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# Study of genetic diversity among maize hybrids using SSR markers and morphological traits under two different irrigation conditions

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## ABSTRACT

**Shiri, M. R., Choukan, R., and Aliyev, R. T. 2014.** Study of genetic diversity among maize hybrids using SSR markers and morphological traits under two different irrigation conditions. **Crop Breeding Journal 4 (1): 65-72.**

**Genetic diversity of 38 maize hybrids was studied using 12 SSR primers and morphological traits under two different irrigation conditions. The 38 hybrids were evaluated in two trials, one under well-watered (WW) conditions and one under drought-stress (DS) conditions, using an RBCD design with three replications for two years (2008-09) in Moghan, Iran. The total number of PCR-amplified products was 40 bands, all of them polymorphic. Primer Phi031 generated the highest number of bands (6). Among the studied primers, UMC2359, PHI031 and UMC1862 showed the maximum polymorphism information content (PIC) and the greatest diversity. These were the most informative primers and thus could be used to assess the diversity of maize hybrids. To determine the genetic relationship among maize hybrids, cluster analysis was performed based on both morphological traits (using the Ward method) and SSR markers (using the CLINK method). Maize hybrids were divided into three main groups based on SSR markers. Principal coordinate analysis (PCoA) of a similarity matrix of hybrids showed that the first 13 coordinates explained 84.73% of the total variance, whereas the first two coordinates explained only 28.14% of total variance. Cluster analysis of morphological traits divided the maize hybrids into two groups under both WW and DS conditions. Grouping hybrids based on morphological data under WW and DS conditions yielded different groups. Generally, results indicated that SSR markers are able to more efficiently classify closely related maize hybrids than morphological traits.**

**Keywords: drought stress, genetic relationship, molecular markers, simple sequence repeats**

## INTRODUCTION

Maize (*Zea mays* L.) is a widely grown crop in most parts of the world due to its adaptability and productivity (Gerpacio and Pingali, 2007). It is one of the most important crops in Iran, accounting for over 4.13% of its total cropped area (FAO, 2013). Maize breeding faces unique challenges resulting from the narrow genetic background of commercial cultivars (Choukan *et al.*, 2004).

There are various techniques for studying the genetic variability of crop germplasm, such as performing pedigree analysis, studying morphological traits or using molecular markers (Pejic *et al.*, 1998). Morphological traits are the strongest determinants of the agronomic value and taxonomic classification of plants. Compared with other methods, morphological evaluation is direct, inexpensive and easy. However, errors can occur; furthermore, morphological estimations are more dependent on the environment and more subjective than other measurements.

Several studies have used morphological characterization to determine the genetic diversity of maize genotypes (Choukan *et al.*, 2004; Galarreta and Alvarez, 2001; Sanchez *et al.*, 2000; Doebley *et al.*, 1985; Bretting *et al.*, 1990; Crossa *et al.*, 1995). Neutral, DNA-based molecular markers are a more precise and environment-independent way of evaluating the genetic diversity of a particular species. Among available DNA-based markers, simple sequence repeats (SSR) have been used extensively to assess maize genetic diversity (Adetimirin *et al.*, 2008; Smith *et al.*, 1997; Phelps *et al.*, 1996; Taramino and Tingey, 1996; Pinto *et al.*, 2003; Warburton *et al.*, 2002).

It has been shown that eukaryote genomes are densely interspersed with simple sequences which consist of stretches of tandemly repeated nucleotide motifs which can be as short as 4, 3, 2 and even 1 nucleotid (Morgante and Olivieri, 1993). SSR markers allow detecting polymorphisms at the DNA level, which facilitates separating genotypes into

well-defined groups based on genetic distance estimates (Enoki *et al.*, 2002).

Several studies have used SSR markers to characterize maize such as Sharma *et al.* (2010) in India; Beyene *et al.* (2006) in Ethiopia; Smith *et al.* (1997) in USA; Nikhou *et al.* (2013) (who assessed hybrids from several countries) in Iran; and Choukan *et al*. (2006) also in Iran. Senior *et al.* (1998) reported that microsatellite markers in maize showed high levels of polymorphism and can be used for studying the crop's genetic variation. By sequencing alleles, a complex pattern of mutation was found to exist in microsatellite regions.

Compared with maize wild relatives, the crop's genetic diversity has been increasingly narrowed due to domestication and modern breeding (Choukan *et al.*, 2004). Narrow genetic diversity is problematic when breeding for adaptation to biotic and abiotic stress. In order to broaden genetic variation for use in future maize breeding, the genetic diversity of maize germplasm needs to be investigated. In the present study, genetic diversity among 38 maize hybrids was examined based on SSR markers and morphological traits under two different irrigation conditions.

## MATERIALS AND METHODS

Seed of 20 maize elite inbred lines was obtained from Iran's Seed and Plant Improvement Institute. Using a line × tester mating design, 18 female inbred lines and two male testers (K3653/2 and K3615/1) were crossed through controlled pollination to produce 36 hybrid progenies. The resulting 36 late-maturing maize hybrids, along with two checks (KSC704 and KSC700), were planted in two trials under well-watered (WW) and drought-stressed (DS) conditions during the grain-filling period, using an RBCD design with three replications in Pars Abad-e-Moghan (39° 41' N 47° 32' E; 40-50 m above sea level), Ardebil Province, during two years (2008-09). Each plot consisted of four 5-m rows with 75 and 18 cm row and hill spacing, respectively. The pedigrees of the studied hybrids are given in Table 1. In the WW environment, irrigation was applied nine times based on the crop's water requirements during its growth period, but in the DS environment, irrigation was applied six times from sowing to the end of flowering and then was withheld completely from the end of flowering to physiological maturity in order to create water stress.

**Table 1. Pedigrees of the 38 maize hybrids used in this study.**

| | Hybrids | | Hybrids |
|---|---|---|---|
| 1 | KLM77008/1-3-3-1-2-2-1× K3653/2 | 20 | KLM77008/1-3-3-1-2-2-1 × K3615/1 |
| 2 | KLM77012/4-1-1-4-1-2-1 × K3653/2 | 21 | KLM77021/4-1-2-1-2-1-2 × K3615/1 |
| 3 | KLM77021/4-1-2-1-2-1-2 × K3653/2 | 22 | KLM77029/8-1-1-1-2-1-5 × K3615/1 |
| 4 | KLM77029/8-1-1-1-2-1-5 × K3653/2 | 23 | KLM77029/8-1-1-1-2-2-2 × K3615/1 |
| 5 | KLM77029/8-1-1-1-2-2-2 × K3653/2 | 24 | KLM76004/3-5-1-2-2-1-1-1 × K3615/1 |
| 6 | KLM76004/3-5-1-2-2-1-1-1 × K3653/2 | 25 | KLM76012/1-3-1-1-1-2-1-1 × K3615/1 |
| 7 | KLM76012/1-3-1-1-1-2-1-1 × K3653/2 | 26 | K74/2-2-1-3-1-1-1-1 × K3615/1 |
| 8 | K74/2-2-1-3-1-1-1-1 × K3653/2 | 27 | K74/2-2-1-4-4-1-1-1 × K3615/1 |
| 9 | K74/2-2-1-4-4-1-1-1 × K3653/2 | 28 | K74/2-2-1-19-1-1-1-1 × K3615/1 |
| 10 | K74/2-2-1-19-1-1-1-1 × K3653/2 | 29 | K74/2-2-1-21-2-1-1-1× K3615/1 |
| 11 | K74/2-2-1-21-2-1-1-1 × K3653/2 | 30 | K74/2-2-1-21-3-1-1-1 × K3615/1 |
| 12 | K74/2-2-1-21-3-1-1-1 × K3653/2 | 31 | K74/1 × K3615/1 |
| 13 | K74/1 × K3653/2 | 32 | K3545/7 × K3615/1 |
| 14 | K3545/7 × K3653/2 | 33 | K3544/4 × K3615/1 |
| 15 | K3544/4 × K3653/2 | 34 | K3640/6 × K3615/1 |
| 16 | K3640/6 × K3653/2 | 35 | KLM75010/4-4-1-2-1-1-1 × K3615/1 |
| 17 | KLM75010/4-4-1-2-1-1-1 × K3653/2 | 36 | KLM76010/1-13-1-2-1-1 × K3615/1 |
| 18 | KLM76010/1-13-1-2-1-1 × K3653/2 | 37 | KSC700 |
| 19 | KLM77012/4-1-1-4-1-2-1 × K3615/1 | 38 | KSC704 |

Data were recorded for grain yield, number of kernels per row, kernel row number, total number of kernels per ear, 1000-kernel weight, ear length, grain hectoliter weight, grain index (grain weight to ear weight ratio), ear perimeter, kernel thickness, kernel depth, kernel width, plant height, ear height, plant height 30 days after planting, stem diameter, internode length, tassel length, number of tassel branches, number of leaves above the ear, number of leaves below the ear, total number of leaves per plant, node number, ear leaf length, ear leaf width, ear leaf area, days to 50% anthesis, days to 50% silking, days to 50% browned silk, anthesis-silking interval, relative growth rate, days to 50% maturity, grain-filling rate, grain-filling period, ear cob percent, chlorophyll-a content, chlorophyll-b content and chlorophyll-(a+b) content.

### DNA extraction and SSR assay

Total genomic DNA was extracted from two to three young fresh leaves at the 4-5-leaf stage using the cetyltrimethylammonium bromide (CTAB) method according to Saghai-Maroof *et al.* (1984), with minor modifications. The quantity and quality

of DNA were evaluated using a UV-spectrophotometer. Based on repeat unit and bin location to provide uniform coverage of the entire maize genome, 12 SSR primers were chosen from the maize GDB database (Maize Genetics and Genomics Database, 2010). Amplification reaction products were separated on a 6% denaturing polyacrylamide gel. The amplified fragments were detected by the silver staining method as described by Bassam *et al.* (1991). For subsequent statistical analysis, in order to obtain a binary matrix, polymorphic bands amplified by SSR markers were scored as present (1) or absent (0). The generated data matrices were subjected to statistical analysis using SPSS (Ver. 18) and POPGEN analytical software.

## RESULTS AND DISCUSSION

In this research, 36 of the 38 studied hybrids were developed by crossing 18 maternal lines with 2 paternal lines, that is, 18 hybrids had K3653/2 as the male parent and 18 had K3615/1 as the male parent. Besides their similarity with respect to the male parent, each pair of the 36 hybrids had the same female parent. This resulted in a good similarity between hybrids, which could be used to determine the correctness of clustering by the molecular and morphological methods.

### Cluster analysis of the studied maize hybrids based on morphological traits under well-watered and drought-stressed conditions

Cluster analysis was performed to generate a dendrogram using the WARD method on a square Euclidean distance matrix based on data recorded for morphological traits under well-watered and drought-stressed conditions. To determine the desired number of clusters, we used the formula $\sqrt{n/2}$ (n= number of hybrids) and cut the dendrogram where the largest distinction was created. In this study, the number of clusters determined by using the $\sqrt{n/2}$ formula was 4-cluster, and 2-cluster was the derived place where cutting the dendrogram created the greatest distinction. Moreover, canonical discriminant function analysis was employed to determine the number of genotype groups in cluster analysis. Discriminant function analysis was performed for the 2, 3, 4 and 5 group states, and the greatest distinction was obtained by the two-group state under both conditions (Table 2).

- The first group included 17 hybrids, whose male parent was line K3615/1. This group was divided into three subgroups, with hybrids 26, 29, 27, 28, 36, 22, and 19 in the first subgroup, hybrids 33, 35, 30, 34, 23, 31, 21, 32, and 24 in the second subgroup, and only hybrid 20 in the third subgroup of cluster-I (Fig. 1).

**Table 2. Determination of group number accuracy for cluster analysis with discriminant function analysis.**

| Group number | Eigenvalue | Canonical correlation | Wilks' lambada | P value |
|---|---|---|---|---|
| **Well-watered conditions** | | | | |
| 2 | 52.097 | 0.991 | 0.019 | 0.000 |
| 3 | 57.136 | 0.991 | 0.001 | 0.000 |
| 4 | 61.892 | 0.992 | 0.000 | 0.000 |
| 5 | 84.069 | 0.994 | 0.000 | 0.000 |
| **Drought-stressed conditions** | | | | |
| 2 | 32.165 | 0.985 | 0.030 | 0.000 |
| 4 | 45.389 | 0.989 | 0.000 | 0.000 |
| 5 | 47.454 | 0.990 | 0.000 | 0.000 |
| **SSR markers** | | | | |
| 2 | 423.039 | 0.999 | 0.002 | 1.02E-10 |
| 3 | 217.957 | 0.998 | 0.005 | 1.43E-18 |
| 4 | 195.564 | 0.997 | 0.005 | 7.88E-09 |
| 5 | 10.833 | 0.957 | 0.085 | 0.043155 |

**Based on the two-group state, group combinations under well-watered conditions were as follows:**

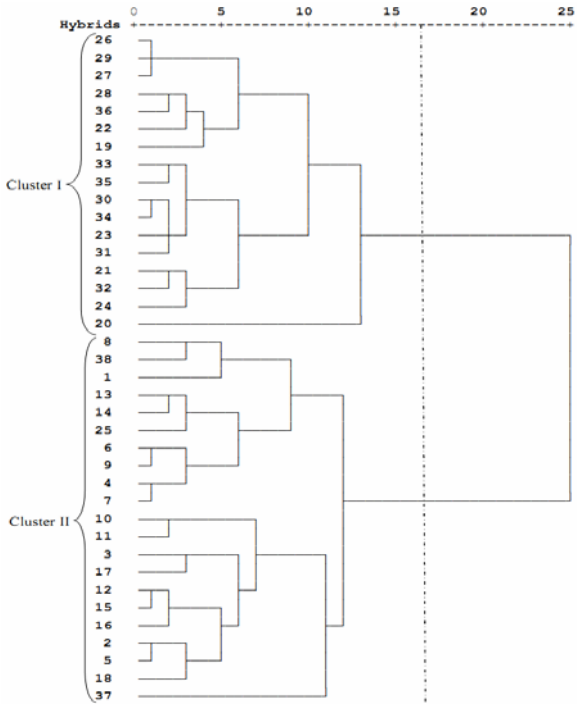


**Fig. 1. Dendrogram generated by the WARD method for 38 maize hybrids based on morphological traits under well-watered conditions.**

- The second group included 21 hybrids (8, 38, 1, 14, 25, 6, 9, 4, 7, 10, 11, 3, 17, 12, 15, 16, 2, 5, 18, 37) and the male parent of 17 of them was line K3653/2. Despite having a different male parent (K3615/1) from the other hybrids, hybrid 25 was placed in this cluster by cluster analysis based on the characteristics studied under WW conditions. Despite having similar morphological characteristics, KSC704 (hybrid 38) and KSC700 (hybrid 37) were placed very far from each other in

cluster-II. The maternal parents (KSC704 and KSC700) of these two hybrids were genetically very close. The second group can be divided into four subgroups, with hybrids 8, 38, and 1 in the first subgroup, hybrids 13, 14, 25, 6, 9, 4, and 7 in the second subgroup, hybrids 10, 11, 3, 17, 12, 15, 16, 2, 5, and 18 in the third subgroup, and only hybrid 37 in the fourth subgroup of cluster-II (Fig. 1).

Under WW conditions, cluster analysis based on morphological traits should recognize similarities and differences between hybrids in order to place them in separate groups.

Based on morphological data measured under drought-stressed conditions, 38 maize hybrids were divided into two groups (Fig. 2). The first group included 22 hybrids, 8 of which had line K3615/1 as the male parent. This group was divided into four subgroups, with hybrids 8, 10, 3, 9, 32, 27, 29, and 26 making up the first subgroup of cluster-I. Line K3653/2 was the male parent of hybrids 8, 10, 3, and 9, and line K3615/1 was the male parent of hybrids 32, 27, 29, and 26. Hybrids 6, 11, 14, and 16 made up the second subgroup and line K3653/2 was the male parent of all of them. Hybrids 17, 18, 21, 28, 15, 33, and 34 made up the third subgroup; line K3653/2 was the male parent of hybrids 17, 18, and 15, and the rest of these hybrids had line K3615/1 as the male parent. Hybrids 37, 38, and 13 were included in subgroup four (Fig. 2). Hybrids 37 and 13 had the same female parent and were genetically very similar to the female parent of hybrid 38. Because of their genetic similarity, it seems logical that these three hybrids should be in the same subgroup.

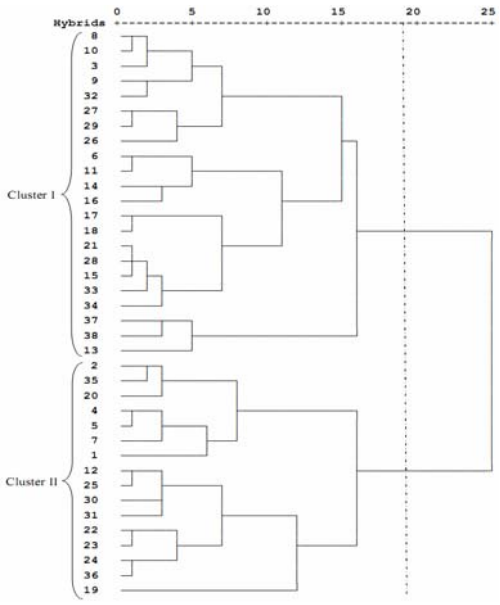


**Fig. 2. Dendrogram generated by the WARD method for 38 maize hybrids based on morphological traits under drought-stressed conditions.**

The second group included 16 hybrids, 6 of which had line K3653/2 as the male parent. The second group was divided into three subgroups, with hybrids 2, 35, 20, 4, 5, 7, and 1 in the first subgroup. Hybrids 12, 25, 30, 31, 22, 23, 24, and 36 were included in the second subgroup (Fig. 2); line K3653/2 was the male parent of hybrid 12, and line K3615/1 was the male parent of the rest of the hybrids. Only hybrid 19 was located in the third subgroup of cluster-II.

Generally, under DS conditions at the grain-filling stage, placing the studied hybrids in different groups and subgroups by cluster analysis did not match their pedigree data; therefore, cluster analysis did not distinguish similarities and differences between hybrids very well.

The Mantel coefficient was used to study the degree of conformity between cluster analyses performed under both conditions. The Mantel coefficient between the distance matrix under DS conditions and the distance matrix under WW conditions was 0.43. This shows that these two matrices had a moderate level of conformity. Because morphological traits are influenced by environmental conditions, the results of cluster analyses performed under the two conditions were somewhat different from each other.

Similarity matrices can be compared based on similarity criteria and also on the manner in which hybrids are grouped in dendrograms. The degree of similarity between dendrograms constructed based on phenotypic data under WW and DS conditions was moderate; as a result, only hybrids 1, 2, 4, 5, 7, 12, 23, 24, 25, 30, 31, and 35 were placed in similar groups under both conditions. Therefore, it can be concluded that the degree of similarity among these hybrids was so high that they were grouped together despite the change in environmental conditions and in traits. In general, it can be said that grouping hybrids yielded different results under the two different conditions because phenotypic data were influenced by environmental conditions.

**Polymorphism revealed by SSR markers**

A total of 40 bands generated by 12 SSR primers were polymorphic. Primers PHI031, UMC1877 and UMC2359 with 6, 5 and 5 bands, and primers UMC1501, UMC1447, UMC1447, BNLG1617, PHI080 and UMC1432 with 2 bands had the highest and lowest number of bands, respectively. The average number of amplified and polymorphic bands per primer was 3.33 (Table 3).

The mean number of alleles per SSR marker found in this study (3.33) was lower than those detected by Beyene *et al.* (2005), who reported an

**Table 3. Number of alleles, number of effective alleles, bin location, polymorphic index content (PIC), Shanan Index, Nei Index and motif for SSR markers.**

| Primers | Motif | Bin location | Alleles (no.) | Effective alleles (no.) | PIC | Shanan Index | Nei Index |
|---|---|---|---|---|---|---|---|
| UMC1862 | (GA)8 | 1.11 | 5 | 3.50 | 0.72 | 1.39 | 0.71 |
| NC133 | GTGTC | 2.05 | 4 | 1.93 | 0.48 | 0.91 | 0.49 |
| UMC1501 | (AAG)5 | 3.05 | 2 | 1.89 | 0.47 | 0.66 | 0.45 |
| UMC1719 | (GCG)5 | 4.10-11 | 4 | 2.80 | 0.64 | 1.13 | 0.65 |
| UMC1447 | (CTT)4 | 5.03 | 2 | 1.54 | 0.35 | 0.53 | 0.36 |
| PHI031 | GTAC | 6.04 | 6 | 4.48 | 0.78 | 1.62 | 0.77 |
| BNLG1617 | AG (16) | 6.05 | 2 | 1.41 | 0.29 | 0.47 | 0.25 |
| UMC1333 | (CAG)4 | 7.03 | 3 | 2.00 | 0.49 | 0.87 | 0.51 |
| UMC1545 | (AAGA)4 | 7.00 | 3 | 2.97 | 0.66 | 1.09 | 0.65 |
| PHI080 | AGGAG | 8.08 | 2 | 1.29 | 0.23 | 0.39 | 0.17 |
| UMC2359 | (AAAAG)4 | 9.07 | 5 | 4.70 | 0.79 | 1.58 | 0.78 |
| UMC1432 | (AG)6 | 10.02 | 2 | 1.85 | 0.46 | 0.65 | 0.47 |
| Mean | - | - | 3.33 | 2.53 | 0.53 | 0.94 | 0.52 |

average of 4.9 with a range of 3-10 alleles in 62 traditional Ethiopian highland maize accessions, and by Pabendona *et al.* (2009), who found an average of 4.47 alleles with a range of 2-8 alleles/SSR locus in 39 Indonesian maize accessions. In another study, Yao *et al.* (2007) found 2-9 alleles and an average of 6.1 alleles per locus in 54 maize landraces from southwest China. The mean number of alleles (3.33) found in our study, however, was higher than the 3.13 average alleles per locus per SSR locus reported by Nikhou *et al.* (2013). These findings are consistent with the results of Smith *et al.* (1997), who found an average of 3.38 alleles per SSR locus. Such considerable differences in the number of detected alleles may arise from differences in: (1) the diversity and number of the genotypes tested, and (2) the number and diversity of SSR primers examined.

PIC varied from 0.23 to 0.79, with an average of 0.53 (Table 2). Regarding the importance of PIC for primer efficiency, it is interesting that UMC2359 had the highest PIC (0.79). Four primers—PHI031 (0.78), UMC1862 (0.72), UMC1545 (0.66) and UMC1719 (0.62)—had the highest PIC after UMC2359. The average PIC value obtained in this study was lower than those of maize landraces from: Japan (0.69) using 60 SSRs (Enoki *et al.*, 2002); India (0.60) using 42 SSRs (Sharma *et al.*, 2010); Ethiopia (0.61) using 20 SSRs (Beyene *et al.*, 2006); as well as that of US maize inbred lines (0.62) using 131 SSR (Smith *et al.*, 1997). Our results were consistent with the results of Choukan *et al.* (2006), who found an average PIC value of 0.54 per SSR locus. The reason for this lower PIC value may be that Iranian maize has narrow genetic diversity.

## Principal coordinate analysis (PCoA) of the studied maize hybrids based on SSR markers

Principal coordinate analysis is used to explain genetic variation, show the variation pattern in a multidimensional pattern and do a better interpretation of the relationship between individuals (Khayyam Nikoyie *et al.*, 2009). The relative variance of each coordinate indicates the importance of the related coordinate of total variance and is expressed as a percentage.

All the data obtained using 12 SSR primers were used in PCoA with simple matching coefficients of similarity. PCoA of a similarity matrix of hybrids showed that the first 13 coordinates explained 84.73% of the total variance, whereas the first two coordinates explained only 28.14% of total variance. The first coordinate explained 17.61% and the second one explained 10.53% of the total variance (Table 4). A scatterplot of hybrids was constructed based on the two main coordinates. Maize hybrids were grouped into three different clusters according to their similar characteristics in the PCoA biplot (Fig. 3).

**Table 4. Eigenvalues, variance and agglomerative variance in principal coordinate analysis for SSR markers.**

| Coordinates | Eigenvalues | Variance (%) | Agglomerative variance (%) |
|---|---|---|---|
| 1 | 7.04 | 17.61 | 17.61 |
| 2 | 4.21 | 10.53 | 28.14 |
| 3 | 3.47 | 8.67 | 36.82 |
| 4 | 2.89 | 7.22 | 44.03 |
| 5 | 2.57 | 6.42 | 50.46 |
| 6 | 2.38 | 5.96 | 56.42 |
| 7 | 2.18 | 5.45 | 61.87 |
| 8 | 2.05 | 5.13 | 67.00 |
| 9 | 1.69 | 4.22 | 71.22 |
| 10 | 1.49 | 3.72 | 74.94 |
| 11 | 1.47 | 3.67 | 78.60 |
| 12 | 1.26 | 3.16 | 81.76 |
| 13 | 1.19 | 2.97 | 84.73 |

## Cluster analysis of studied maize hybrids based on SSR molecular data

Cluster analysis was performed to develop a dendrogram based on the Complete Linkage (CLINK) hierarchical algorithm on a Jaccard similarity matrix (Fig. 4). Given that a higher cophenetic correlation coefficient (r = 0.915) indicates the usefulness of the CLINK on a Jaccard similarity matrix, it is clear that this algorithm grouped the hybrids better than other algorithms (Table 5).

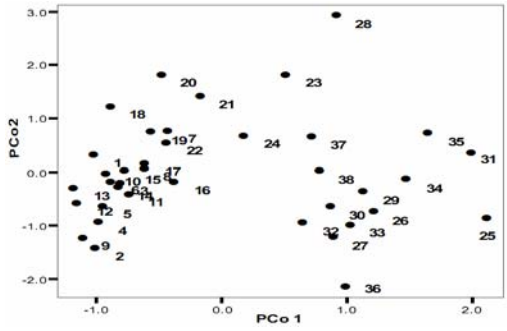


**Fig. 3. Grouping of 38 maize hybrids with the first two PCo extracted from SSR data.**

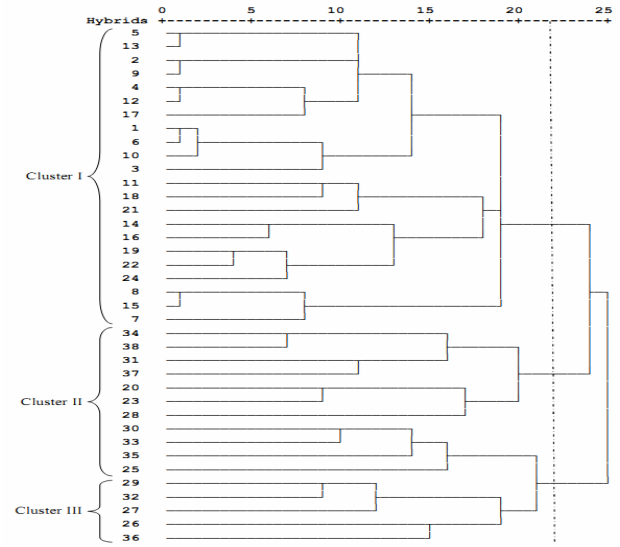


**Fig. 4. Dendrogram generated using the complete linkage (CLINK) method on a Jaccard similarity coefficient for 38 maize hybrids based on SSR amplification polymorphism analysis.**

**Table 5. Cophenetic correlation coefficient of clustering different algorithms in different similarity matrices for 38 maize hybrids based on SSR data.**

| Method | Similarity criteria | | |
|---|---|---|---|
| | Dice | Jaccard | SM |
| UPGMA | 0.802 | 0.854 | 0.845 |
| CLINK | 0.895 | 0.915 | 0.671 |

**SM: simple matching**

The number of clusters was determined by using the $\sqrt{n/2}$ formula (4, the greatest distinction) and cutting the dendrogram (3). Moreover, discriminant function analysis confirmed that the greatest distinction was in the three-group state (Table 1).

Based on SSR data, the first main group consisted of 22 hybrids that were subdivided into five subgroups, with hybrids 5, 13, 2, 9, 4, 12, 17, 1, 6, 10, and 3 located in first subgroup of the first main group. All of the hybrids located in this subgroup had the same male parent (line K3653/2). Therefore, considering the pedigrees of maize hybrids used in this study, this grouping seems logic. The second subgroup included hybrids 11, 18, and 21, and the first two hybrids had the same male parent. Hybrids in the third subgroup (hybrids 14 and 16), fourth subgroup (hybrids 19, 22 and 21) and fifth subgroup (hybrids 8 and 15) had the same male parent.

The second main cluster included seven hybrids that were divided into two subgroups. The first subgroup included hybrids 37, 38, 31 and 34. The female parent of hybrid 37 was genetically very similar to the female parent of hybrid 38. Therefore, the fact that hybrid 37 (KSC700) and hybrid 38 (KSC704) were in same group indicated that the molecular method was highly efficient in diversity studies. Hybrids 20, 23 and 28 had the same male parent and were in the second subgroup of cluster-II. The third cluster, which consisted of nine hybrids (hybrids 30, 33, 35, 25, 29, 32, 27, 26, and 36), was divided into three subgroups. All nine hybrids had the same male parent (Fig. 4).

In general, only hybrids 21, 19, 22, and 24 of the 38 studied hybrids were placed in groups that could not be predicted based on similarities in pedigree data. Therefore, the molecular method is a reliable and accurate way of evaluating genetic diversity that breeding programs can use with confidence. In general, this research indicated microsatellite markers are very suitable for differentiating and recognizing various maize hybrids, and can be used not only for grouping maize hybrids but also for identifying various genes conferring resistance to biotic and abiotic stresses. This is in agreement with other studies (Legesse *et al*., 2006; Reif *et al*., 2003; Smith *et al*., 1997) that found that SSR markers are efficient for classifying closely related lines.

In this research, the Mantel coefficient was used to determine the degree of correlation between the similarity matrix and the distance matrix. The molecular data similarity matrix was converted into a distance matrix by using the formula $D_{ij} = 1 - GS_{ij}$ (where Dij is the distance between the two genotypes and $GS_{ij}$ is the degree of similarity between the two genotypes). The matrix of similarities between molecular data and phenotypic data had a Mantel coefficient of 0.019 under both WW and DS conditions. This shows there was very little similarity between molecular data and phenotypic data, while the similarity matrix of phenotypic data under both WW and DS conditions had a Mantel coefficient of 0.423 (which shows the data had a moderate level of similarity). These results were consistent with those of Beyene *et al.* (2005) and Pabendona *et al.* (2009), who found little similarity between phenotypic and molecular data.

In addition to comparing similarity matrices based on similarity criteria, another comparison can be made based on the manner of grouping hybrids in dendrograms. Similarity between the dendrogram of molecular data and that of phenotypic data under WW conditions was greater than their similarity under DS conditions; the reason for this may be that under WW conditions, all changes in genotypic traits are revealed due to the optimal conditions, which leads to a greater similarity between these two. On the other hand, under DS conditions, plants cannot reveal all possible changes in their traits because they are influenced by drought stress.

## CONCLUSIONS

There are differences of opinion regarding whether data obtained from morphological evaluations are better than data from molecular evaluations for estimation of heredity. Some researchers believe most molecular markers determine the majority of individual diversities with respect to the non-coding parts of the genome. Therefore, from this point of view, it may not be possible to establish a direct relationship between molecular and morphological diversities. Other researchers are of the opinion that determining diversity based solely on morphological data is misleading and does not yield the information needed to estimate the relationships between genotypes or between populations in applied studies. Nevertheless, it must be noted that both morphological and molecular methods have advantages and disadvantages. For example, most (but not all) molecular data have a known genetic basis and Mendelian inheritance, and the total number of data items obtained from a study depends on the size of the genome and the number of markers used. On the other hand, morphological traits are easily measured and, in contrast to molecular markers, show changes related to the coding parts of the genome and to environmental effects. Diversities determined by DNA markers are mostly silent diversities and morphological data include genetic changes in addition to non-inheritable environmental changes; therefore, simultaneous use of both types of data will provide better and more comprehensive description and interpretation of biological diversity (Mohammadi and Prasanna, 2003). In recent years, due to the significant advances in DNA marker technology, these markers have been increasingly used as efficient tools that complement morphological methods in studying and determining genetic diversity levels and the relationships among individuals, species, and populations.

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