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Phenotypic and Genetic Diversity and Their Relationship to F_1 Performance For Yield Traits in Some Maize Inbred Lines

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ABSTRACT

The present study of this investigation was aimed to use RAPD markers and phenotypic distances based on yield traits to assess the genetic diversity among six inbred lines (three Egyptian and three American inbred lines) widely used in maize breeding programs and to evaluate the association of the genetic diversity with their F_1 performance. Five primers succeeded to evaluate six inbred lines of maize using RAPD technique. This technique was efficient in detecting polymorphism with an average of 91.3% and determining genetic diversity among the six studied inbred lines. But they were not effective enough to distinguish some inbred lines by unique markers. The molecular distances and phenotypic distances were found to range from 0.355 to 0.600 and from 27.37 to 164.46, respectively. According to cluster analysis and principal coordinate analysis, the parental inbred lines divided into three and two groups based on molecular and phenotypic distances**,** respectively. Also, using principal coordinate analysis based on two types of distances observed that the separation between the American inbred lines was higher than separation between the Egyptian inbred lines. On the other hand, poor correlation (r = 0.297) among molecular and phenotypic distances were found. This poor correlation also found for two types of distance with specific combining ability and mid parents heterosis and better parent heterosis. Coefficient of determination for F_1 performance against molecular distance and phenotypic distances were extremely low and ranged from 0.0002 to 0.189 and from 0.0001 to 0.351, respectively. This result demonstrated the reliability impairment of regression models in predicting for F_1 performance.

Keywords: Maize, Genetic Diversity, RAPD, Molecular Distance, Phenotypic Distance, Principal Coordinate Analysis, Heterosis, Combining Ability.

INTRODUCTION

Maize,(*Zea mays* L.) is one of the most economically important crops and the third nutrition crop after rice and wheat all over the world (Frova *et al.*, 1999) . Considering maize in Egypt, it is among the major cereal crops, where occupies third place after wheat and rice. Maize is very essential either for the human food or animal feeding and a common ingredient for industrial products. Also, maize is used as a feed for livestock whether fresh, silage or grains. The maize grains also have many industrial uses, including transformation into plastics and fabrics. Thus, there is a critical need to increase the production of maize to face the gap between production and consumption. In this respect, National Maize Research Program, breeders and geneticists who are interested in maize improvement need conclusive information related to the identification of inbred lines, single and three-ways crosses. The need for varietal identification or verification of varietal identity arises throughout the sequence of events from breeding, through variety release, pure-seed multiplication, varieties registration and sowing, seed quality control, processing of the harvested grain and marketing.

The introduction of Plant Breeder's Rights has brought even more exacting requirements for genotype and distinctness testing in seed certification (Cooke,1999). To achieve this goal, it is essential to use stable international technique that will identify morphological characters at different growth stages. The international reorganization descriptor of UPOV, (2009) was followed to differentiate between the tested inbred lines, cross and three-ways crosses. Since morphological attributes may be influenced by genotype environment and traditionally, morphological comparisons formed the basis of genetic purity evaluations, but this is expensive and unreliable, and cannot provide information on the purity of specific genetic attributes that relate to grain quality of the variety (Baird *et al.*, 1995). This makes the development of new techniques for genetic purity determination and identification even more essential.

As one of the genetic markers developed in the 1980's, the use of isozymes for identification of varieties is now a mature technology. The past restrictions related to pedigree data, physiological, morphological and cytological markers for assessing genetic diversity in cultivated and wild plant species have largely been circumvented by the development of DNA markers such as restriction fragment length polymorphisms (RFLPs)(Botstein *et al.*, 1980), random amplified polymorphic DNAs (RAPDs) (Williams *et al.*, 1990) as well as simple sequence repeats (SSRs), microsatellites (Tautz, 1989). RAPDs technique has great benefits, since no prior information about the target sequences is required for the design of primers requires just nanogram quantity of DNA, results are immediately understand through agarose gels, as well as the whole genome is screened(Williams *et al.*, 1990). Even so, the nature of DNA sequences involved in RAPD fragments is little known, as many different types of sequences are most likely involved (Schierwater, 1995). These techniques have been used to reveal the relationship between molecular distances and heterosis in rice, maize, wheat and etc (Bernardo, 1992; Zhang *et al.*, 1994; Diers *et al.*, 1995; Liu *et al.*, 1999; Benchimol *et al.*, 2000). Also, phenotypic

distances were calculated between plants by applying the Euclidean metric of Excoffier *et al., (*1992) based on morphological traits can be used as a measure of genetic diversity. As well as, Principal Coordinate Analysis (PCoA) was used for the purpose of estimating the genetic similarity and quantitative variation in crops (Rahim *et al.,* 2008; Sesli and Yeğenoğlu 2010). This analysis was discriminated the genotypes into various groups based on the geographic origin comparatively in a better way than the UPGMA clustering (Selvaraj *et al.* 2010). Genetic diversity in relation to hybrid performance was studied in several crops. The performance of hybrid can be reflected by three parameters viz., mean value of hybrids, heterosis and specific combining ability.Therefore, there is a requirement to study all these parameters with genetic diversity for best perception for these relationships. (Soni and Khanorkar, 2013).

Hence, the present study was aimed to use Molecular Distance (MD) based on RAPD analysis and Phenotypic Distances based on yield traits to assess the genetic diversity among six maize inbred lines widely used in maize breeding programs and to evaluate the association of the genetic diversity with F_1 performance for hybrids were obtained from single crosses between the six studied maize inbred lines.

MATERIALS AND METHODS

The present investigation was carried out at the Experimental Station Farm, Faculty of Agriculture, Mansoura University, Dakahlia Governorate during the two successive maize growing seasons of 2012 and 2013. Six parental inbred lines of maize (*Zea mays* L) were used in this investigation. Three Egyptian inbred lines R39, Inb1021 and Inb1004 as well as, three American inbred lines P97, B73 and Oh43. Sources and characters of parental inbred lines are presented in Table 1.

In 20th June 2012 growing season, the seeds of all parental inbred lines were sown and the growing plants were crossed according to a half diallel crosses mating design to obtain 15 F_1 single crosses. In 10th June 2013 growing season, all 21 genotypes which included six parental inbred lines and their 15 F_1 single crosses were cultivated using the dry method (Afir) in rows. The experimental design was randomized complete block design with three replicates. One row which is the three meter long and contains 12 plants is repeated 3 times, the distance between the plant and other 25 cm and between each row and other one 70 cm. Data from all genotypes were recorded on three plants chosen at random from each plot for yield traits: Number of rows per ear (Nr/e); Number of kernels per row (Nk/r); 100-Kernel weight (100-Kw(g)), Grain yield per plant (Gy/p(g)) and shelling percentage (S%). Specific combining ability (SCA) was recorded according to Griffing's Approach, method-4, model-1 (fixed effects) of Griffing (1956). Estimates of heterosis (%) were computed as the percent deviation of F_1 mean performance over that either mid or better parent according to Mather and Jinks (1982).

Total DNA was extracted from fresh leaf tissue using the Gene-JET Plant Genomic DNA Purification Mini Kit (#0791) from company (Thermo Scientific). Genomic DNA was used as a template for PCR (Polymerase Chain Reaction) amplification using five randomly selected primers (Operon Technology, USA) (Table 2). The amplification was performed in a System PTC 200 Bio-Rad cycler.RAPD reactions were done in a volume of 15 µL containing 1x PCR buffer (75 mM Tris-HCl pH 9.0, 50 mM KCl, 2.0 mM $MgCl_2$, 20 mM $(NH_4)_2SO_4$, 0.3 mM of each dNTP (dCTP, dGTP,dTTP, dATP), 0.4 µM of 10-mer primers, 0.7 U of Taq-polymerase (Biotools), and 20 ng of template DNA. Amplifications were carried out in a PTC-100 with the following program: 1 initial denaturation step at 94 °C for 2 min followed by 47 cycles at 94 °C for 1 min, 38 °C for 1.45 min, and 72 °C for 2 min and a final cycle at 72 °C for 7 min. The amplified products were separated by electrophoresis in1.4% agarose gel in 1 x TAE buffer (Tris-acetate 0.04 M and EDTA 0.01 M pH 7.5), containing 0.15 µg/µL of ethidium-bromide (Carvalho *et al.*, 2004). 5 µL of amplified products and 5 µL sterile water in a 0.2 mL micro centrifuge tube along with 2µL of gel tracking dye. Run the gel for (20 min.)at (100v). Gel photography were carried out using the Bio-Rad gel documentation system.

Table 1: Names, sources and characters of the maize six parental inbred lines.

No.	Name	Sources	Grain color	Grain type	Cob color
1	R39	Egyptian inbred line	Red	Flint	White
2	P97	American inbred line	Yellow	Dent	Red
3	B73	American inbred line	Yellow	Dent	Red
4	Inb1021	Egyptian inbred line	Yellow	Flint	White
5	Inb1004	Egyptian inbred line	Yellow	Flint	White
6	Oh43	American inbred line	Yellow	Dent	White

Table 2: Names and sequences for the five randomly selected primers that were successful in generating reproducible and reliable amplicons.

Name	Sequence (5´→ 3´)
0P- A03	AGTCAGCCAC
0P- B05	TGCGCCCTTC
0P-B09	TGGGGGACTC
0P- B11	GTAGACCCGT
0P- B16	TTTGCCCGGA

Molecular data obtained by RAPD experiments were analyzed by Gel Analyzer (version 3) program. These DATA scoring clear amplification products (amplicons) as present (1) or absent (0) for each primer and entered in the form of a binary data matrix. Cluster analysis by Molecular Distances (MD) (de Souza *et al.*, 2012) and Principal Coordinate (PCo) analysis of the binary data were performed by computational package MVSP (version 3.1) using Nei & Li coefficients (Nei and Li, 1979). Also, using the same program (Kovach, 2001) cluster analysis by Euclidean Phenotypic Distances PD and PCo analysis were performed based on yield traits data according to Nei, (1987).

Regression analysis and simple correlations were used to explain relationships between Molecular Distances (MD) and Phenotypic Distances (PD) and also with specific combining ability (SCA), heterosis over mid–parents (H_{MP}%) and heterosis over better parent (H_{BP}%) for yield traits (Rizkalla *et al.*, 2012 and El-Zanaty *et al.*, 2013). Coefficient of determination (R^2) was used to test the reliability of the regression models. An R^2 closer to 1.00 revealed more reliability.

RESULTS

Data of the amplified fragments of DNA (amplicons) using those five 10-mer randomly primers succeeded for evaluating six inbred lines of maize (*Zea mays* L) (Fig. 1 and Table 3). In total, five primers amplified 35 amplicons ranged from 114 bp (0P-B09) to 1064 bp (0P- A03). Among these; 32 amplicons (91.3%) were polymorphic. On average, seven amplicons per primer were observed with maximum of eight and minimum of six amplicons. The primers 0P-B11 and 0P- B16 were found to produce 100 % polymorphic fragments and the lowest polymorphism (83.3%) was exhibited by primer 0P- B05 . Inbred lines specific markers generated from RAPD-PCR analysis are presented in Table 3. Thirteen out of 35 amplicons were found to be useful as unique markers (eight positive and three negative unique markers). All primers generated various unique markers, the highest number of unique markers was generated by primer 0P- B11 (three positive and one negative unique markers), while the lowest number (one positive unique markers) was generated by primers 0P- B05. On the other hand, the largest number of unique markers was scored for American inbred lines (eight unique markers). American inbred line Oh43 was identified by the highest number of positive unique markers (3) and one negative marker. R39 is the only Egyptian inbred line which showed unique markers (two positive and one negative unique markers), while Inb1021 and Inb1004 did not show unique markers.

Range of estimates that indicate the performance of the parental inbred lines and their single crosses (Table 4) according to Soni and Khanorkar, (2013). The values of H_{MP}, H_{BP} % and SCA showed variation from trait to another. The highest significant values of H_{MP}% and H_{BP}% for grain yield per plant were 562.2%, 462.2% respectively. While, the lowest to number of rows per ear (-7.7 and -14.3 % respectively). As well as, the highest significant positive and negative values of SCA effect for grain yield per plant were 66.5 and -33.6, respectively while, the SCA ranges for the other traits were close together.

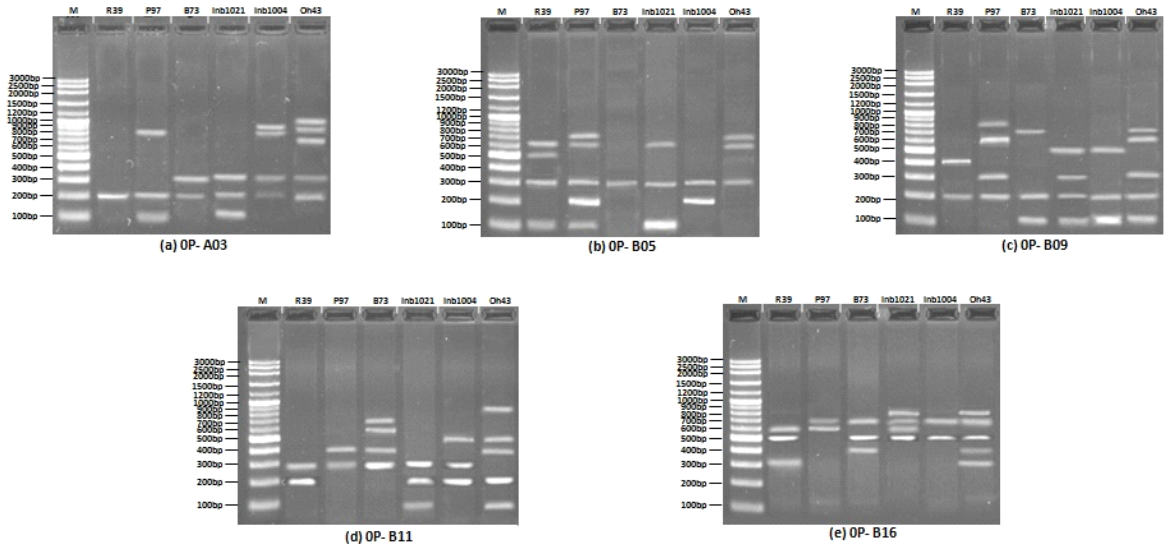

Figure 1: RAPD -PCR products of six accessions of the maize produced with five primers as listed in Table 3, lane M is 3 kb ladder and lanes 2 to 7 represent six parental inbred lines of maize accessions as listed in Table 1.

Table 3: Various parameters to efficiency of the five randomly selected primers for RAPD analysis in the six studied maize parental lines

Primer	Mol. size range (bp)	Monomorphic bands	Number of polymorphic bands polymorphic	Unique marker Unique (+)	Unique (-)	Grand Total	Total Number of band	Polymorphism (%)
0P- A03	115-1064	1	4	2 Oh43 (673, 1064 bp)	--	2	7	85.7
0P- B05	117- 727	1	4	1 R39 (478 bp)	--	1	6	83.3
0P-B09	114-845	1	5	2 R39 (380 bp) P97 (845 bp)	--	2	8	87.5
0P- B11	117-910	-	4	3 B73 (568, 720 bp) Oh43 (910 bp)	1 Oh43 (275 bp)	4	8	100.0
0P- B16	287-840	-	4	--	2 R39 (697 bp) P97 (477 bp)	3	6	100.0
Total		3	21	8	3	11	35	
			Mean				7	91.3

Table 4: Range of the mean values (MV) of parental inbred lines (P) as well as their single crosses (C), heterosis over mid parent (H_{MP}%), heterosis over better parent (H_{BP}%) and specific combining ability (SCA) for yield traits.

Traits	Range MV	H_{MP}%	H_{BP}%	SCA
Nr/e	P) 12.0 to 16.0 C) 12.0 to 20.0	-7.7 to 53.9**	-14.3 to 42.9**	-3.5** to 2.2*
Nk/r	P) 14.0 to 30.0 C) 32.3 to 41.0	37.8** to 143.4**	7.8 to 123.9**	-3.1** to 2.2*
100-Kw(g)	P) 12.5 to 18.1 C) 19.8 to 30.9	35.1** to 115.8**	23.1** to 105.1**	-2. 3** to 2.9**
Gy/p(g)	P) 27.8 to 121.7 C) 133.7 to 287.1	32.5* to 562.2**	21.8 to 462.2**	-33.6** to 66.5**
S %	P) 67.1 to 84.1 C) 75.4 to 90.7	-6.7** to 17.2**	9.3** to 8. 5**	-4.9** to 6.2*

***, **Significant at 0.05 and 0.01 levels probability, respectively**

The results presented in Table 5 and Fig. 2 showed that Molecular distance (MD) matrix and UPGMA clustering dendrogram based on binary data. The Molecular distance (MD) were found to range from 0.355 to 0.600 for studied inbred lines. The lowest MD was 0.355 reported between Inb1021 and Inb1004 as they localized at the same sub-cluster (Fig. 2). While, the highest MD was 0.600 observed between R39 and Oh43 as they were found in different clusters. Also, these results indicated that the parental inbred lines divided into three groups with different degrees of MD. The first group (A) comprises the two inbred lines R39 and P97; the second group (B) is comprised by inbred line Oh43 only; and last group (C) comprises the three inbred lines B73, Inb1021 and Inb1004. The mean of MD recorded between all combinations of these three groups was 0.476 the highest mean MD was 0.513 between A×(B,C) combination, and the lowest mean was 0.463 between BxC combinations.

Table 5: Molecular distance (MD) matrix for studied parental inbred lines of maize based on RAPD markers.

MD	R39	P97	B73	Inb1021	Inb1004
P97	0.500				
B73	0.565	0.556			
Inb1021	0.379	0.394	0.429		
Inb1004	0.538	0.533	0.360	0.355	
Oh43	0.600	0.538	0.529	0.400	0.459

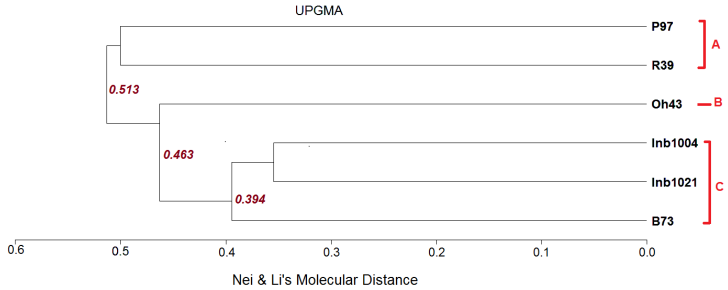

Figure 2: UPGMA clustering dendrogram showing relationship among the six parental lines of maize based on Molecular Distance (MD) according Vaillancourt *et al.*, 1995.

Also, phenotypic distances (PD) matrix and UPGMA clustering dendrogram for six parental lines based on yield traits are shown in (Table 6 and Fig. 3). Phenotypic distances (PD) based on morphological data were found to range from 27.37 (between R39 and Oh43) to 164.47 (between B73 and Oh43).On the other hand, the parental inbred lines divided into two groups A and B with mean of phenotypic distances was 118.83. The first group (A) included two subgroups(c) and (d), the first subgroup (c) involved the two inbred lines R39 and Oh43 as well as, the other subgroup (d) included the two inbred lines Inb1021 and P97. The second group (B) comprises the two inbred lines B73 and Inb1004.

As well as, the results of principal coordinate (PCo) analysis based on RAPD analysis and yield traits data are shown in (Figs. 4 and 5), respectively. In addition to the cluster analysis, explained by PCo analysis given clear indications for genetic diversity. The results showed that the first and second eigenvectors were 31.96 and 23.80% for PCo based on RAPD analysis and were 93.60 and 4.58% for PCo analysis based on yield traits data, respectively. Based on the breadth of the triangle connecting between the American inbred lines and comparing triangle connecting between the Egyptian inbred lines which indicates the degree of separation, the separation between American inbred lines P97, B73 and Oh43 was higher than separation between Egyptian inbred lines R39, Inb1021 and Inb1004.

Insignificant positive correlation (r = 0.297) was found among MD and PD are presented in Fig. 6. In agreement with this result, a poor correlation between molecular and phenotypic distances was found (Dillmann *et al.*, 1997; Sant *et al.*, 1999 and Yadav *et al.*, 2010).

Table 6: Phenotypic distances (PD) matrix for six studied parental inbred lines of maize.

PD	R39	P97	B73	Inb1021	Inb1004
P97	38.05				
B73	144.20	110.69			
Inb1021	42.21	41.02	118.24		
Inb1004	112.48	77.06	40.65	90.69	
Oh43	27.37	61.55	164.47	53.47	132.79

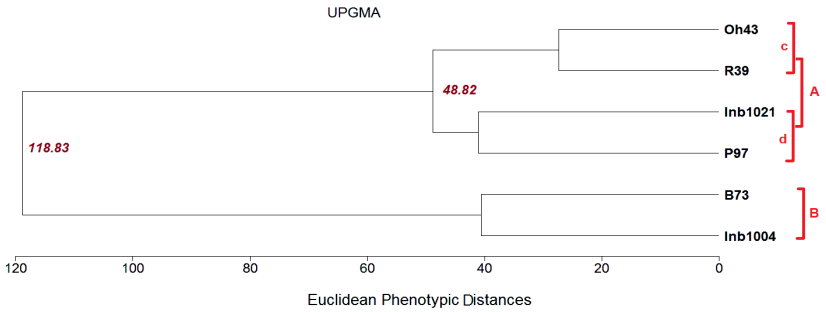

Figure 3: UPGMA clustering dendrogram showing relationship among six parental lines of maize based on phenotypic distances PD according to Sneath and Sokal, (1973).

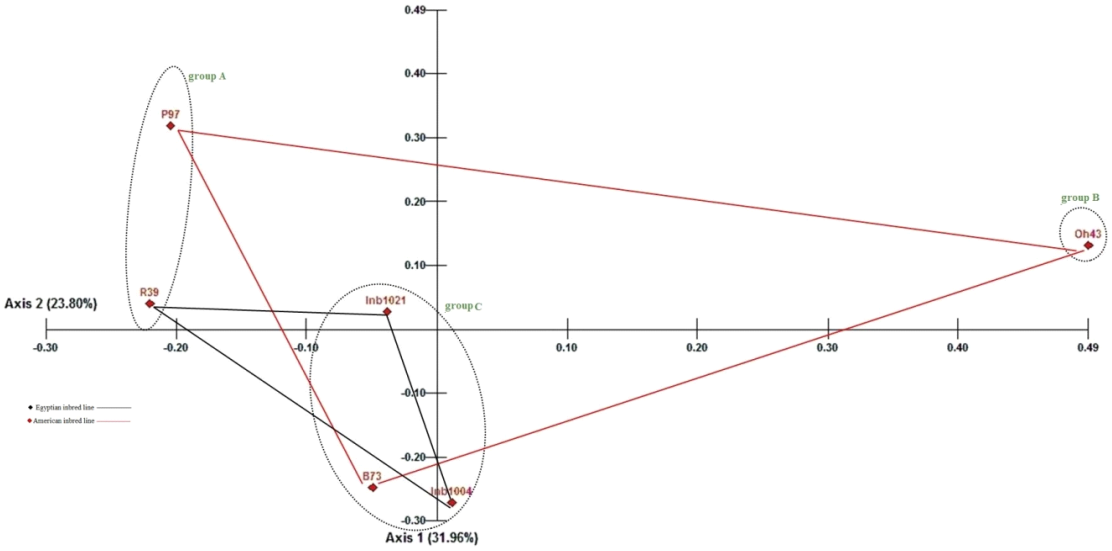

Figure 4: Principal Coordinate (PCo) analysis for six parental lines of maize based on RAPD analysis.

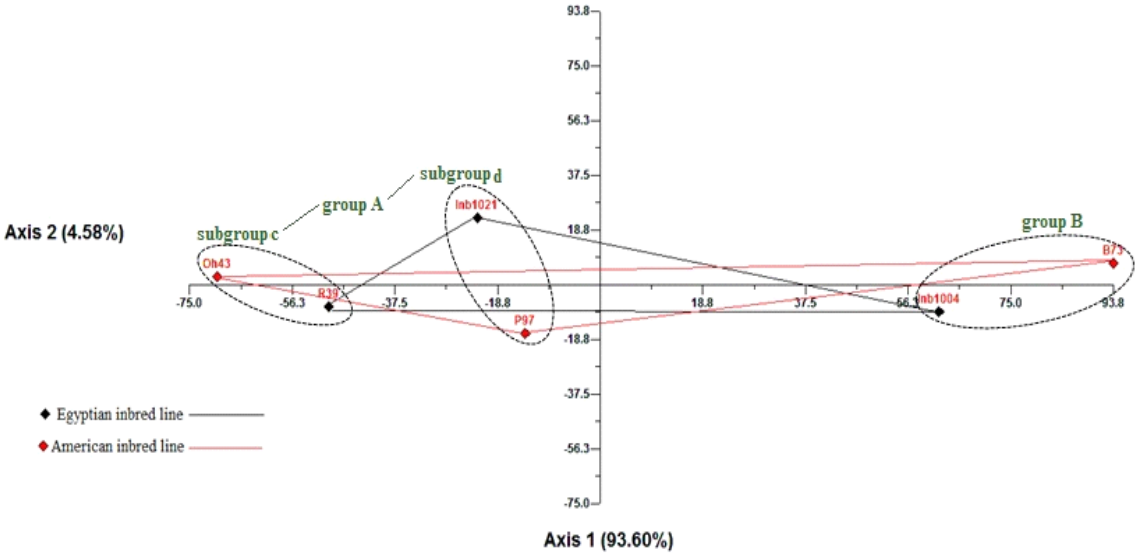

Figure 5: Principal Coordinate (PCo) analysis for six parental lines of maize based on yield traits

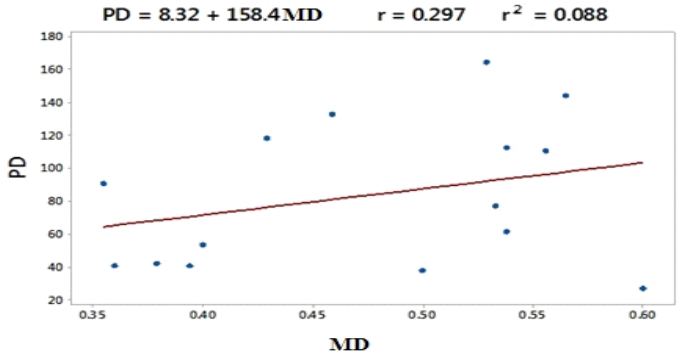

Figure 6: Relationship between molecular distance (MD) and phenotypic distances (PD).

The results were presented in Table 7 showed that correlation coefficients of MD with, SCA, H_{MP}% and H_{BP}% ranged from -0.097 (S% for H_{MP}%) to 0.351 (S% for H_{BP}%). These correlations were extremely low, positive and insignificant for all studied yield traits, except in S % traits for H_{MP}% which was extremely low, negative and insignificant. Also, correlation coefficients of PD with, H_{MP}%, H_{BP}% and SCA ranged from -0.593 (Gy/p(g) for H_{BP}%) to 0.442 (Nr/e for SCA). These correlations were low, negative and insignificant except in Nr/e, Gy/p(g), S% for SCA and Nr/e for H_{BP}% which were positive as well as, Nr/e,

Gy/p(g) for H_{BP}% were moderately significant (at 5% probability). Also, From the graphs (Fig. 7) indicated clear difference relationship between MD against heterosis (H_{MP} and H_{BP} %) and between PD against heterosis (H_{MP} and H_{BP}%), this difference was not clear for the relation of the two types of distances (MD and PD) with SCA. Coefficient of determination (R^2) of MD with, H_{MP}%, H_{BP}% and SCA ranged from 0.0002 (Nr/e for SCA) to 0.189 (Gy/p(g) for H_{MP}%) as well as, R^2 of PD with, H_{MP}%, H_{BP}% and SCA which ranged from 0.0001 (Nr/e for SCA) to 0.351 (Gy/p(g) for H_{BP}%).

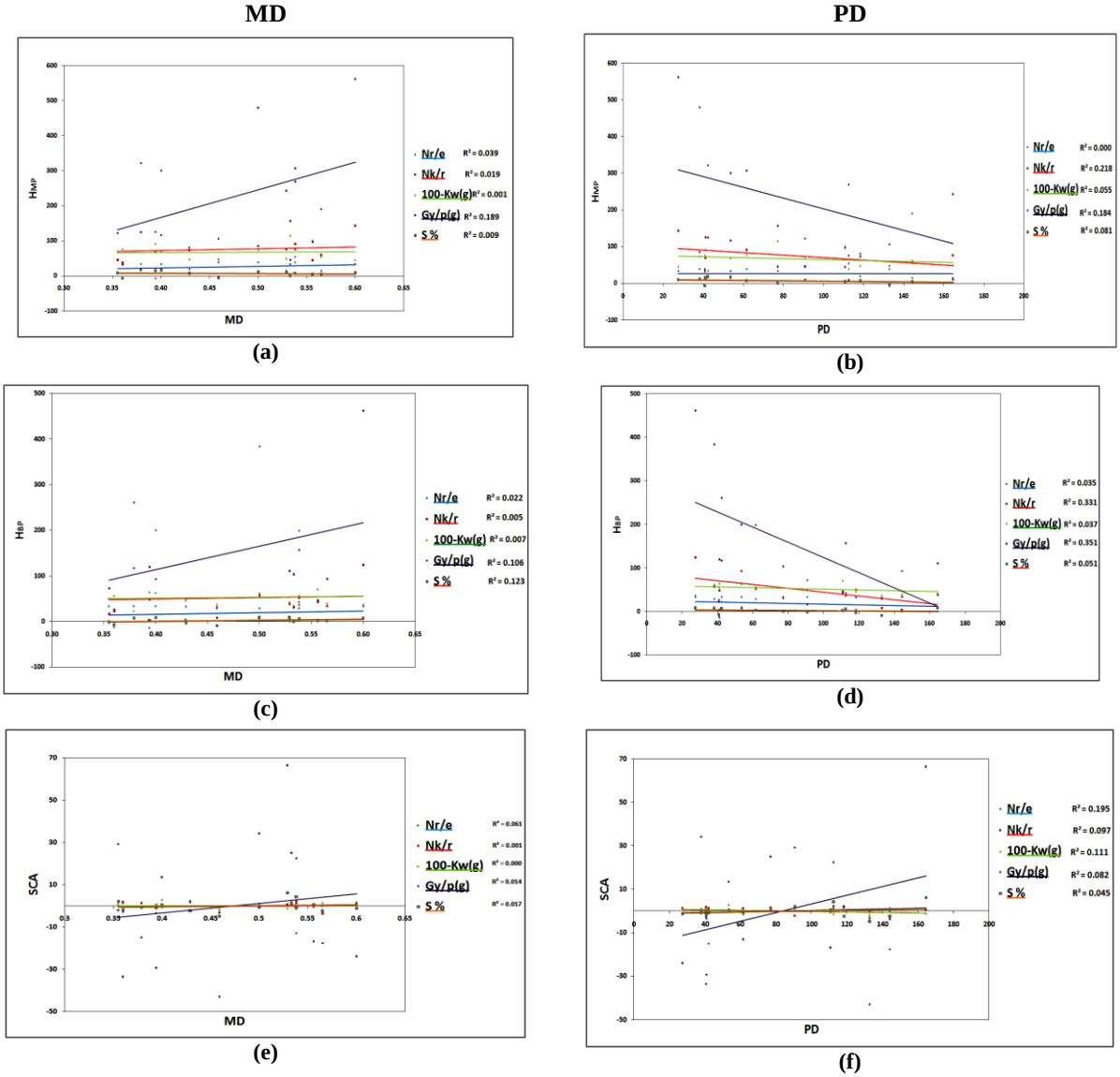

Figure 7: Correlations of heterosis over mid–parent H_{MP}% (a,b), heterosis over better parent H_{BP}% (c,d) and specific combining ability SCA (e,f) (Y-axis) against MD (a,c,e) or PD (b,d,f) (X-axis).

Table 7: Correlation coefficients (r) between molecular distance (MD) and phenotypic distances (PD) with specific combining ability (SCA) , heterosis over mid parent (H_{MP}%), and heterosis over better parent (H_{BP}%) for yield traits.

Yield traits	(Distance) D	D *vs.* SCA	D *vs.* H_{MP}%	D *vs.* H_{BP}%
Nr/e	**MD**	0.248	0.198	0.150
	PD	0.442	0.012	-0.188
Nk/r	**MD**	0.037	0.140	0.073
	PD	-0.312	-0.467	-0.576*
100-Kw(g)	**MD**	0.016	0.035	0.084
	PD	-0.334	-0.235	-0.194
Gy/p(g)	**MD**	0.121	0.435	0.326
	PD	0.287	-0.429	-0.593*
S %	**MD**	0.131	-0.097	0.351
	PD	0.213	-0.285	-0.226

*** Significant at 0.05 levels probability.**

DISCUSSION

The selected primers were used in this study succeeded in the production of various amplicons effective enough to reveal usable level of DNA polymorphism. But they were not effective enough to distinguish some inbred lines by unique markers. However, the obtained high level of polymorphism depends upon the degree of divergence between all studied inbred lines. This finding was in agreement with those stated by Bruel *et al.,* (2006).

The results presented in Table 5 and Fig. 2 indicated that the parental inbred lines divided into three groups with different degrees of molecular distance. The information about genetic diversity of maize genotypes is necessary for identifying diverse inbred lines combinations that result in segregating progeny with high genetic variability for selection. In this respect Lanza *et al.,* (1997) reported that RAPD technique can be used as a good alternative to determine genetic diversity in maize.

From data of PD and UPGMA clustering dendrogram based on MD (Table 6 and Fig. 3) it is clear that clustering of inbred lines based on PD using morphological data was sufficient to determine genetic diversity in maize. However, evaluating studied inbred lines in multi locations could make the method even more efficiency and give reliable results; because the discrimination ability increased with increasing heritability for traits.

PCo analysis based on RAPD analysis (Fig. 4) is more pronounced compared with PCo analysis based on yield traits data (Fig. 5). This result also suggests a relationship between the binary data matrix of RAPD analysis and yield traits data. On the other hand, the results of PCo analysis based on RAPD analysis and yield traits data were almost similar to that of UPGMA cluster analysis. Also, these results indicating that the studied inbred lines can be divided into the same groups in UPGMA cluster analysis (A, B, C for PCo analysis based on RAPD analysis and A_c, A_b, B PCo analysis based on yield traits data). Based on that, the cluster analysis was supported by principal components analysis (PCA). These results were in agreement with those obtained by Singh *et. al.,* 2015.

The absence of significant correlation among MD and PD (Fig.6) may be explained by the fact that (91.3%) of amplicons were polymorphic from any area of genome, including sequences without selection pressure, as in the case of sequences that do not code for any morphological trait (Joyce *et al.,* 1999). It is evident from (Fig. 6) that molecular markers can be used as grouping characters would by default, require acceptance of their use as distinguishing characters, at least for the different inbred lines. Therefore, the selection through molecular markers is considered the alternative way to deal with the poor correlation among molecular and phenotypic distances.

Results of correlation coefficients of MD with, SCA, H_{MP}% and H_{BP}% (Table 7) demonstrate the reliability impairment of regression models in predicting for F_1 performance. From the above mentioned data, the poor correlation between molecular and phenotypic distances with F_1 performance can be explained by the fact that hybrids had been evaluated at a one location, since the heterotic response of a gene pool does not depend on the distance between parents alone, but also on the adaptation to different environments (de Souza *et al.,* 2012).

CONCLUSION

The results of this study suggest that the RAPD technique was efficient in detecting polymorphism and determining genetic diversity among the six studied inbred lines. Nonetheless, the molecular distance did not correlate significantly with the phenotypic distance as well as, the two types of distance did not correlate significantly with the heterosis and specific combining ability for yield studied traits.

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التنوع الوراثى والمظهرى وعلاقته بأداء الجيل الأول لصفات المحصول فى بعض السلالات المرباه من الذرة الشامية

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تهدف هذه الدراسة إلى إستخدام معلمات RAPD الجزيئية والمسافات المظهرية المعتمدة على صفات المحصول فى تقييم التنوع الوراثى بين ستة سلالات من الذرة الشامية المرباه داخلياً(ثلاثة سلالات مصرية والثلاثة سلالات الأخرى أمريكية) والتى تستخدم بكثرة فى برامج تربية وتحسين الذرة وكذا تقييم إرتباط التنوع الوراثى مع آداء الجيل الأول الهجين بين هذه السلالات . وقد نجحت خمسة بوادئ فى إظهار التباين الجزيئى بين الستة سلالات من خلال تكنيك الـ RAPDوالذى أبدى كفاءة وضحت فى تقدير تباين حزم بمتوسط 91.3 %وإظهار التنوع الوراثى بين السلالات المختبرة ولكن هذه البوادئ لم تكن فعالة بشكل كافى فى تمييز بعض السلالات المرباه داخلياً من خلال إظهار معلمات متفردة فيها .تراوحت تقديرات المسافات الوراثية والمظهرية بين 0.335 و 0.600 وبين 27.27 و 164.46 على التوالى , ومن خلال التحليل العنقودى وتحليل التناسق الأساسى المعتمدان على المسافات الوراثية والمظهرية تبين أن السلالات الأبوية تنقسم إلى ثلاثة أقسام تبعاً للمسافات الوراثية وقسمين تبعاً للمسافات المظهرية. ومن خلال تحليل التناسق الأساسى لكلا النوعين من المسافات تبين أن الإنعزال الوراثى بين السلالات الأمريكية كان أكبر من الإنعزال الوراثى بين السلالات المصرية . ومن جهة أخرى تبين إنخفاض قيمة الإرتباط (r= 0.297) بين تقديرات المسافات الوراثية والمسافات المظهرية هذا الإرتباط الضعيف وجد أيضا بين نوعى المسافات مع القدرة الخاصة على التآلف وكذا مع قوة الهجين منسوباً لمتوسط الأبوين والأب الأفضل . كذلك فإن قيم معامل التقدير (R^2) المقدرة لأداء الجيل الاول مع المسافات الوراثية والمسافات المظهرية كانت منخفضة حيث تراوحت بين 0.0002 إلى 0.189 وبين 0.0001إلى 0.351 على التوالى لكلا النوعين من المسافات وهذا يشير إلى ضعف مستوى الإعتماد على هذه النتائج لتقديرات الإنحدار فى التنبؤ بآداء الجيل الاول الهجين .