J Plant Mol Breed (2017) 5(1): 49 - 60
DOI: 10.22058/JPMB.2017.31701.1081

RESEARCH ARTICLE

Genetic Diversity Analysis of Maize Hybrids Through Morphological Traits and Simple Sequence Repeat Markers

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ABSTRACT: Comparing different methods of estimating the genetic diversity could define their usefulness in plant breeding programs. In this study, a total of 18 morphological traits and 20 simple sequence repeat (SSR) loci were used to study the morphological and genetic diversity among 20 maize hybrids selected from different countries, and to classify the hybrids into groups based on molecular profiles and morphological traits. To collect morphological data, a field experiment was carried out using an RBCD design with three replications in Moghan, Ardabil, Iran. The highest estimates for genetic coefficients of variation were observed in anthesis-silking interval, followed by grain yields, leaf chlorophyll rates, kernel row numbers, and ear heights. The total number of PCR-amplified products was 84 bands, all of which were polymorphic. Among the studied primers, NC009, BNLG1108, BNLG1194, PHI026 and PHI057 showed the maximum polymorphism information content (PIC) and the greatest diversity. To determine the genetic relationship among maize hybrids, the cluster analysis was performed based on both morphological traits (using the Ward method) and SSR markers (using the CLINK method). The cluster analysis of morphological traits divided the maize hybrids into five groups. Furthermore, Maize hybrids were divided into seven main groups based on SSR markers. Principal coordinate analysis (PCoA) of a similarity matrix of hybrids for SSR data showed that the first 15 coordinates explained 97.21 % of the total variance, whereas the first two coordinates explained only 33.14% of the total variance. Generally, results indicated that SSR markers were able to classify closely related maize hybrids more efficiently than morphological traits.

Keywords: Agronomical Traits, Genetic Relationship, SSR, Maize hybrid

INTRODUCTION

Maize is one of the most important cereal crops having wider adaptability under varied agro-climatic conditions. Globally, maize is known as the queen of cereals because it has the highest genetic yield potential among cereals. It is cultivated on nearly 150 million hectare in about 160 countries having a wide range of management practices, soil type, climate biodiversity and management practices that comprises 36% (782 m t) of global grain production. Maize is the third most important food crop after rice and wheat in Iran and is grown on more than 230,000 ha (19). Moreever maize uses as an important animal food and serves as the basic raw material in numerous industrial products that including starch, oil, protein, alcoholic beverages, food sweeteners, pharmaceutical, cosmetic, film, textile, gum, package, paper industries and so on (6). The basis for genetic improvement is genetic diversity.

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Received: 17 November 2015 / Revised: 23 March 2017
Accepted: 07 June 2017

Awareness of genetic diversity among elite breeding materials or adapted cultivars has an important role in the improvement of crop plants. Genetic variation within a population can be assessed based on: i) the number (and percentage) of polymorphic genes in the population, ii) the number of alleles for each polymorphic gene and iii) the proportion of heterozygous loci per individual (11). Various techniques are used for studying the genetic variability of crop germplasm includingpedigree analyses, morphological traits or use of molecular markers (15). Due to the influence of environmental factors evaluating based on plant phenotypes probably not be a reliable measure of genetic differences. Recent techniques, such as DNA-based markers, provide more reliable and effective tools for measuring genetic diversity in crop germplasm and assessing evolutionary relationships. DNA-based molecular marker systems are able to detect differences in genetic information carried by different individuals, therefore they are effective for identification and characterization of novel germplasm (12). Simple sequence repeats (SSRs) are simple, tandemly repeated nucleotide sequence motifs flanked by unique sequences. SSR markers, which can be assayed through PCR, are used to detect a high level of allelic diversity. Furthermore, the genotyping of these co-dominant markers can be automated (1, 2, 23). Numerous SSR markers have been identified in maize, and it has been used to assess genetic diversity. Regarding maize, most research efforts have been directed toward the development of microsatellite marker systems for genetic mapping and germplasm analysis (4, 5, 16, 18, 20, 24). Senior et al. (1998) reported that microsatellite markers in maize show a high level of polymorphism, and can be used for the investigation of genetic variation in this plant(17). By sequencing the alleles, it was found that there is a complex pattern of mutation in the microsatellite regions. Compared with maize wild relatives, the genetic diversity of the crop has been increasingly narrowed due to domestication and modern breeding (4). 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 20 maize hybrids was examined based on SSR markers and morphological traits and their efficiency was compared in classifying maize genotype.

MATERIALS AND METHODS

Twenty maize hybrids collected from different countries were used in this study (Table 1). The hybrids were grown in Ardabil Agricultural and Natural Resources Research Center (Pars Abad-e-Moghan, 39° 41' N 47° 32' E, 40-50 m above sea level, Ardabil, Iran,) in a randomized complete block design with three replications. Each hybrid was grown in two-row plots. Each plot consisted of two rows each raw with 16 hills, with 75 cm row spacing and 36 cm between hills. When the plants reached the fourth and fifth leaf stages, the plots were thinned, leaving two plants per hill, to achieve a final plant density of approximately 74000 plants/ha. The fertilizer was applied at rates of 140 kg ha^{-1} of N and 160 kg ha^{-1} P_2O_5 during planting. An additional 140 kg ha^{-1} of N was top-dressed 40 days after planting.

Data were recorded for grain yield (tons per hectare), the number of kernels per row, the kernel row number, 1000-kernel weight (gr), grain hectoliter weight, kernel depth (mm), plant height (cm), ear height(cm), stem diameter (cm), the number of leaves above the ear, the number of leaves below the ear, the total number of leaves per plant, leaf chlorophyll rate, days to 50% emergence, days to 50% silicon, days to 50% anthesis, anthesis-silking interval and days to 50% maturity.

Variance components were estimated based on expected mean squares in the analysis of variance using expectations as below (21):

$$\hat{\delta}_g^2 = \frac{Ms_g - Ms_e}{r}$$

$$\hat{\delta}_p^2 = \hat{\delta}_g^2 + \hat{\delta}_e^2$$

Where, $\hat{\delta}_g^2$, $\hat{\delta}_p^2$ and $\hat{\delta}_e^2$ are variance components for genotypes, phenotypes and errors, respectively, MSe and MSg are the observed values of the mean squares for error and genotype, respectively and r is the number of replications. The genotypic coefficient of variation (CVg), the environment coefficient of variation (CVe), phenotypic coefficient of variation (CVp), Broad sense heritability (h^2), Genetic advance (GA) and Genetic advance percentage (GA%) were calculated using the

Table 1. Names and origin of 20 maize hybrids used in this study

Code	Crosses or Hybrid names	Origin
1	KLM77007/7-2-6-3-1-2-1×K18	Developed in Iran
2	K166B×K19	Developed in Iran
3	K166B×K18	Developed in Iran
4	XTO3×A679	Developed in Iran
5	KSC705(K3640/3×MO17)	Developed in Iran
6	KSC704(B73×MO17)	Developed in Iran
7	KSC706(K2347/4 ×MO17)	Developed in Iran
8	KERMESS	Developed in KWS seed company derived from the Germanic-Italian Germplasm
9	KUADRO	Developed in KWS seed company derived from the Germanic-Italian Germplasm
10	KENDRAS	Developed in KWS seed company derived from the Germanic-Italian Germplasm
11	KALIMERAS	Developed in BC seed company derived from the Yugoslavia-Croatian Germplasm
12	BC582	Developed in BC seed company derived from the Yugoslavia-Croatian Germplasm
13	BC612	Developed in BC seed company derived from the Yugoslavia-Croatian Germplasm
14	BC712	Developed in BC seed company derived from the Yugoslavia-Croatian Germplasm
15	DKC6315	Developed in Monsanto seed company derived from the American Germplasm
16	DKC6589	Developed in Monsanto seed company derived from the American Germplasm
17	DKC6677	Developed in Monsanto seed company derived from the American Germplasm
18	DKC6876	Developed in Monsanto seed company derived from the American Germplasm
19	HIDO	Developed in Turkey
20	MAY-70	Developed in Turkey

following formulas:

$$C.V_g = \frac{\sqrt{\hat{\delta}_g^2}}{\overline{X}} \times 100 \qquad C.V_p = \frac{\sqrt{\hat{\delta}_p^2}}{\overline{X}} \times 100$$

$$C.V_e = \frac{\sqrt{\hat{\delta}_e^2}}{\overline{X}} \times 100 \qquad h^2 = \frac{\hat{\delta}_g^2}{\hat{\delta}_p^2}$$

$$G.A. = K \times h_i^2 \times \hat{\delta}_p^2 \qquad G.A.\% = \frac{G.A.}{\overline{X}} \times 100$$

Where, $\overline{X}$ is the mean value of the particular trait of interest and K: constant=2.06 at 5% selection intensity. To cluster of the hybrids into similar groups based morphological data, cluster analysis was performed using Ward's hierarchical algorithm based on the squared Euclidean distance. Prior to the calculation of the squared Euclidean distance, the data were standardized.To determine the desired number of clusters, the dendrograms was cut where the largest distinction was created. To identify the patterns of morphological variation, a principal component analysis (PCA) was conducted. The number of principal components that underwent interpretation was set on the basis of Kaiser's criterion, according to which those variables whose eigenvalues were greater than 1 were chosen. Also, Statistical calculations were carried out with the use of the SPSS software (18).

Total genomic DNA was extracted from two to three young fresh leaves at the 4-5 leaf stage using kits developed by the Iranian biological resource center based on spin column methods. The quantity and quality of DNA were evaluated by a UV- Spectrophotometer. Twenty SSR primers, were chosen based on repeat unit and bin location to provide uniform coverage of the entire maize genome from the MaizeGDB database (10). Amplicions were separated on a 6% denaturing polyacrylamide gel. The amplified fragments were detected by the silver staining method as described by Panaud et al. (1996) (14). Regarding subsequent

statistical analysis, in order to obtain a binary matrix, polymorphic bands amplified by SSR markers were scored as present (1) or absent (0). Some important parameters of marker efficiency are PIC, Nei's index and Shannon's information index. Nei and Shannon's coefficients were calculated using POPGEN software. The PIC parameter measures the diversity of alleles in each gene locus, which is $1 - \sum f_i^2$(17). In this formula, f_i is the frequency of $_i$-th allele in a locus. In addition, Cluster analysis using CLINK (Complete-linkage clustering) hierarchical algorithm based on the Jaccard dissimilarity criteria and principal coordinate analysis (PCoA) were performed on molecular data by SPSS (18) software.

RESULTS and DISCUSSION

Variability analysis based on morphological traits

Significant differences were observed among hybrids for all traits except the 1000-kernel weight, stem diameter, the number of leaves above the ear and the number of leaves below the ear (Table 2). This considerable variability provides a good opportunity for improving traits of interest in maize breeding programs. The highest phenotypic coefficients of variation were recorded for anthesis-silking interval (44.06%) followed by grain yield (16.09%), leaf chlorophyll rate (10.89%) and days to 50% emergence (10.19%). The highest estimates for genetic coefficients of variation were observed in anthesis-silking interval (34.25%), followed by grain yield (12.91%), leaf chlorophyll rate (8.13%), kernel row number (6.78%) and ear height (6.41%), which indicate the presence of exploitable genetic variability for these traits. Heritability estimates were greater for such traits due to days to 50% anthesis, days to 50% silking, ear height, kernel row number, grain yield and anthesis-silking interval. Hence, it is assumed that phenotypes of days to 50% anthesis, days to 50% silking, ear height, kernel row number, grain yield and anthesis-silking interval are largely determined by their genotypes. The genetic advance (5% selection intensity) was the highest for anthesis-silking interval, leaf chlorophyll rate, kernel row number and grain yield, and the lowest for the number of leaves below the ear and stem diameter (Table 2). This implies that progress in improving grain yield could be achieved through the simple selection of anthesis-silking interval, leaf chlorophyll rate, and kernel row number.

Heritability is not a very useful measure alone, but together with genetic advance, it is valuable (26). For the number of days to 50% silking and days to 50% anthesis, high heritability was associated with low genetic advance, indicating the influence of dominant and epistatic gene effects on these traits. High heritability of grain yield and anthesis-silking interval, coupled with high genetic advancements, indicated that additive gene effects were important in determining these traits (Table 2). Crop improvement for these traits is assumed to be possible by simple selection, due to high heritability coupled with high genotypic variation and additive gene effects (26).

Principal component analysis for studied maize hybrids based on morphological traits

Principal component analysis (PCA) was conducted to sum up the significant information from the data. PCA also lowered the number of traits responsible for the maximum percentage of overall variation of the experimental data. So, PCA was conducted using 18 traits by generating a genetic correlation matrix (Tables 3) to determine which traits were the major sources of variation within the germplasm panel. The first six principal components with eigenvalue higher than one, accounted for 82.3% of the total phenotypic variation. PC1 had an eigenvalue of 3.92 and accounted for 21.80% of the total variation and this represented an equivalent of three variables and indicated that days to 50% silking, days to 50% anthesis and days to 50% maturity were important contributing variables in distinguishing these accessions. PC2 had an eigenvalue of 2.62 contributing 14.58% of the total variation and had a number of leaves below the ear and the stem diameter as the main contributing traits. PC3 had an eigenvalue of 2.32 and contributed 12.90% of the variation. Also, the most important trait was the number of leaves above the ear. PC4 with an eigenvalue of 2.28 contributed 12.66% of the total variation and had kernel depth and grain yield as the main contributing traits. PC5 with an eigenvalue of 1.88 contributed only 10.42% of the total variation and majorly 1000-kernel weight as the contributing trait. PC6 had an eigenvalue of 1.78 contributing 9.89% of the variation and had plant height and ear height as the main contributing traits (Table 3).

Cluster analysis of the studied maize hybrids based on morphological traits

The cluster analysis, using WARD method based on the squared Euclidean distance criteria, was conducted for

Table 2. Analysis of variance and genetic parameters for different agronomic traits in 20 maize hybrids

Traits	MSt	Max	Min	Mean	Sd	h^2	CVg	CV_P	CVe	GA	GA%
Grain yield	6.87**	12.52	7.96	10.77	1.51	0.64	12.91	16.09	9.61	2.30	21.32
Number of kernels per row	12.13**	45.02	36.90	39.46	2.01	0.47	4.35	6.33	4.59	2.44	6.17
Kernel row number	3.99**	18.27	14.27	15.65	1.15	0.64	6.78	8.44	5.03	1.75	11.21
1000-kernel weight	1159.2ns	378.8	298.8	341.85	19.63	0.20	3.79	8.39	7.49	12.07	3.53
Grain hectoliter weight	33.77**	87.20	74.87	81.99	3.36	0.59	3.68	4.81	3.09	4.77	5.82
Kernel depth	0.01**	1.22	1.01	1.11	0.06	0.36	4.05	6.76	5.42	0.06	5.00
Plant height	142.22**	222.3	193.9	207.3	6.92	0.39	2.70	4.30	3.35	7.23	3.49
Ear height	145.40**	123.08	92.07	100.4	6.99	0.66	6.41	7.87	4.56	10.81	10.77
Stem diameter	0.02 ns	2.45	2.11	2.24	0.09	0.11	2.05	6.21	5.86	0.03	1.40
Number of leaves above the ear	0.25 ns	9.67	8.67	9.12	0.30	0.10	2.25	7.07	6.70	0.13	1.48
Number of leaves below the ear	0.29 ns	6.33	5.13	5.62	0.30	0.02	1.34	9.36	9.27	0.02	0.39
Total number of leaves per plant	0.56**	15.47	14.07	14.74	0.39	0.40	2.39	3.79	2.95	0.46	3.09
Leaf chlorophyll rate	70.01**	58.33	42.50	52.82	4.83	0.56	8.13	10.89	7.24	6.61	12.51
Days to 50% emergence	0.44**	6.33	4.33	5.07	0.38	0.33	5.85	10.19	8.35	0.35	6.91
Days to 50% silking	12.56**	68.67	61.33	64.67	2.05	0.74	2.99	3.49	1.79	3.42	5.28
Days to 50% anthesis	12.30**	65.33	58.67	62.07	2.02	0.78	3.12	3.53	1.64	3.53	5.69
Anthesis-silking interval	2.90**	4.67	0.00	2.60	0.98	0.60	34.25	44.06	27.72	1.43	54.84
Days to 50% maturity	25.80**	120.67	110.33	115.45	2.93	0.52	2.22	3.08	2.13	3.82	3.31

MSt: mean square of hybrids in analysis of variance, Sd: standard deviation, CVg: genotypic coefficient of variation, CVp: phenotypic coefficient of variation, CVe: environmental coefficient of variation, h^2: Broad sense heritability, GA: Genetic advance, GA%:Genetic advance percent. ns and **: Not significant, significant at 1% level of probability, respectively.

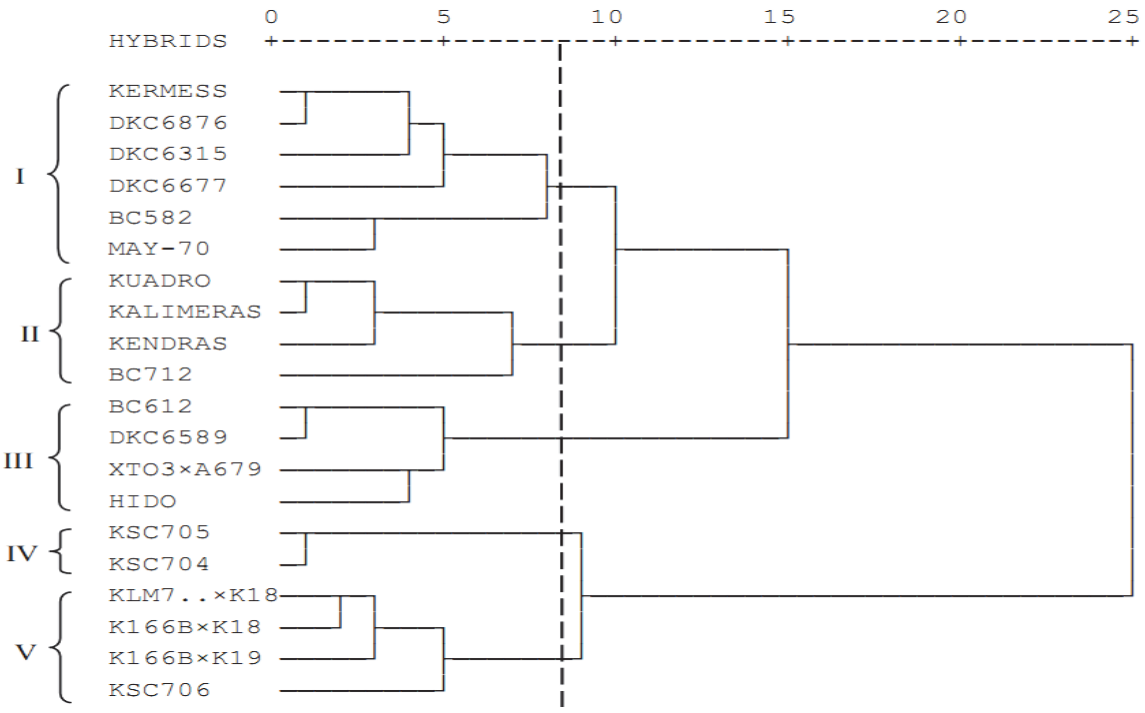

Figure1. Dendrogram of 20 maize hybrids constructed using WARD cluster analysis of squared Euclidean distance values obtained by morphological markers.

measuring genetic diversity and relatedness among the studied hybrids (Fig. 1). The studied maize hybrids were grouped into five clusters, showing the existence of considerable genetic diversity among 20 maize hybrids. The highest genetic distance was found between two hybrids, KSC706 and KERMESS, where they held the first and last positions of the dendrogram. The KSC706 was derived from the Iranian germplasm and the KERMESS from the German-Italian germplasm. On the other hand, the lowest genetic distance was found between the maize hybrids KERMESS and DKC6876 in the same group. These two hybrids originated from the German-Italian and American germplasm, respectively.

Six of the studied hybrids were located in group I, which developed from different germplasms. Three of them named as DKC6677, DKC6876 and DKC6315 derived from the American germplasm, whereas KERMESS, BC582 and May-70 originated from the German-Italian, Yugoslavia-Croatian, Turkey germplasm, respectively. Hybrids KUADRO, KALIMERAS, KENDRAS (derived from the American germplasm) and BC712 (originated from Yugoslavia-Croatian gerplasm) comprised cluster II. Hybrids namely BC612, DKC6589, XTO3×A679 and HIDO formed cluster III. They derived from the Yugoslavia-Croatian, American, Iranian and Turkish germplasms, respectively. Only two hybrids, KSC 705 and KSC 704, formed cluster IV and four hybrids namely KLM77007/7-2-6-3-1-2-1×K18, K166B×K19, K166B×K18 and KSC706 consisted cluster V, developed in Iran (Fig. 1).

Because some of the hybrids of the same geographical region were in different groups and placing the studied hybrids in different groups and subgroups by the cluster analysis did not match their pedigree data as well. It can be concluded that the genotypes present in the same region were genetically distant from each other or the cluster analysis based on morphological markers could not distinguish similarities and differences among hybrids very well.

Polymorphism revealed by SSR markers

All of SSR markers were polymorphic across the 20 analysed hybrids and a total of 84 alleles were detected. The number of alleles varied from two to seven per locus

with an average of 4.2. Primers NC009, BNLG1194 and BNLG1890 had the highest allele number with 7, 6 and 6 allele numbers, respectively. Nevertheless, BNLG1335 and BMC2136 with 2 bands had the lowest number of alleles (Table 4). In this study, the average value of total alleles per locus is similar to that found by Jambrović et al. (2008) who analyzed 15 maize inbreds from Eastern Croatia using microsatellites(8). But it is slightly lower than the 4.47 alleles obtained by Pabendona et al. (2009), in a study of the genetic diversity of thirty nine Indonesian maize accessions(13). Beyene et al. (2005) reported an average of 4.9 alleles per locus in a study which included 62 traditional Ethiopian highland maize accessions(3). The mean value of the present study, however, was higher than the 3.33 average alleles per SSR locus as reported by Shiri (2015) for thirty-eight Iranian maize hybrids(20). Such considerable differences in the number of detected alleles may arise from differences in: (i) the diversity and number of genotypes tested and; (ii) the number and diversity of SSR primers examined. The average PIC value for the 20 SSR was 0.65; the lowest value was found in the UMC2210 marker(0.37), which corresponds to the fact that this locus showed only 3 alleles, while the highest value was obtained by the NC009 marker(0.82), a dinucleotide repeat that allowed the amplification of 7 alleles. BNLG1108(0.78),

Table 3. Loadings of PCA for the measured morphological traits of maize hybrids

Traits	PC1	PC2	PC3	PC4	PC5	PC6
Grain yield	-0.21	0.41	0.06	0.68	0.19	-0.10
Number of kernels per row	0.23	-0.22	0.09	0.57	0.15	-0.49
Kernel row number	-0.23	0.23	-0.03	0.24	-0.73	-0.05
1000-kernel weight	-0.07	0.33	-0.01	0.15	0.85	-0.22
Grain hectoliter weight	-0.63	0.13	0.41	0.24	-0.20	0.13
Kernel depth	-0.25	-0.19	-0.13	0.82	-0.08	-0.03
Plant height	-0.30	0.21	0.45	-0.01	-0.04	0.72
Ear height	0.57	-0.01	0.08	-0.17	0.11	0.75
Stem diameter	-0.20	-0.71	-0.15	0.23	-0.24	0.17
Number of leaves above the ear	-0.21	-0.03	0.87	-0.16	-0.03	0.13
Number of leaves below the ear	-0.10	0.86	-0.16	0.10	-0.10	0.32
Total number of leaves per plant	-0.24	0.65	0.55	-0.04	-0.10	0.35
Leaf chlorophyll rate	0.45	0.09	0.58	0.55	-0.10	-0.02
Days to 50% emergence	0.01	0.08	-0.17	0.45	0.63	0.21
Days to 50% silking	0.91	-0.18	-0.23	-0.01	0.02	0.07
Days to 50% anthesis	0.93	0.13	0.06	0.05	0.07	0.08
Anthesis-silking interval	-0.04	-0.65	-0.61	-0.12	-0.10	-0.02
Days to 50% maturity	0.92	0.05	-0.01	-0.18	0.01	-0.21
Eigenvalues	3.92	2.62	2.32	2.28	1.88	1.78
Proportion	21.80	14.58	12.90	12.66	10.42	9.89
Cumulative	21.80	36.38	49.28	61.94	72.36	82.25

Table 4. Allele numbers, Effective Allele numbers, Bin location, Polymorphic index content (PIC), Shanan Index, Nei Index and Motif for SSR markers

Primers	Motif	Bin Location	Allele No.	Effective Allele No.	PIC	Shanan and Nei Index
BNLG1643	(AG)24	1.08	4	3.49	0.71	1.31, 0.71
BNLG 1016	(AG)20	1.04	4	2.46	0.59	1.05, 0.59
MMC0401	(GGA)2, (AG)27	2.05	3	2.40	0.58	0.95, 0.58
BNLG1335	(AG)21	2.07	2	1.88	0.47	0.66, 0.47
BMC2136	(CA)31	3.04	2	2.00	0.50	0.69, 0.50
BNLG1108	(AG)21	3.08	5	4.60	0.78	1.57, 0.78
PHI026	(CT)	4.05	5	4.40	0.77	1.54, 0.77
BNLG1890	(AG)26	4.11	6	3.36	0.70	1.45, 0.70
BNLG105	-	5.02	3	3.00	0.67	1.10, 0.67
BNLG609	-	5.06	5	3.67	0.73	1.42, 0.73
NC009	(AG)	6.04	7	5.56	0.82	1.80, 0.82
UMC1006	(GA)19	6.02	4	3.09	0.68	1.23, 0.68
BNLG155	-	7.03	4	3.20	0.69	1.26, 0.69
PHI057	GCC	7.01	5	4.22	0.76	1.53, 0.76
BNLG1194	(AG)33	8.01-02	6	4.52	0.78	1.62, 0.78
UMC2210	(AAAAT)4	8.05	3	1.59	0.37	0.68, 0.37
BNLG619	-	9.07-08	3	2.06	0.51	0.85, 0.51
BNLG127	-	9.03	5	3.40	0.71	1.32, 0.71
PHI084	GAA	10.04	4	2.21	0.55	0.89, 0.55
BMC1152	(AG)24	10.02	4	3.11	0.68	1.18, 0.68
Mean	-	-	4.2	3.21	0.65	1.20, 0.65
Sd	-	-	1.32	1.06	0.12	0.34, 0.12

BNLG1194(0.78), PHI026(0.77) and PHI057(0.77) primers had the highest PIC after NC009. These values were similar to those found by Xu et al. (2004), they reported PIC values of 0.28 to 0.81 with a mean value of 0.63 in the fifteenth Chinese maize inbred lines (25). Smith et al. (1997) also reported a mean PIC value of 0.62 for SSR in a collection of US maize inbreds (23). The average PIC value obtained in this study was lower than maize landraces from Japan(0.69) using 60 SSRs (5) and higher than maize landraces from India(0.60) using 42 SSRs(20), Ethiopia(0.61) using 20 SSRs(3),US maize inbreed lines(0.62) using 131 SSR(23) and Iranian maize inbreed lines (0.54) using 131 SSR(4).

Regarding the importance of PIC for primer efficiency, five primers -NC009, BNLG1108, BNLG1194, PHI026 and PHI057- were the most informative primers and thus could be used to assess the diversity of maize genotypes.

Principal coordinate analysis for studied maize hybrids based on SSR markers

Principal coordinate analysis was used to explain genetic variation and show the variation pattern in a multidimensional pattern and to provide a better interpretation of the relationship between individuals (9). The relative variance of each coordinate indicated the importance of the related coordinate of total variance which was expressed as a percentage. The data obtained using 20 SSR primers were used in principal coordinate analysis with simple matching coefficients of similarity. Principal coordinate analysis (PCoA) on a similarity matrix of hybrids showed that the first 15 coordinates explained 97.21 % of the total variance, whereas the first two coordinates explained only 33.14% of the total variance. The first coordinate explained 17.50% and the second one explained 15.64% of the total variance (Table 5). A scatterplot of hybrids was constructed based on the first two main coordinates. Maize hybrids were grouped into six different clusters according to their similar characteristics in the PCoA biplot (Fig. 2).

Cluster analysis of studied maize hybrids based on SSR molecular data

The cluster analysis, using complete linkage method based on Jaccard dissimilarity criteria, was conducted for measuring genetic diversity and relatedness among the studied hybrids (Fig. 3). The studied maize hybrids were grouped into seven clusters, indicating the existence of considerable genetic diversity among 20 maize hybrids. The highest genetic distance was found between two hybrids, KSC705 and KENDRAS, where they held the first and last positions of the dendrogram. The KSC705 was derived from the Iranian Germplasm and the KENDRAS was derived from the German-Italian one. On the other hand, the lowest genetic distance was found between the maize hybrids KERMESS and KALIMERAS in the same group. These two hybrids originated from the German-Italian.

Four of the studied hybrids, including KENDRAS, KALIMERAS, KERMESS and KUADRO were located in group I, originated from the German-Italian germplasm. Hybrids BC612, BC712 and BC582 formed the cluster II. All of the hybrids of this cluster were derived from the Yugoslavia-Croatian gerplasm. Hybrids namely K166B×K19 and XTO3×A679 comprised the cluster III that were developed in Iran. Only two hybrids, HIDO and MAY-70, formed the cluster IV, which were developed in Turkey. Hybrids DKC6315 and DKC6589,

Table 5. Eigen values, variance and agglomerative variance of PCoA for the SSR data

Coordinates	Eigen values	% of Variance	Agglomerative variance %
1	13.82	17.50	17.50
2	12.36	15.64	33.14
3	8.62	10.91	44.05
4	6.61	8.37	52.42
5	6.35	8.04	60.46
6	4.88	6.18	66.64
7	4.04	5.12	71.76
8	3.86	4.88	76.64
9	3.56	4.51	81.15
10	2.93	3.71	84.86
11	2.70	3.42	88.28
12	2.27	2.88	91.16
13	2.16	2.73	93.89
14	1.59	2.01	95.90
15	1.04	1.31	97.21

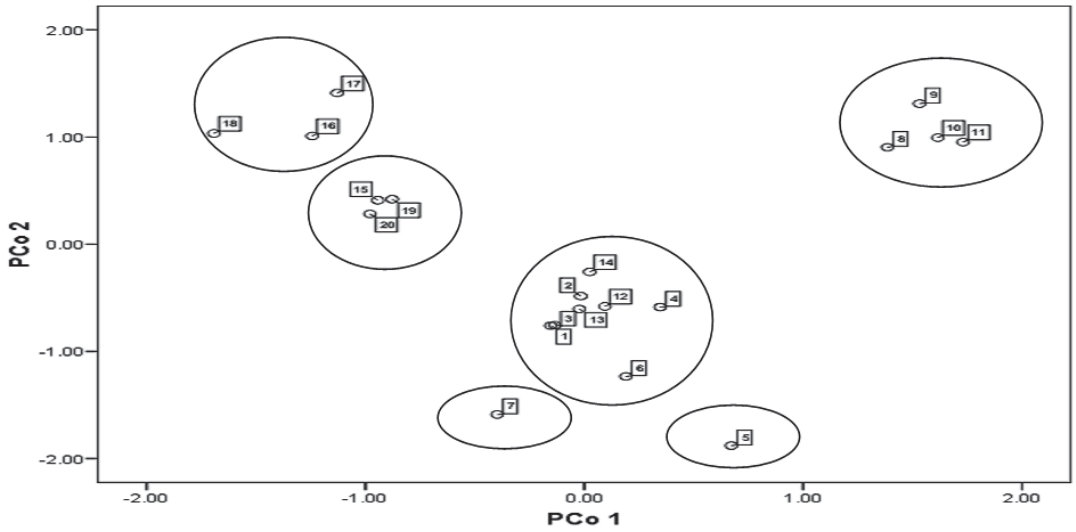

Figure2. PCO grouping of 20 maize hybrids

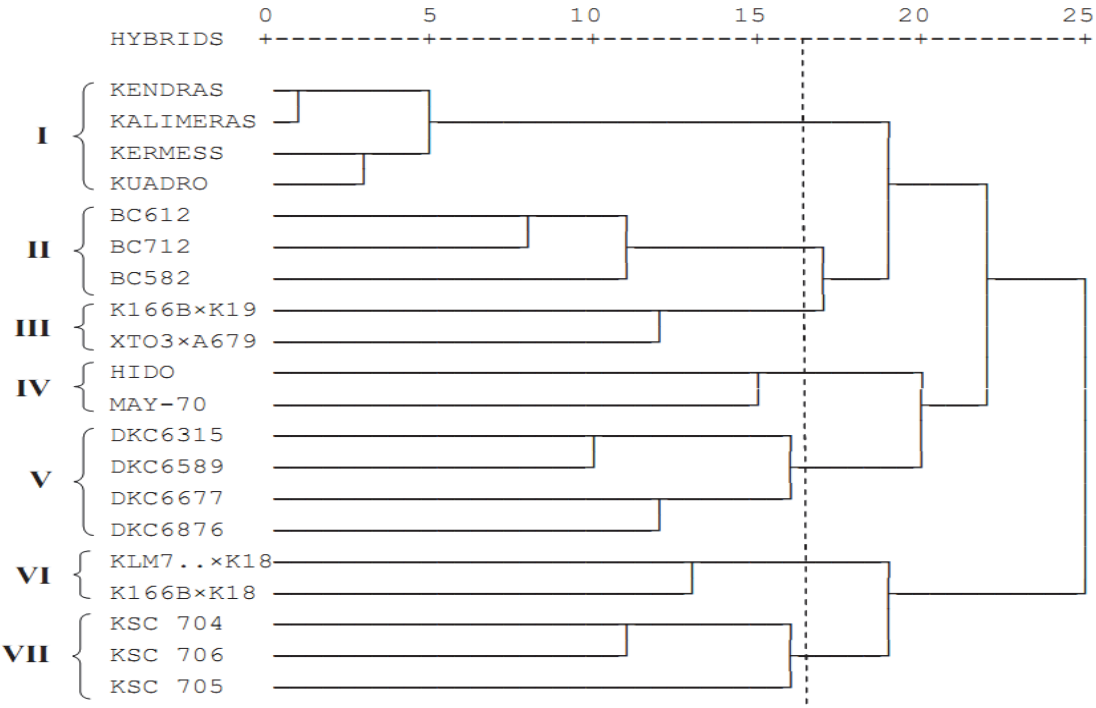

Figure3. Dendrogram of 20 maize hybrids constructed using CLINK cluster analysis of Jaccard dissimilarity values obtained by SSR markers.

DKC6677 and DKC6876, derived from American germplasm, formed the cluster V. Two Iranian hybrids named KLM77007/7-2-6-3-1-2-1×K18 and K166B×K18 and three Iranian hybrids named SC704, SC705 AND SC706 formed cluster VI and VII, respectively (Fig. 3). The results obtaniaed form grouping the studied maize hybrids by PCoA biplot, had close similarity with results of cluster analysis(Fig. 2 and 3). So, SSR marker could detect the differences and similarities among hybrids very well and hybrid clustering was in full conformity with the origin of their developments. The results of the present study showed that hybrids are clustered according to their geographic origin using SSRs more than morphological markers.

CONCLUSION

The results of this study showed that variability was exist for the most of morphological traits. The comparison of morphological and molecular characterization data is ofhave an immense importance for estimating the extent of genetic diversity present in the set of genotypes. In addition, although morphological trait analysis is a useful tool in studying the genetic differences reflected by phenotypic expression, its results may not always reflect the real genetic variation because of genotype×environment interactions and the unknown genetic control of polygenic morphological and agronomic traits (22). Furthermore, the characterization of genotypes based on polymorphisms at the DNA level with molecular markers is a powerful tool for the estimation of genetic divergence (7).In the present study, among the 20 polymorphic SSR markers, 18 polymorphic SSR markers exhibited PIC values higher than 0.5, thereby suggesting their suitability for genetic diversity studies. Generally, in both SRR and morphological analysis, 20 maize hybrids were classified into different groups. However, some differences could be found between two dendrograms. Since morphological variation is influenced by environmental conditions, good accuracy levels can be achieved by the application of molecular markers for grouping the genotypes. The present study revealed that SSR markers could be successfully utilized for inferring genetic diversity and genetic relationships among a variety of maize genotypes.

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تجزیه و تحلیل تنوع ژنتیکی هیبریدهای ذرت با استفاده از صفات مورفولوژیکی و نشانگرهای SSR

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چکیده

مقایسه روش‌های مختلف تخمین تنوع ژنتیکی می‌تواند سودمندی آنها در برنامه‌های اصلاح نباتات را تعیین کند. در این تحقیق از ۱۸ صفت مورفولوژیکی و ۲۰ جایگاه توالی تکراری ساده (SSR) برای مطالعه تنوع ژنتیکی و مورفولوژیکی بین ۲۰ هیبرید ذرت انتخاب شده از کشورهای مختلف و دسته‌بندی هیبریدها بر اساس پروفایل‌های مولکولی و صفات مورفولوژیکی استفاده شد. برای جمع‌آوری داده‌های مورفولوژیکی، یک آزمایش مزرعه‌ای در قالب طرح بلوک کامل تصادفی با سه تکرار در مغان، اردبیل، ایران انجام شد. بیشترین مقدار ضرایب ژنتیکی تنوع در فاصله زمانی گل تاجی تا کاکل و سپس در عملکرد دانه، میزان کلروفیل برگ، تعداد ردیف دانه و ارتفاع بلال مشاهده شد. تعداد کل محصولات واکنش زنجیره‌ای پلیمراز ۸۴ باند بوده و همه آنها چندشکل بودند. در میان آغازگرهای مطالعه شده، NC009، BNLG1108، BNLG1194، PHI026 و PHI057 حداکثر محتوای اطلاعات چندشکلی (PIC) و بیشترین تنوع را نشان دادند. برای تعیین روابط ژنتیکی میان هیبریدهای ذرت، تجزیه و تحلیل خوشه‌ای بر اساس صفات مورفولوژیکی (با استفاده از روش Ward) و نشانگرهای SSR (با روش CLINK) انجام شد. تجزیه و تحلیل خوشه‌ای صفات مورفولوژیکی هیبریدهای ذرت را به پنج گروه تقسیم کرد. بر اساس نشانگرهای SSR این هیبریدها در ۷ گروه دسته‌بندی شدند. تجزیه و تحلیل مختصات اصلی (PCoA) ماتریس شباهت حاصل از داده‌های SSR نشان داد که ۱۵ مولفه اول ۹۷/۲۱ درصد کل واریانس بین هیبریدها را تبیین می کنند، درحالیکه دو مولفه اول تنها ۳۳/۱۴ درصد کل واریانس را تبیین می‌کنند. بطور کلی، نتایج نشان داد که نشانگرهای SSR نسبت به صفات مورفولوژیکی ازکارایی بیشتری برای دسته‌بندی هیبریدهای ذرت خویشاوند برخوردار می‌باشند.

کلمات کلیدی: صفات زراعی، روابط ژنتیکی، SSR، هیبرید ذرت