thirty bread wheat (*Triticum aestivum* L.) genotypes collected from Regional Agriculture Research Institute (RARI), Bahawalpur were sown in polythene bags of (6``L x 4``W) in the glass house of the Department of Plant Breeding and Genetics, Bahauddin Zakariya University, Multan during 2012-2013. The experiment was carried out in two factor factorial complete randomized design (CRD) with three replications. After 21-days of planting, treatments with different soil moisture conditions (T_1=40%, T_2=60%, T_3=80%, and T_4=100%) were applied until 50% mortality appeared. Hoagland solution was applied to strengthen the weaker plants to obtain data regarding the survival rate. Next day the data for survival rates of different treatments were recorded by following formulae.

Survival rate (%): The dead plants per genotypes were counted and the data for their survival rate was calculated by using the following formula:

$$SR = \frac{Number\ of\ alive\ plants}{Total\ number\ of\ plants\ grown} * 100$$

In order to conduct the data for root shoot architecture the plant seedlings were up taken from the polythene bags following thorough washing with distilled water. The following seedling parameters were recorded, shoot length (cm, SL), root length (cm, RL), Shoot fresh weight (gm, SFW), Shoot dry weight (gm, SDW), Root fresh weight (gm, RFW), Root dry weight (gm, RDW), Chlorophyll rate (%, CR) with chlorophyll meter.

2.2 Genotypic characterization:

Fourteen diverse wheat genotypes were used to extract genomic DNA from young leaf tissues as described by Sofalian et al. (2009). DNA concentration was estimated using spectrophotometer. DNA concentration was calculated using following formula

Concentration of DNA µl/ml= OD at 260x 50 x DF

To characterize the 14 wheat varieties 10 ISSR primers (UBC-807, 808, 809, 810,811,812, 813, 815, 816, and 817) were used to conduct the PCR reaction. The PCR reaction was performed in 20µl volume. The PCR products were separated and scored by agarose gel electrophoresis (Ahmad et al. 2014)

2.3 Statistical Analysis

To find out significant difference among genotypes, analysis of variance was performed as described by Steel et al. (1997). Principal component analysis (PCA) was performed on the basis of correlation matrix to determine diverse genotypes (Ogunbayo et al. 2005). By eigen value as determined by Kaiser (1960) statistically significant principal components (PC_S) were selected. Genotypes were further grouped on the basis of ward's linkage cluster analysis (Sneath and Sokal, 1973).

III. RESULTS

Analysis of variance showed significant genetic differences for all the characters under all the treatments except SR which showed no significant differences under $T_2$60% and $T_3$80% soil moisture conditions (Table 1-4). SR showed lowest heritability under all the treatments (Table 1-4) whereas highest heritability estimates were observed in RL, SL and RFW (0.98) under $T_1$40%, RL and SL (0.99) under $T_2$60%, RFW (0.99) $T_3$80% and RL (0.99) under $T_4$100% soil moisture conditions. Values of genetic advance were ranged between (2.36 for SL and 0.15 for SFW) under $T_1$40%, between (3.05 for SL and 0.17 for SR) under $T_2$60%, between (2.91 for SL and 0.13 for SFW) under $T_3$80% and between (3.86 for SL and 0.13 for SR) under $T_4$100% soil moisture conditions (Table 1-4). Observed heritability was higher than 70% of all parameters except SR exhibiting heritable deviation of genotypes.

TABLE 1

MEAN VALUES AND ANALYSIS OF VARIANCE FOR 8 CHARACTERS AMONG 30 WHEAT GENOTYPES IN T_1 40% SOIL MOISTURE CONDITIONS.

Parameters	MS(Rep)	MS(V)	F. value	h^2	G.A	CV (%)
RL	0.107	238.16	71.69**	0.98	2.28	1.92
SL	0.103	235.37	79.20**	0.98	2.36	4.68
RFW	0.001	3.41	79.21**	0.98	0.27	2.38
SFW	0.001	1.02	24.52**	0.96	0.15	3.08
RDW	0.003	2.59	29.99**	0.97	0.23	8.01
SDW	0.001	1.14	30.99**	0.97	0.15	19.72
CR	0.131	222.47	54.82**	0.98	2.19	2.24
SR	0.171	8.96	1.69*	0.44	0.19	10.26

TABLE 2

MEAN VALUES AND ANALYSIS OF VARIANCE FOR 8 CHARACTERS AMONG 30 WHEAT GENOTYPES IN T_2 60% SOIL MOISTURE CONDITIONS.

Parameters	M.S(Rep)	M.S(V)	F. value	h^2	GA	CV (%)
RL	0.075	313.16	133.22**	0.99	2.64	1.23
SL	0.120	418.79	112.28**	0.99	3.05	2.58
RFW	0.002	1.71	30.21**	0.97	0.18	2.01
SFW	0.006	3.22	15.88**	0.94	0.25	4.91
RDW	0.002	1.89	25.66**	0.96	0.19	4.00
SDW	0.002	2.17	39.07**	0.97	0.22	6.01
CR	0.179	267.39	48.15**	0.98	2.41	2.00
SR	0.155	7.91	1.65^{NS}	0.41	0.17	7.69

TABLE 3

MEAN VALUES AND ANALYSIS OF VARIANCE FOR 8 CHARACTERS AMONG 30 WHEAT GENOTYPES IN T_3 80% SOIL MOISTURE CONDITIONS

Parameters	MS(Rep)	MS (V)	F. value	h^2	GA	CV (%)
RL	0.209	264.97	40.85**	0.97	2.38	1.59
SL	0.190	386.18	65.50**	0.98	2.91	2.13
RFW	0.001	3.83	179.53**	0.99	0.29	0.99
SFW	0.001	0.82	20.61**	0.95	0.13	1.65
RDW	0.002	1.43	22.08**	0.95	0.17	2.59
SDW	0.001	1.07	27.11**	0.96	0.15	3.01
CR	0.141	152.42	34.68**	0.97	1.80	1.43
SR	0.406	20.37	1.62^{NS}	0.41	0.27	11.76

TABLE 4
MEAN VALUE AND ANALYSIS OF VARIANCE FOR 8 CHARACTERS AMONG 30 WHEAT GENOTYPES IN T_4 100 % SOIL MOISTURE CONDITIONS.

Parameters	MS(Rep)	MS(V)	F. value	h^2	GA	CV (%)
RL	0.068	416.47	1.96**	0.99	3.05	0.74
SL	0.457	687.98	48.53**	0.98	3.86	2.43
RFW	0.001	3.72	87.29**	0.98	0.28	1.09
SFW	0.003	3.97	41.89**	0.97	0.29	2.10
RDW	0.002	1.67	25.41**	0.96	0.18	2.09
SDW	0.002	1.69	26.22**	0.96	0.19	2.76
CR	0.398	396.74	32.15**	0.97	2.90	1.98
SR	0.054	3.27	1.93**	0.49	0.13	3.95

3.1 Principal component analysis

The data matrix was standardized to make the variable traits unit less for computing PCA (Principal Component Analysis). Individual accession component scores were accounted by following character loading. The sum of Eigen values resulted in total number of variables. Eight PCs were accounted to analyze the available genetic variation in the wheat genotypes. Out of eight PCs, 1st PC accounted maximum variation for the studied traits. In treatment (T140%, T260%, T380%, and T4100%) contribution of 1st PC was 81.415%, 81.955%, 87.775%, and 84.731% of the variability in different genotypes estimated for root shoot architecture components (Table 5). In case of treatment T140% the first PC was more related to SFW, RDW, CR, SL, RFW, SDW, RL and SR. Under $T_2$60% soil moisture conditions the PC1 was more related to SR while rests of the attributes were not contributing to cause variability.

TABLE 5
PRINCIPAL COMPONENTS (PCS) FOR 8 CHARACTERS IN 30 WHEAT GENOTYPE IN T_1 40%SOIL MOISTURE CONDITIONS.

Traits	PC1
Eigen value	6.513
Proportion of variance	6.513
Cumulative variance	81.415
Eigen vectors	
PC1	
SFW	0.994
RDW	0.993
CR	0.993
SL	0.991
RFW	0.988
SDW	0.976
RL	0.798
SR	0.054

Among thirty wheat genotypes, fourteen diverse genotypes were selected on the basis of accession component scores. To analyze genetic differences the selected genotypes were analyzed with molecular markers. The characterization and genetic identification of fourteen wheat accession were carried out by 10 ISSR primers (Table 6). The PCR amplification results of ISSR primers indicated characteristic differences among genotypes. A total of 74 DNA fragments were amplified, whereas 66 fragments were polymorphic and 8 fragments were monomorphic. Therefore, out of 74 DNA fragments 72.7% were polymorphic. The amplified DNA fragments were ranged from 4 (UBC-809) to 11 (UBC-808). The lowest level of polymorphisms (72.7%) was represented by ISSR primer UBC-808 and markers UBC-807, UBC-809, UBC-811, UBC-816, and UBC-817 showed 100% polymorphism (Table 7). PIC values were ranged from 0.32 to 0.81.

TABLE 6
PRINCIPAL COMPONENTS (PCS) FOR 8 CHARACTERS IN 30 WHEAT GENOTYPES IN T_2 60% SOIL MOISTURE CONDITIONS.

Traits	PC1
Eigen value	6.556
Proportion of variance	6.566
Cumulative variance	81.955
Eigen vectors	
	PC1
SR	0.215
SFW	-0.995
SL	-0.993
RL	-0.992
SDW	-0.991
RFW	-0.988
RDW	-0.981
CR	-0.792

TABLE 7
PRINCIPAL COMPONENTS (PCS) FOR 8 CHARACTERS OF 30 WHEAT GENOTYPES IN T_3 80% SOIL MOISTURE CONDITIONS.

Traits	PC1
Eigen value	7.022
Proportion of variance	7.022
Cumulative variance	87.775
Eigen vectors	
	PC1
RFW	-0.996
SDW	-0.994
RDW	-0.993
SFW	-0.988
RL	-0.979
CR	-0.948
SL	-0.936
SR	-0.585

TABLE 8
PRINCIPAL COMPONENTS (PCS) FOR 8 CHARACTERS OF 30 WHEAT GENOTYPES IN T_4 100% SOIL MOISTURE CONDITIONS.

Traits	PC1
Eigen value	6.778
Proportion of variance	6.778
Cumulative variance	84.731
Eigen vectors	
	PC1
RFW	-0.991
SFW	-0.988
RDW	-0.986
RL	-0.981
SL	-0.979
SDW	-0.931
CR	-0.926
SR	-0.446

3.2 Cluster analysis

Using ward's linkage clustering method experimental data was analyzed by cluster analysis. In treatment T140% the dendogram classified the thirty wheat genotypes into five clusters. The genotype 11903 present in cluster 5 and showed dissimilarity with rest of the genotypes under T1 conditions, which showed genetic differences between 11903 and other genotypes under limited water conditions. But under T4 moisture conditions 11903 showed similarity with 11935 which showed expression of different genes under different environmental conditions. Similarly, genotypes explained less variation under T4 100% water conditions because they were grouped in 4 clusters but under limited moisture condition genotypes were grouped in 5 clusters which showed variation among genotypes under different water regimes.

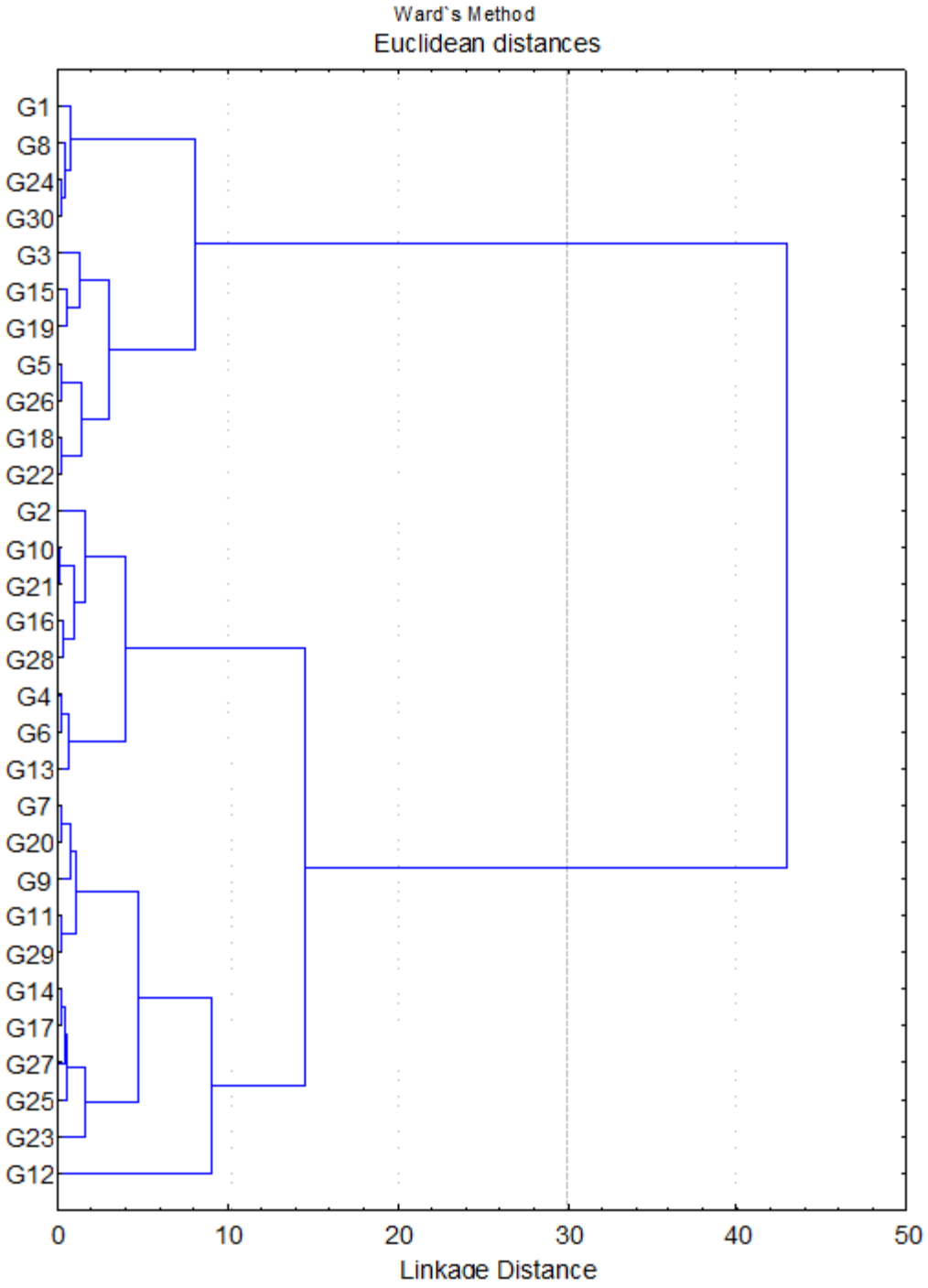


FIGURE 1: DENDOGRAM RESULTING FROM CLUSTER ANALYSIS OF 30 WHEAT GENOTYPES IN T_1 40% SOIL MOISTURE CONDITIONS

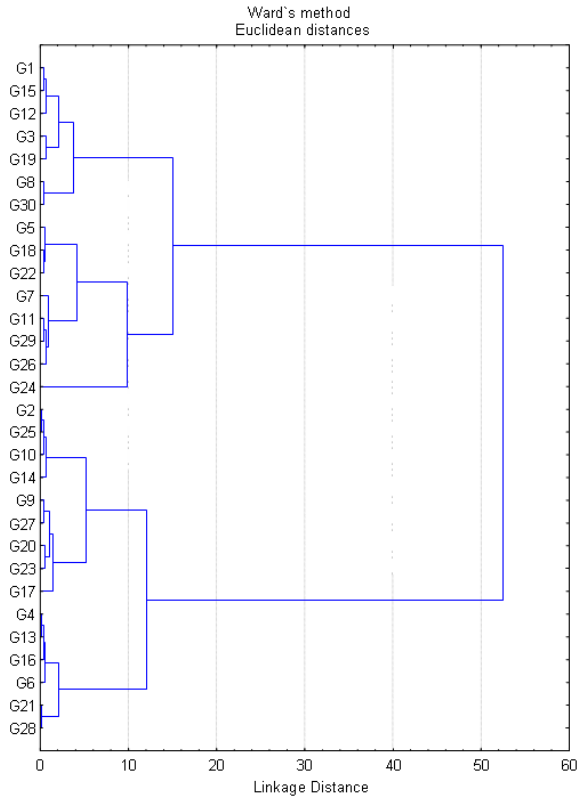
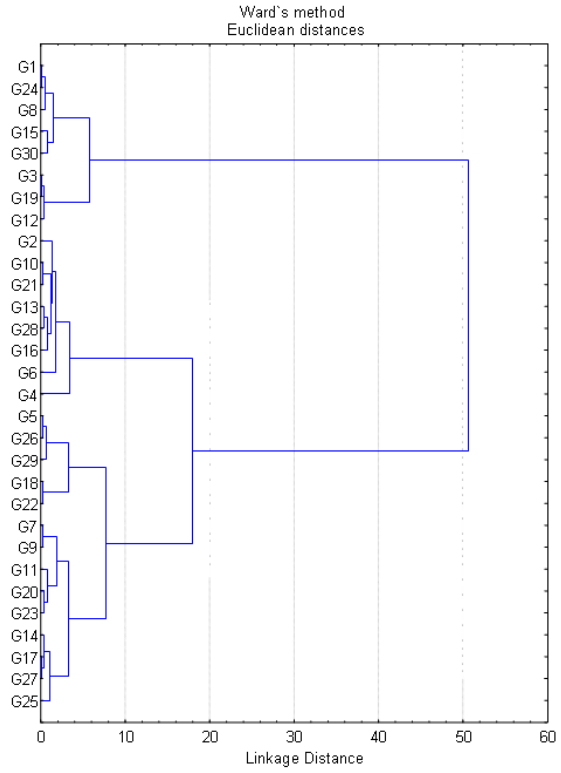


FIGURE 2: DENDOGRAM RESULTING FROM CLUSTER ANALYSIS OF 30 WHEAT GENOTYPES IN T_2 60% SOIL MOISTURE CONDITIONS.



DENDOGRAM RESULTING FROM CLUSTER ANALYSIS OF 30 WHEAT GENOTYPES IN T_3 80% SOIL MOISTURE CONDITIONS.

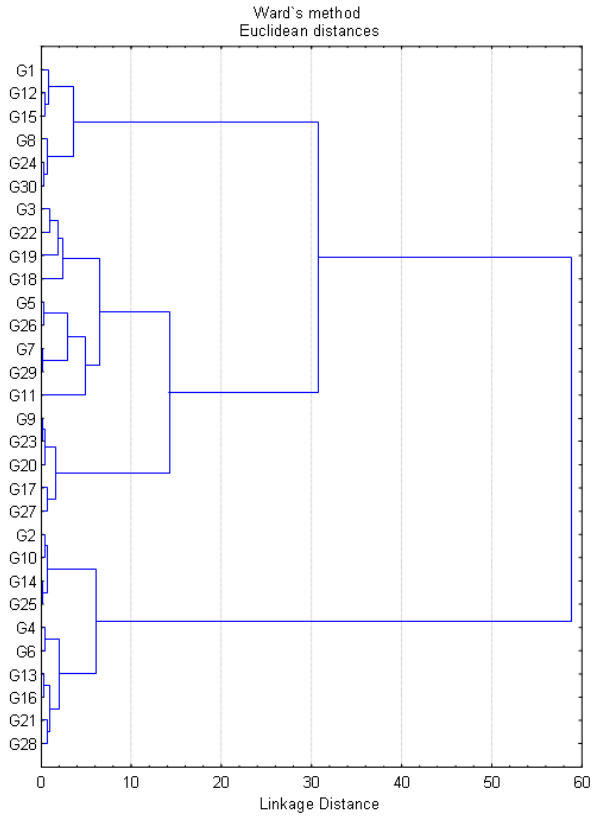
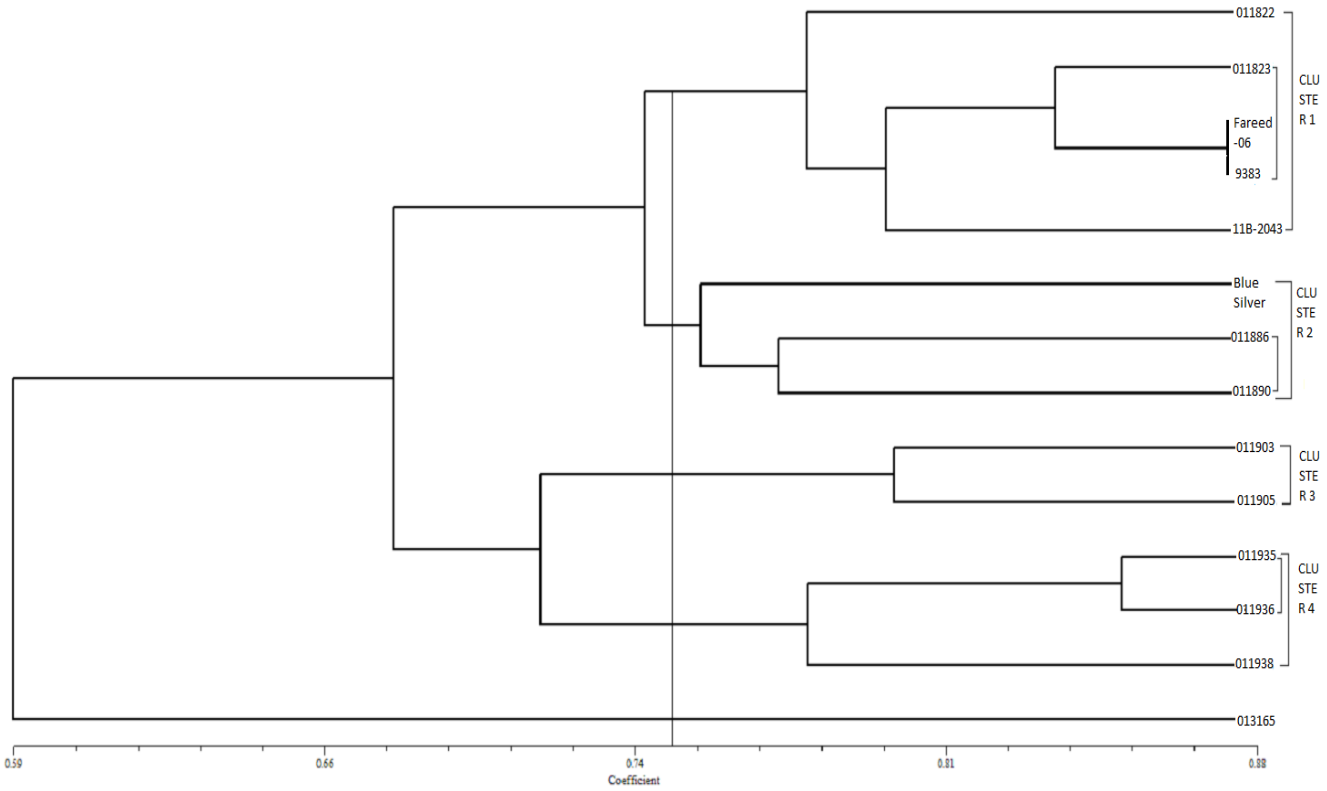


FIGURE 4: DENDOGRAM RESULTING FROM CLUSTER ANALYSIS OF 30 WHEAT GENOTYPES IN T_4 100% SOIL MOISTURE CONDITIONS.

To distinguish varieties from one another at molecular level DNA finger printing can be used. Among wheat varieties similarity values showed substantial differences (Table 7). The genetic similarity ranged from 0.53 to 0.88 with an average of 71%. High genetic similarity was observed between 9383 and Fareed-06 (0.88). The low genetic similarity between 013165 and 011936 (0.53) was observed. To observe the genetic association among genotypes based on ISSR marker analysis. A dendogram classified the 14 wheat genotypes into 4 clusters (Fig.2). The first cluster contain genotypes 011822, 011823, Fareed-06, 9383 and 11B-2043. The genetic similarity between genotypes 011822, 011823 and Fareed-06, 9383 were 0.76 and 0.74. Second cluster include 011886, 011890 and Blue silver. The genetic similarity between 011886 and 011890 was 0.77. Third cluster contain 011930 and 011905 with genetic similarity 0.80. Cluster four contains genotypes 011935, 011936 and 011938.



3.3 Relation between the phenotypic characterization and ISSR loci data:

Cluster analysis was performed separately for each treatment and for markers data to study the genetic diversity in wheat. The dendogram constructed on the basis of phenotypic data showed maximum similarity between 9383 and Fareed-06 genotypes. The dendogram generated on the basis of molecular markers data also grouped the Fareed-06 and 9383 in the same cluster which showed their genetic closeness.

The genotype 11903 (Iran) showed no association with rest of the genotypes under T1 conditions but showed maximum similarity with 11935 (Japan) under T4 conditions. But molecular data showed association between 11903 and 11905 genotypes both have Iranian origin. Most notable is the location of genotypes 11903 and 11935 which are located in the nearby clusters on the basis of molecular fingerprinting. This may be concluded that both these genotypes which belong to different geographical regions may share some parents having similar allelic combinations that express only under normal conditions as represented here by phenotypic and molecular characterization.

Molecular characterization grouped the genotypes into 4 clusters similarly phenotypic evaluation under normal conditions also allocated genotypes into 4 clusters whereas under T1 (moisture stress) conditions genotypes were grouped into 5 clusters this may be due to varying degree of drought tolerance in different genotypes. The observed similarity among the dendogram between phenotypic data and molecular data give an evidence for the presence of relationship between seedling traits under different water regimes and molecular data.

Results obtained by principal component analysis also resembled with the cluster analysis which showed that under T1 conditions all the traits such as, shoot fresh weight, root dry weight, shoot length, chlorophyll, root fresh weight, shoot dry weight, root length and survival rate contributed for diversity. Cluster analysis showed 5 clusters under T1 conditions, so more genes are involved under drought conditions. PCA under T2 was only related to SR which also showed less heritability and genetic advance values, therefore no selection should be carried out under T2 conditions.

TABLE 11
ISSR MARKERS USED AMPLIFIED PRODUCTS AND ANALYSIS OF GENETIC DIVERSITY OF WHEAT GENOTYPES.S

Primer	Total amplified band	No. of monomorphic band	No. of polymorphic bands	Percentage of polymorphic bands	Polymorphism information content (PIC)
UBC-807	10	-	10	100	0.55
UBC-808	11	3	8	72.7	0.55
UBC-809	4	-	4	100	0.38
UBC-810	10	1	9	90	0.77
UBC-811	5	-	5	100	0.50
UBC-812	7	1	6	85.7	0.75
UBC-813	6	1	5	83.3	0.32
UBC-815	8	2	6	75	0.81
UBC-816	6	-	6	100	0.55
UBC-817	7	-	7	100	0.46
Total	74	8	66		
Minimum	4	1	4	72.7	0.32
Maximum	11	3	10	100	0.81
Average	7.4	0.8	6.6		

IV. DISCUSSION AND CONCLUSION

Dwindling environmental conditions and rapid increase in world's population has created serious threats to world food security. To combat with hunger and diminishing water resources is a greatest challenge being faced by scientists today. Decreasing water resources has created alarming situation to sustainable food production. Wheat is the leading cereal crop being consumed by humans across the globe. Limited water supply may decrease wheat yields upto 90% (Dhanda et al. 2004). Different morpho-physiological traits can be studied to evaluate the performance of plants under limited water conditions (Inou et al. 2004). Understanding of the genomic regions controlling these important traits will contribute in the genetic improvement of wheat to cope with number of stresses particularly low moisture (Frova et al. 1999). Moreover, the association among different plant traits should be determined either it is genetic or phonetics, heritable or non heritable.

In the following study wheat genotypes were evaluated under different water regimes. The study showed significant variation among genotypes and treatments (different water levels) which demonstrated the contribution of genetic attributes (Birsin 2005). Heritability values were higher than 70% for all the parameters except SR. Awan et al. (2007) and Haidar et al. (2012) also observed significant differences among genotypes and higher values of heritability. The traits SL, RL, SFW, SDW, RFW, RDW, CR showed greater magnitudes of heritability along with higher values of genetic advance were under the control of additive genetic effects. Heritability also provides the estimation of genetic advance, either the selection under certain environment is heritable or non heritable. Magnitude of heritability determines the simplicity of selection (Khan et al. 2008). To undertake selection in succeeding generation, heritability should accompany substantial amount of genetic advance, which is the indicative of potential to which the trait can be improved under certain environment, therefore higher values of heritability and genetic advance in this study provides an opportunity to breeders to fix these traits with full strength and ease in coherent selection programs (Eid 2009). Lower values for coefficient of variation also demonstrated higher precision levels of the study. Noorka et al. (2007) also observed lower values of coefficient of variation.

Sardana et al. (2007) demonstrated that high heritability may not always lead to high genetic gain, unless sufficient genetic variability existed in the germplasm. Therefore, to account variation among genotypes principal component analysis was performed (Panthee et al. 2006). As the results indicates that the first PC accounted maximum variation for the studied traits

such as SFW, RDW, CR, SL, RFW, SDW, RL and SR but other PCs have not played an important role in accounting variation**.** Mohammadi and Prasanna (2003) explained that if there is high correlation among the data set then first few PCs expresses maximum variation but it decreases with the decresae in correlation among original data set. Gulnaz et al. (2012) observed four significant PCs in a set of seven PCs. Similarly results were also reported by Ahmad et al. (2012). Most of the variation has been accounted by first PC so other PCs were not given due importance in the following study. Eigen values showed continuous decrease, which exhibits that major amount of variation has been accounted by the first few principle components (Leilah and Al-Khateeb 2005). High positive association among root and shoot parameters as depicted by this study provide an opportunity to breeders to breed for these traits at the same time. Furthermore, genetic control of these traits should be identified to enhance breeding accuracy.

To explore diversity at genetic level, 14 most diverse genotypes were selected on the basis of accession component score which were further analyzed with ISSR markers. The PCR results showed characteristics differences among genotypes. Assessment of genetic diversity in wheat has been carried out by different molecular marker systems. Najaphy et al. (2012) observed that for evaluating genetic diversity of wheat genotypes ISSR markers provide sufficient polymorphisms and reproducible fingerprint profiles. Sofalian et al. (2003) reported high level of polymorphism of wheat landraces based on ISSR markers as compared to other markers. The amplified DNA fragments were ranged from 4 (UBC-809) to 11 (UBC-808). Carvalho et al. (2009) observed 12.9 polymorphic bands per primer using 8 ISSR primers in 48 wheat accessions. Nagaoka and Ogihara (1997) found that 3.7 polymorphic bands per ISSR primer. Presence of high polymorphism in wheat genotypes using ISSR markers indicates high efficiency of this marker technique. The lowest level of polymorphisms (72.7%) was represented by ISSR primer UBC-808 (Table 6). Abou-Dief et al. (2013) identified 112 amplified DNA fragments, of which 17 were monomorphic (15.2%) and 95 fragments showed polymorphism (84.8%). PIC values were ranged from 0.32 to 0.81. PIC index has been widely used to explore genetic diversity among genotypes (Tatikonda et al. 2009; Talebi et al. 2010; Thudi et al. 2010).

In self pollinated crops like wheat genetic variation is vital for stress tolerance. Joshi et al. (2004) observed genetic diversity between parents is essential to derive transgressive segregants from a cross. To start a wheat hybridization program in which parents have high heritability along with high molecular diversity, cluster analysis should be carried out to exclude similar parents from the breeding material. Therefore, PCA should be followed by cluster analysis so that genotypes can be grouped in similar and distinct groups (Ahmad et al. 2012). Ayed et al. (2010) demonstrated that cluster analysis is a successful strategy for selection of genotypes to initiate a wheat hybridization programme on the basis of certain morphological traits. Using ward,s linkage clustering method experimental data was analyzed by cluster analysis. Ahmad et al. (2012) identified 2 clusters and 3 subclusters by ward,s linkage clustering method.

Rana and Bhat (2005) estimated 74% genetic similarity by cluster analysis. Similarly, Aliyu and Fawal (2000) highlighted the efficiency of cluster analysis to identify and group crop accessions on the basis of genetic similarity using dendrogram. Multivariate analysis is a valid system to study germplasm collection (Ghafoor et al. 2001; Ahmad et al. 2012). Ijaz and Khan, (2009) classified the 63 genotype into three clusters. Salem et al. (2008) showed the cluster analysis of seven wheat varieties into two major clusters and three sub cluster. The dendrogram represents a number of dissimilar groups. Within the same cluster individuals are similar but there have significant differences with other cluster (Finsten 1996).

In the following study some genotypes occupy different clusters under different water conditions which showed expression of different genes under different environmental conditions. Similarly, under T4 100% water conditions genotypes were grouped in 4 clusters except 5 as under different environments which showed variation among genotypes under different water regimes. Moisture stress induces the expression of large number of genes (Shinozaki and Yamaguchi-Shinozaki, 2007). Drought tolerance is a veryd trait which is controlled by many genes and their expressions are influenced by various environmental elements. As these traits are controlled by different QTLs so it may be due to the response of different QTLs to different environments. On the other hand it may be due to the pleiotropic effect by the co-location of QTLs for different traits at a single locus or cluster of closely linked genes (Landjeva et al. 2008).

The following study has depicted the influence of different moisture regimes on the trait expression. Molecular and phenotypic characterization also explored the genetic differences among genotypes. Moreover the genetic diversity dissected in this study using ISSR markers should be explored with SSR or SNP markers to identify QTLs controlling these important traits. Because the seedling growth in wheat is under the control of many loci as concluded by Landjeva et al. (2008) while studying on the International Triticeae Mapping Initiative (ITMI) recombinant inbred population, and find QTLs located on different chromosomes.

The results of the following study have demonstrated the involvement of different genetic components which are controlling seedling traits. Traits which showed high heritability and genetic advance should be given due importance to start a breeding program. We conclude that only one level of moisture deficit is not a suitable strategy to breed for drought tolerance. As the study depicted that different plant traits are influenced by different water levels. So, phenotypic evaluation should be done at different water levels to select best genotypes having drought tolerance.

REFERENCES

[1] Abou-Dief, M.H., Rasheed, M.A., Salaam, M.A.A., Mostafa, E.A.H., Ramadan, W.A. 2013. Characterization of twenty wheat varieties by ISSR markers. Middle-East J. Sci. Res. 15 : 168-175.

[2] Ahmad, M.Q., Khan, S.H., Azhar, F.M. 2012. Decreasing level of genetic diversity in germplasm and cultivars of upland cotton (*Gossypium hirsutum*) in Pakistan. J. Agric. Social Sci. 8: 92–96.

[3] Aliyu, B.N., Fawal, Q. 2000. Inheritance of pubescence in crosses between *V. unguiculata* and *V. rhomboidea*. Nigerian J. Genet. 15: 9–14.

[4] Awan, S.I., Shahid. N., Malik, M.F.A., Ali, S. 2007. Analysis of variability and relationship among seedling traits and plant height in semi dwarf wheat (*Triticum aestivum* L.). J. Agric. Social Sci. 2: 59–62.

[5] Ayed, S., Karmous, C., Sliml A., Amara, H.S. 2010. Genetic variation of durum wheat landraces using morphological and protein markers. Afr. J. Biotechnol. 9: 8277-8282.

[6] Birsin, M.A. 2005. Effects of Removal of Some Photosynthetic Structures on Some Yield Components in Wheat. Tarim. Bilimleri. Dergisi., 11: 364-367.

[7] Brown-Guedira, G., Thompson, J., Nelson, R., Warburton, M. 2000. Evaluation of genetic diversity of soybean introductions and North American ancestors using RAPD and SSR markers. Crop Sci. 40: 815-823.

[8] Carvalho, A., Lima-Brito, J., Macas, B., Guedes-Pinto, H. 2009. Genetic diversity and variation among botanical varieties of old Portuguese wheat cultivars revealed by ISSR assays. Biochem. Genet. 47: 276-294.

[9] Ceccarelli, S., Grando, S. 1997. Drought as a challenge for the plant breeder. *In* "Drought Tolerance in Higher Plants: Genetical, Physiological and Molecular Biological Analysis", pp. 71-77. Springer.

[10] Dhanda, S.S., Sethi, G.S., Behl, R.K. 2004. Indices of drought tolerance of wheat genotypes at early stage of plant growth. J. Agron. Crop Sci. 190: 6-12.

[11] Eid, M.H. 2009. Estimation of heritability and genetic advance of yield traits in wheat (*Triticum aestivum* L.) under drought condition. Int. J. Genet. Mol. Biol. 1: 115-120.

[12] Frova, C., Villa, M., Sari, G.M., Krajewski, P., Fonzo, N.D. 1999. Genetic analysis of drought tolerance in maize by molecular markers. I. Yield components. Theor. Appl. Genet. 99: 280-288.

[13] Finstein, A., Mohammad, H., Singh, H. 1996. Genetic divergence in selected durum wheat genotypes of Ethiopian plasm. Afr. Crop Sci. J. 15: 62-72

[14] Ghafoor, A., Sharif, A., Ahmad, Z., Zahid, M.A., Rabbani, M.A. 2001. Genetic diversity in Black gram (*Vigna mungo* L.). Field Crops Res. 69: 183–190.

[15] Gulnaz, S., Khan, S.H., Shahzad, M., Ashfaq, M., Sajjad, M. 2012, Genetic evaluation of spring wheat (*Triticum aestivum*) germplasm for yield and seedling vigor traits. J. Agric. Social Sci. 8: 123–1281.

[16] Haider, Z., Khan, A.S., Zia, S. 2012. Correlation and path co-efficient analysis of yield components in rice (*Oryza sativa* L.) under simulated drought stress condition. Int. J. Bot. Res. 2: 1-12.

[17] Ijaz, S., Khan, I.A. 2009. Molecular characterization of wheat germplasm using microsatellite markers. Genet. and Mol. Res. 8: 809-8 15.

[18] Inoue, T., Inanaga, S., Sugimoto, Y., Eneji, A.E. 2004. Effect of drought on ear and flag leaf photosynthesis of two wheat cultivars differing in drought resistance. Photosynthetica. 42: 559–565.

[19] Joshi, B.K., Mudwari, A., Bhatta, M.R., Ferrara, G.O. 2004. Genetic diversity in Nepalese wheat cultivars based on agro-morphological traits and coefficients of parentage. Nepal Agric. Res. J. 5: 7-17.

[20] Khan, I.A., Habib, S., Sadaqat, H.A., Tahir, M.H.N. 2004. Selection criteria based on seedling growth parameters in maize varies under normal and water stress conditions. Int. J. Agric. Biol. 6: 252-256.

[21] Kaiser, H.F. 1960. The application of electronic computers of factor analysis. Edu. Psychological Measurement, 20: 141- 151.

[22] Landjeva, S., Lohwasser, U., Borner, A. 2010 .Genetic mapping within the wheat D genome reveals QTL for germination, seed vigour and longevity, and early seedling growth Euphytica. 171:129–143.

[23] Leilah, A., Al-Khateeb, S. 2005. Statistical analysis of wheat yield under drought conditions. J. Arid Environ. 61: 483-496.

[24] Melchinger, A. 1993. Use of RFLP markers for analysis of genetic relationships among breeding materials and prediction of hybrid performance. Int. J. Crop Sci. 621-628.

[25] Mohammadi, S., Prasanna, B. 2003. Analysis of genetic diversity in crop plants—salient statistical tools and considerations. Crop Sci. 43: 1235-1248.

[26] Najaphy, A., Parchin, R.A., Farshadfar, B. 2012.Comparison of phenotypic and molecular characterizations of some important wheat cultivars and advanced breeding lines. Aus. J. Crop Sci. 6: 326-332.

[27] Nagaoka, T., Ogihara, Y. 1997. Applicability of Comparative inter-simple sequence repeat polymorphisms in wheat for use as DNA markers in comparison to cultivars using RAPD and ISSR markers. Theo. Appl.Genetics. 94: 597-602.

[28] Noorka, I.R., Khaliq, I., Kashif, M. 2007. Index of transmissibility and genetic variation in spring wheat seedlings under water deficit conditions. Pak. J. Agric. Sci. 44: 604-607.

[29] Ogunbayo, S.A., Ojo, D.K., Guei, R.G., Oyelakin, O., Sanni, K.A. 2005. Phylogenetic diversity and relationship among forty rice accessions using morphological and RAPD techniques Afr. J. Biotechnol. 4: 1234-1244.

[30] Panthee, D., Kc, R., Regmi, H., Subedi, P., Bhattarai, S., Dhakal, J. 2006. Diversity analysis of garlic (*Allium sativum* L.) germplasms available in Nepal based on morphological characters. Genetic Resourc. Crop Evol. 53: 205-212.

[31] Rana, M.K., Bhat, K.V. 2005. RAPD markers for genetic diversity study among Indian cotton cultivars. Current Sci. 88: 1956–1961.

[32] Reynolds, M., Condon, A., Rebetzke, G., Richards, R. 2004. Evidence for excess photosynthetic capacity and sink-limitation to yield and biomass in elite spring wheat. Proc. 4th. Int. Crop Sci. Cong, Brisbane.

[33] Salem, K.F.M., El-Zanaty, A.M., Esmail, R.M. 2008. Assessing Wheat (*Triticium aestivum* L.) Genetic Diversity Using Morphological characters and microsatallite markers. World J. Agric. 4: 538-544.

[34] Sardana, S., Mahajan, R.K., Gautam, N.K., Ram, B. 2007. Genetic variability in pea (*Pisum sativum* L.) germplasm for utilization. SABRAO J. Breed. Genet. 39: 31-41.

[35] Shao, H., Chu, L.J., Manivannan, P., Panneerselvam, R., Shao, M. 2009. Understanding water deficit stress-induced changes in the basic metabolism of higher plants biotechnologically and sustainably improving agriculture and the eco environment in arid regions of the globe. Critical Rev. Biotechnol. 29: 131-151.

[36] Shinozaki, K., Yamaguchi-Shinozaki, K. 2007. Gene networks involved in drought stress response and tolerance. J. Exp. Bot. 58: 221‑227.

[37] Singh, B.B., Mai-Kodomi, Y., Terao, T. 1999. A simple screening method for drought tolerance in cowpea. Ind. J. Genet. 59: 211-220.

[38] Sofalian, O., Chaparzadeh, N., Javanmard, A., Hejazi, M.S. 2003. Study the genetic diversity of wheat landraces from northwest of Iran based on ISSR molecular markers. Int. J. Agric. Biol. 10: 466-468.

[39] Sneath, P.H.A., Sokal, R.R. 1973. Numerical taxonomy: The principles and Practice of Numerical Classification. W.H. Freeman and Co., San Francisco.

[40] Steel, R.G.D, Torrie, J.H., Dicky, D.A. 1997. Principles and Procedures of Statistics: A Biometrical Approach, 3rd edition. McGraw Hill Book Co., Inc., New York.

[41] Thompson, J.A., Nelson, R.L. 1998. Utilization of diverse germplasm for soybean yield improvement. Crop Sci. 38: 1362-1368.

[42] Talebi, R, Haghnazari, A, Tabatabaei, I. 2010. Assessment of genetic diversity within international collection of *Brassica rapa* genotypes using inter simple sequence repeat DNA markers. Biharean Biol. 4: 145-151.

[43] Thudi, M., Manthena, R., Wani, S.P., Tatikonda, L, Hoisington, D.A., Varshney, R.A. 2010. Analysis of genetic diversity in pongamia (*Pongmia pinnatal L*. Pierre) using AFLP markers. J. Plant Biochem. Biotechnol. 19: 209-216.

[44] Tatikonda, L, Wani, S.P., Kannan, S., Beerelli, N., Sreedevi, T.K., Hoisington, D.A., Devi, P., Varshney, R.A. 2009. AFLP- based molecular characterization of an elite germplasm collection of *Jatropha curcas* L. a biofuel plant. Plant Sci. 176: 505-513.

[45] Tomar, S.M.S., Kumar, G.T. 2004. Seedling survivability as a selection criterion for drought tolerance in wheat. Plant Breed. 123: 392-394.