Journal of Applied Biological Sciences 1 (2): 63–70, 2007

Estimating Genetic Variation among Dent Corn Inbred Lines and Topcrosses Using Multivariate Analysis

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Received : 21 October 2006
Accepted : 25 January 2007

Abstract

Identification of suitable parental lines and high yielding hybrids in maize breeding and genetics program is crucial. The aims of this research were to: (1) determine the genetic variation between dent corn inbred lines from diverse backgrounds and topcrosses created by crossing each inbred line with the tester line 'FrMo 17', by using multivariate analyses, and (2) identify appropriate parents and topcrosses for future breeding and genetics program. Field evaluations were conducted in two different environments, Samsun and Tokat, during 2001-2002 growing season. Tasseling time, plant height, ear height, ear length, row number per ear, grain number per ear, single ear yield, 1000 grain weight and total grain yield were evaluated. Based on the field evaluation results, inbred lines, H49, Y582A and Yildiz32, had relatively high yielding genotypes when compared to the other genotypes, yet their combining ability with the tester line was low. The topcrossess developed by using Akpinar55 and Yildiz32 genotypes with the tester line was also identified as relatively high yielding genotypes. The most similar inbred lines, revealed by D^2 multivariate distances, were B 87 and Pool 30a, while the topcrosses 496 x FrMo 17 and 504 x FrMo 17 were the most similar. On the other hand, the most different inbred lines were FrMo 17 and Pool 30 whereas the topcrosses were Pa.401.P x FrMo 17 and Akpinar 10 x FrMo 17. The inbreds Akpinar 55 and Yildiz32 will be used in maize genetics and breeding programs as parents.

Key words: Cluster analysis, maize breeding, heterosis, yield components

INTRODUCTION

Heterosis level of a hybrid mostly depends on the genetic variation among cultivars or populations from which inbred lines are developed [1, 2]. Identification of appropriate parental lines among inbred lines can be determined by topcrossing or diallel crossing methods. Multivariate analyses (i.e. discriminant and/or cluster analysis) can classify inbred lines in different groups. This classification may assist in the correct identifications of inbred lines as parents in hybrid breeding studies.

Determination of genetic variability of parental combinations is an important step for successful breeding and genetic programs. Single character evaluation by statistical analysis methods may cause incomplete and sometimes incorrect interpretations. Hence it is very important to analyze morphological, biochemical and/or molecular traits simultaneously. Principal component and discriminant analysis methods can be used for the combined analysis and provide more reliable conclusions in identification of genetic materials [3, 4]. Cluster analysis is another commonly used multivariate analysis method in identifying genetic variability [5]. Although cluster analysis is not statistically powerful since it does not use experimental error values and is not dependent upon any hypothesis, it still can analyze several factors simultaneously and provides different classes based upon similarity values.

Discriminant analysis and cluster analysis were used by several different research groups for identification of genetic variability in different crop species. Genetic variability among 32 Turkish pop, flint and dent corn races based upon 25 morphological and agronomic traits was evaluated using canonical discriminant analysis, and 68 % of total variation was determined to be provided by two canonical discriminant variants [6]. Similarly, canonical discriminant analysis and cluster analysis were used to determine water use models of 61 *Poa pratensis* L. cultivars [7], to reveal variations in ploidy levels of 56 hybrid potato cultivars [8] and to develop a phenotypic similarity index for soybean cultivars that originated from China and North America [9]. In addition, these methods were used to determine genetic variation in Chinese, South Korean and Japanese soybean cultivars [10], in annually grown Poa populations [11], and also to differentiate wild and domesticated soybean cultivars [12].

The aims of this study were to: (1) determine genetic variation of dent corn inbred lines from diverse background and topcrosses developed by crossing each inbred line with tester line (FrMo17) by using discriminant and cluster analyses, and (2) identify appropriate parents and topcrosses for future breeding and genetics program.

MATERIALS AND METHODS

Plant Material and Experimental Design

Thirty dent corn inbred lines (Table 1) from diverse backgrounds were used in this study. Thirty genotypes were

used to develop topcrosses by crossing each inbred line with the tester line, "FrMo 17". From this crossing effort, thirty topcrosses were developed (Table 2). The single cross hybrid cultivar 'TTM 813' was used as control. Experiments were established in Samsun (Lat. 36°20'E, long. 41°17'N, 4 m above sea level) and Tokat (Lat. 36°43'E, long. 40°19'N, 640 m above sea level) in 2001-2002 growing season. Inbred lines and topcrosses were planted in two separate experiments using completely randomized block designs with three replications. Each experimental plot included five meter long rows spaced 0.70 m apart, with 25 single-plant hills spaced 0.20 m apart. Plots were overplanted and thinned, obtaining a final density of approximately 71420 plants ha^{-1} in each experiment. The soil was silty-loam in Samsun and loam in Tokat. Fertilizers were applied as 220 kg N ha^{-1} and 100 kg P_2O_5 ha^{-1} in both locations. Half of the N was applied when the plants were 40-50 cm tall.

Table 1. Inbred dent corn lines.

Inbred lines			
Number		Number	
1	Fr 634	17	Pool 30
2	A 670	18	Pool 30
3	B 87	19	H 108
4	Fr 43	20	ALKD 187
5	H 49	21	Ada 1.3002
6	H 99	22	A 682
7	Mo 5	23	Akpınar 9
8	ND 300	24	Akpınar 10
9	ND 301	25	Akpınar 55
10	Pa 373	26	Yıldız 26
11	Pa 401 P	27	Yıldız 32
12	Pa 402 P	28	Yıldız 40
13	Pa 870	29	Yıldız 41
14	Y 582 A	30	Yıldız 50
15	496 W	31 (Tester)	FRMo.17
16	504 W		

Data were taken on tasselling time (days from planting to 50 % of plants tasselling), single ear yield (g), and grain yield (kg ha^{-1}). Plant height (cm), ear height (cm), 1000-kernel weight (g), number of kernels per ear, ear length (cm) and number of rows per ear were estimated from a sample of 10 plants from each plot.

Statistical Analyses

The univariate analysis of variance (PROC GLM) was used to evaluate differences between inbred lines and topcrosses, as well as to determine genotype by environment interaction. Following the analysis of variance, the protected Fisher's $LSD_{0.05}$ was calculated among inbreds and topcrosses. Variance components were computed with maximum likelihood estimates of PROC VARCOMP [13] to estimate trait heritability. The error estimate was computed using within inbred line and within F_1 topcross variance, respectively, that includes mainly the environmental variation. The variance components among genotypes (σ^2_G) and within genotypes (σ^2_e) have been computed for the whole set of traits. Phenotypic variance (σ^2_P) was obtained by adding genetic variance and environmental variance ($\sigma^2_P = \sigma^2_G + \sigma^2_e$). Heritability ($H^2 = \sigma^2_G / \sigma^2_P$) was computed for traits showing a significant variation (P <0,05).

Table 2. List of the topcrosses.

Topcrosses			
Number		Number	
1	Fr 634 X FRMo.17	17	Pool 30 X FRMo.17
2	A 670 X FRMo.17	18	Pool 30 X FRMo.17
3	B 87 X FRMo.17	19	H 108 X FRMo.17
4	Fr 43 X FRMo.17	20	ALKD 187 X FRMo.17
5	H 49 X FRMo.17	21	Ada.1 3002 X FRMo.17
6	H 99 X FRMo.17	22	A 682 X FRMo.17
7	Mo.5 X FRMo.17	23	Akpınar 9 X FRMo.17
8	ND 300 X FRMo.17	24	Akpınar 10 X FRMo.17
9	ND 301 X FRMo.17	25	Akpınar.55 X FRMo.17
10	Pa.373 X FRMo.17	26	Yıldız 26 X FRMo.17
11	Pa.401 P X FRMo.17	27	Yıldız 32 X FRMo.17
12	Pa.402 P X FRMo.17	28	Yıldız 40 X FRMo.17
13	Pa.870 X FRMo.17	29	Yıldız 41 X FRMo.17
14	Y 58 2 A X FRMo.17	30	Yıldız 50 X FRMo.17
15	496 W X FRMo.17	31	TTM 813 (Local check)
16	504 W X FRMo.17		

Heterosis was calculated as difference between the mean of the F1 and the average of the best parent (tester line or inbred); significance was tested using $LSD_{0.05}$ for each trait and for each better performing F_1 [14].

Canonical discriminant and cluster analyses were computed using PROC DISCRIM and PROC CLUSTER [15]. Distances (or similarities) between inbred lines and hybrids were estimated using the Mahalanobis distances computed as: $D^2 = (X_i-X_j)^2\ cov^{-1}(X_i-X_j)$. In this model, X_i and X_j are the general means of i and j cultivars. Graphics were established based on the mean of each cultivar [3]. Cluster analysis based on the multivariate Mahalanobis distances was carried out to visualize relationships among inbred lines and among topcrosses and to detect externally isolated groups of genotypes, using the Average Linkage Method (ALM) [15].

RESULTS

Univariate Analysis

Significant variation (P<0.05) was observed for inbred lines and for topcrosses as shown by Fisher's $LSD_{0.05}$ (Table 3). Genotype by environment interaction (GXE) was also significant for all the traits measured in inbred lines used in this study. However, GXE was not significant for yield, thousand kernel weight, plant height, number of rows, kernels per ear and single ear yield in topcrosses (Table 3). The trait showing the highest heterosis was plant height; every F_1 was taller than the tallest parent. Single ear yield, yield, ear height and number of kernels per ear also exhibited high level of heterosis. F_1 heterosis was less marked for thousand kernel weight and number of row per ear. No heterosis was observed for tasseling time and ear length (Table 3). With this experiment, it has been confirmed that several quantitative trait components show F_1 heterosis probably due to dominance at few or several dispersed loci [14].

In inbred lines, broad sense heritability was higher than 0.60 for all the traits measured except for tasseling time (Table 4). The most heritable traits, within inbred lines were plant height, ear height, number of row and kernels per ear; while within

Table 3. Average for yield and yield component traits, least significant difference and significance of the genotype x environment interaction for inbred lines (P2) and hybrids (F1) respectively. For hybrids an asterisk label the significance of the heterosis (hybrid with significantly higher average than the average of the best parent). Line 31 is the tester parent (P1).

	Yield (t/ha)		**1000 kernel weight (g)**		**Plant height (cm)**		**Tasseling time (days)**		**Ear height (cm)**		**Number of rows per ear (Number)**		**Ear lenght (cm)**		**Number of kernel per ear (Number)**		**Single ear yield (g)**	
Number	P2	F1	P2	F1	P2	F1	P2	F1	P2	F1	P2	F1	P2	F1	P2	F1	P2	F1
1 (P2)	2.71	**8.81 ***	283	**315 ***	154	**233 ***	61.7	**59.0**	69	**113 ***	14.6	**14.6**	11.6	**16.7**	243	**455**	33.0	**108 ***
2 (P2)	3.11	**8.79 ***	245	**289**	169	**239 ***	61.0	**57.3**	66	**109 ***	15.2	**14.1**	15.5	**18.5**	372	**501 ***	38.0	**108 ***
3 (P2)	5.08	**9.19 ***	230	**267**	170	**230 ***	60.7	**59.7**	66	**106 ***	12.0	**13.1**	17.3	**20.3**	370	**533 ***	62.0	**113 ***
4 (P2)	4.14	**9.33 ***	275	**266**	155	**226 ***	58.8	**57.5**	64	**107 ***	13.5	**13.7**	16.2	**19.7**	401	**550 ***	51.2	**115 ***
5 (P2)	5.06	**8.22 ***	207	**273**	152	**238 ***	63.2	**59.7**	63	**115 ***	13.7	**13.7**	15.3	**17.2**	468	**525 ***	61.8	**101 ***
6 (P2)	2.87	**7.45**	215	**262**	105	**215 ***	54.2	**57.7**	33	**95**	11.2	**13.0 ***	13.2	**16.0**	295	**483**	64.7	**92**
7 (P2)	4.24	**10.02 ***	222	**295 ***	143	**233 ***	63.7	**58.7**	69	**115 ***	14.1	**13.8**	14.5	**18.2**	449	**582 ***	52.0	**123 ***
8 (P2)	3.50	**8.06 ***	239	**268**	136	**221 ***	50.5	**53.7**	52	**94**	14.8	**13.0**	12.2	**18.2**	315	**505 ***	42.5	**99 ***
9 (P2)	3.87	**8.06 ***	194	**308 ***	147	**215 ***	49.5	**51.7**	66	**104**	13.5	**14.7 ***	13.3	**17.7**	393	**514 ***	47.8	**99 ***
10 (P2)	3.22	**7.72 ***	227	**271**	150	**215 ***	53.7	**56.2**	74	**107 ***	13.6	**13.6**	11.8	**17.2**	305	**444**	38.8	**95 ***
11 (P2)	4.05	**8.64 ***	196	**250**	151	**245 ***	61.8	**58.5**	77	**129 ***	15.3	**15.3**	12.6	**17.8**	424	**598 ***	49.8	**106 ***
12 (P2)	4.33	**10.08 ***	214	**258**	145	**236 ***	57.3	**55.3**	66	**122 ***	17.2	**16.2**	12.4	**18.0**	408	**628 ***	52.8	**124 ***
13 (P2)	2.17	**7.53**	294	**290 ***	130	**218 ***	63.3	**57.8**	55	**107 ***	10.3	**14.1 ***	15.1	**18.3**	196	**502 ***	26.3	**93 ***
14 (P2)	5.41	**8.42 ***	262	**293 ***	195	**255 ***	62.7	**60.0**	103	**137 ***	13.3	**13.7**	12.3	**17.4**	326	**502 ***	66.3	**103 ***
15 (P2)	5.33	**11.28 ***	265	**320 ***	164	**250 ***	60.2	**60.0**	79	**128 ***	12.2	**12.3**	15.0	**19.9**	356	**521 ***	65.3	**139 ***
16 (P2)	6.49	**10.84 ***	303	**313 ***	177	**246 ***	61.0	**60.2**	88	**128 ***	12.1	**12.6**	16.0	**20.0**	355	**521 ***	79.5	**133 ***
17 (P2)	3.23	**9.41 ***	303	**271**	173	**236 ***	60.2	**58.7**	54	**112 ***	17.9	**15.0**	12.4	**18.2**	351	**561 ***	39.5	**116 ***
18 (P2)	5.68	**10.40 ***	257	**262**	170	**229 ***	59.3	**59.0**	62	**106 ***	12.4	**12.8**	18.7	**20.0**	395	**528 ***	69.3	**128 ***
19 (P2)	4.49	**5.71**	239	**238 ***	164	**213 ***	58.8	**60.8**	65	**110 ***	14.3	**12.0**	11.8	**16.1**	355	**429**	54.7	**70**
20 (P2)	3.49	**7.73 ***	212	**245**	150	**222 ***	62.3	**59.5**	78	**114 ***	13.1	**14.8 ***	15.3	**16.6**	364	**521 ***	42.3	**95 ***
21 (P2)	3.68	**8.29 ***	263	**272**	171	**234 ***	59.3	**57.2**	87	**120 ***	14.7	**15.0**	14.3	**17.6**	398	**547 ***	45.0	**102 ***
22 (P2)	3.85	**9.28 ***	305	**311 ***	161	**236 ***	55.5	**57.2**	61	**124 ***	10.0	**13.8 ***	15.8	**18.1**	265	**528 ***	47.0	**114 ***
23 (P2)	1.26	**8.48 ***	270	**284**	121	**218 ***	55.2	**55.8**	39	**103**	12.4	**13.5 ***	17.2	**18.0**	216	**469**	15.3	**104 ***
24 (P2)	2.11	**6.88**	261	**329 ***	135	**208 ***	54.3	**52.3**	52	**90**	11.4	**11.6**	13.2	**20.1**	228	**411**	25.5	**84**
25 (P2)	5.74	**9.90 ***	231	**272**	155	**237 ***	59.8	**57.8**	83	**128 ***	14.0	**14.3**	15.9	**17.7**	449	**557 ***	70.2	**121 ***
26 (P2)	4.64	**9.03 ***	291	**315 ***	185	**241 ***	56.5	**57.7**	89	**121 ***	13.8	**13.9**	13.7	**17.4**	318	**466**	56.8	**111 ***
27 (P2)	6.15	**8.08 ***	248	**247**	201	**238 ***	57.3	**56.7**	101	**111 ***	14.6	**13.9**	17.5	**19.0**	494	**548 ***	75.0	**99 ***
28 (P2)	3.19	**7.93 ***	269	**308 ***	147	**231 ***	62.2	**59.7**	68	**117 ***	16.1	**14.8**	13.2	**17.0**	352	**455**	39.2	**98 ***
29 (P2)	2.11	**8.00 ***	289	**313 ***	160	**235 ***	60.0	**59.7**	67	**114 ***	13.3	**13.3**	13.0	**18.2**	263	**459**	26.2	**98 ***
30 (P2)	3.18	**7.70 ***	232	**288**	157	**223 ***	58.3	**57.7**	67	**111 ***	16.9	**14.6**	14.5	**17.5**	425	**467**	39.2	**95 ***
31 (P1)	6.22	9.13	265	274	191	217	59.1	58.2	96	109	11.4	13.9	18.6	18.2	436	540	76.1	112
LSD.05	9.26	13.6	17.0	19.3	7.8	8.7	1.5	1.2	5.9	2.0	0.7	0.7	1.3	2.0	42.5	45.4	2.1	3.1
GxE	s.	n.s.	s.	n.s.	s.	n.s.	s.	s.	s.	s.	s.	n.s.	s.	s.	s.	n.s.	s.	n.s.

the hybrid population the most heritable trait was number of row per ear (Table 4). Yield was highly correlated (P<0.01) with plant height (r=0.87), ear height (r=0.83), ear length (r=0.75), kernels per ear (r=0.83), yield of single ear (r=0.99) and was less correlated with thousand kernel weight (r=0.39). Correlation analysis showed that number of rows per ear was independent from yield and yield components (data not shown). Thus, because of high heritability of number of rows per ear, it could be possible to select inbred lines and hybrids with both higher yield and number of rows per ear. One example in this experiment was represented by inbred lines number12 and 17 (both have a high value of number of rows per ear) both of which after crossing with tester 31 produced high yielding heterotic F_1's maintaining a high number of rows per ear (Table 3).

Table 4. Heritability for traits of breeding interest showing significant variation among inbred lines and hybrids, respectively.

	Heritability	
Trait	Lines	Hybrids
Yield	0.63	0.46
Ear lenght	0.65	0.35
Number of row per ear	0.87	0.73
Number Kernels per ear	0.73	0.51
Plant height	0.85	0.65
Ear height	0.86	0.66
Yield of single ear	0.62	0.46
1000 kernel weight	0.78	0.57
Tasseling	0.12	0.04

Discriminant Analysis

Discriminant analyses used for revealing general distances between genotypes as numerical values (D^2) indicated which traits could be used to differentiate genotypes. Based on the D^2 values obtained from discriminant analyses, the most different lines were number 17 (Pool 30) and number 31 (Fr Mo 17) while the most similar ones were number 3 (B 87) and number 18 (Pool 30a) (Figure 1 and Figure 2). Traits that allowed the highest level of discrimination among inbred lines were, in decreasing order, number of row per ear, ear height, thousand kernel weight, plant height, kernels per ear, ear length, tasseling time and yield (validated by stepwise discriminant analysis). The first canonical variable (can 1) explained 32 % of the total variation and showed high values for ear length (c= 0.56), ear height (c= 0.55), single ear yield (c= 0.47), yield (c=0.47) and plant height (c= 0.45), but with a low value for number of rows per ear (c= - 0.71) (Figure 2). The second canonical variable (can 2) explained 25% of total variation. It reflected high positive values for kernels per ear (c= 0.70), ear height (c = 0.51), yield (c= 0.45), yield of single ear (0.45), number row per ear (c= 0.35), but negative value for thousand kernel weight (c = -0.70) (Figure 2). Based on the canonical coefficients, better performing inbred lines in terms of yield, single ear yield and ear size were associated in the upper-right quadrant of the scatter plot while the best inbred lines for thousand seed weight and number of rows per ear was associated on the lower quadrant of the scatter plot (Figure 2). Plant height of inbred lines tended to increase with positive values of can1 (Figure 2). Can3 (not shown in the plot) mainly controlled by plant height (c= 0.78), number rows per ear (c= 0.56) and ear height (c= 0.62) and discriminated better the inbred lines 17 and 6.

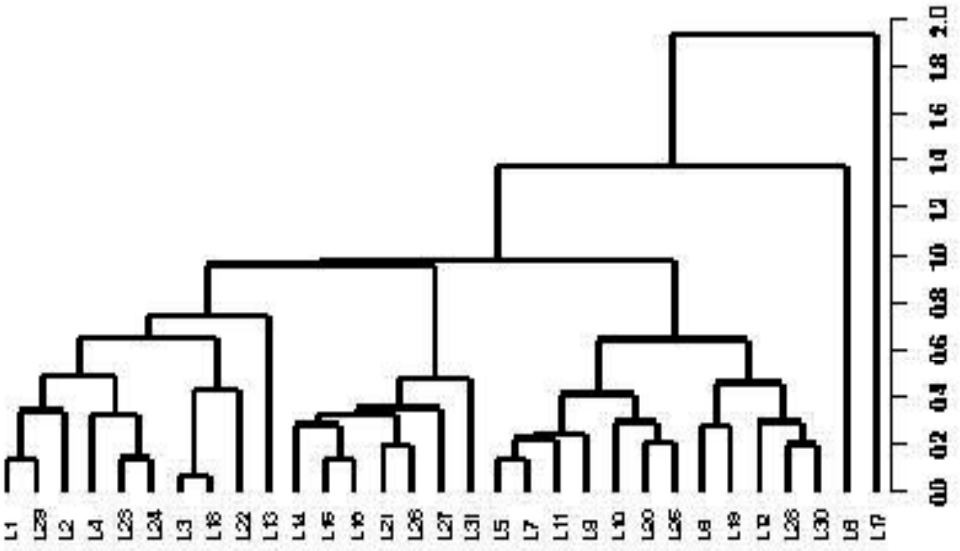

Figure 1. Phenotypic relationships among 31 inbred lines based on cluster analysis (average method) of Mahalanobis multivariate distances.

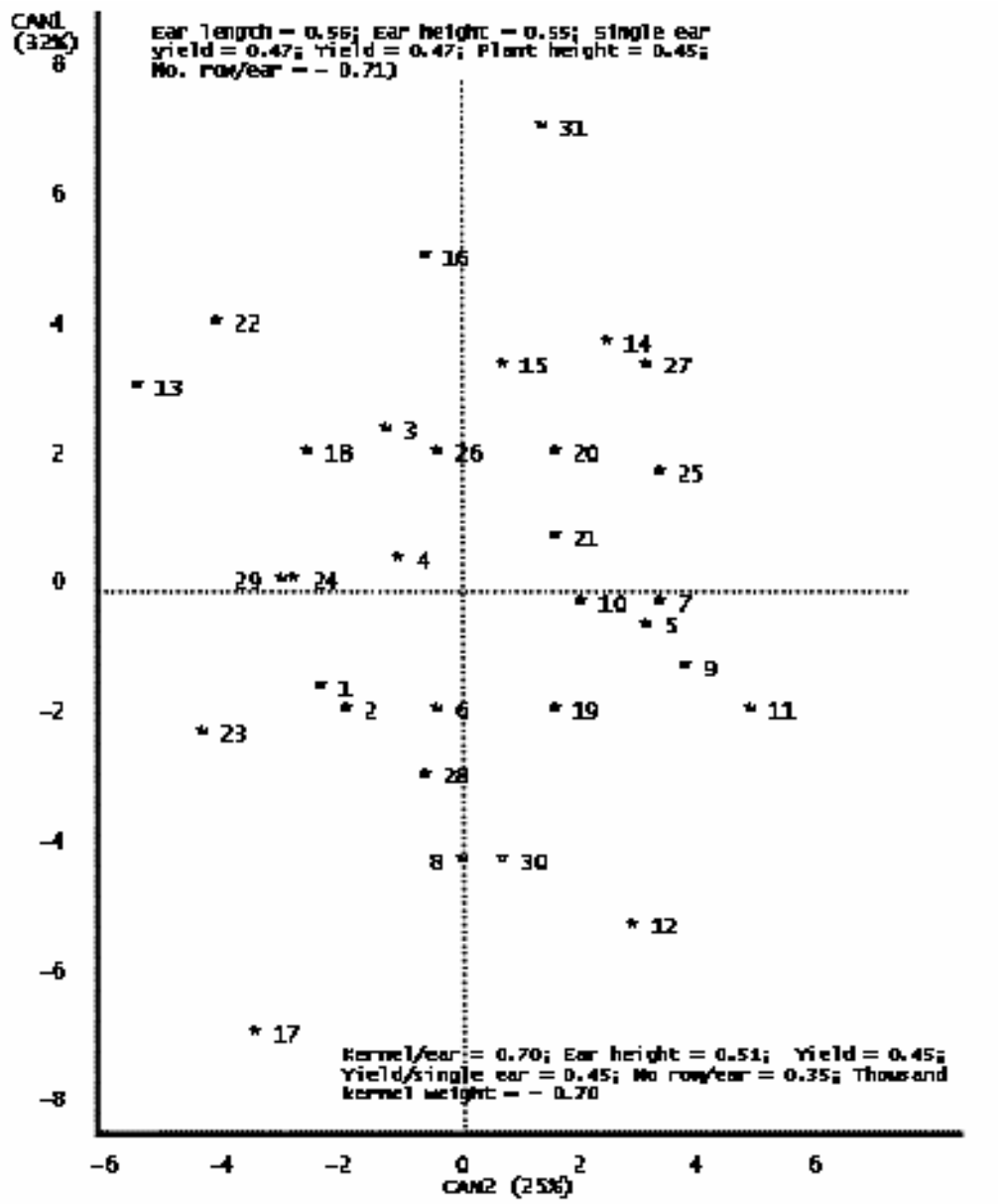

Figure 2. Scatter plot of first and second canonical variables showing discrimination by inbred lines based on nine quantitative traits.

Overall, lines classified into the upper quadrant of Figure 2 had higher yields (yield perse and single ear yield) (Table 3), with the exception of line 13 which was lower in yield but had a high value of thousand seed yield. These lines are consistently classified by the intermediate subclusters, based on multivariate Mahalanobis distances, reported in Figure 1. The tester 31 (FrMo17) showed the best combining ability for yield with inbreds 7, 12, 15, 16 and 18 respectively (F_1s all with yield > 10 t/ha). This result indicates that rather than average trait distances, good combining genes from both parents are crucial for yield heterosis.

The most discriminating traits among F_1 hybrids, in a decreasing order were; number of rows per ear, plant height, thousand kernel weight, ear height, kernels per ear, ear length, yield and tasseling time (validated by stepwise discriminant analysis). From discriminant analysis, it is shown that the first canonical variable (can 1) explains 34 % of the total variation and tends to be large with high values of number of rows per ear (c= 0.72), ear height (c= 0.59), kernels per ear (c= 0.47), plant height (c=0.35), except ear length (c= - 0.41) and thousand kernel weight (c= - 0.33) (Figure 3). The second positive canonical variable (can 2), explaining the 21% of the total variation, is mainly affected by thousand kernels weight (c= 0.74) and ear length (c= 0.40), except kernels per ear (c = - 0.35) (Figure 3). Based on the canonical coefficients, the hybrids with higher values of ear and plant height were classified on the top of the scatter plot (hybrid 14 show the maximum values). Lines with the highest thousand kernel weight were associated in the lower right quadrant of the plot (hybrid 24 shows the maximum value) (Figure 3, Table 3). Hybrids with higher kernels per ear were associated in the upper left quadrant of the plot. However, yield showed low discriminant ability among the F_1s, reflected by a lack of association among F_1s on the basis of yield, at least in the first two canonical variants.

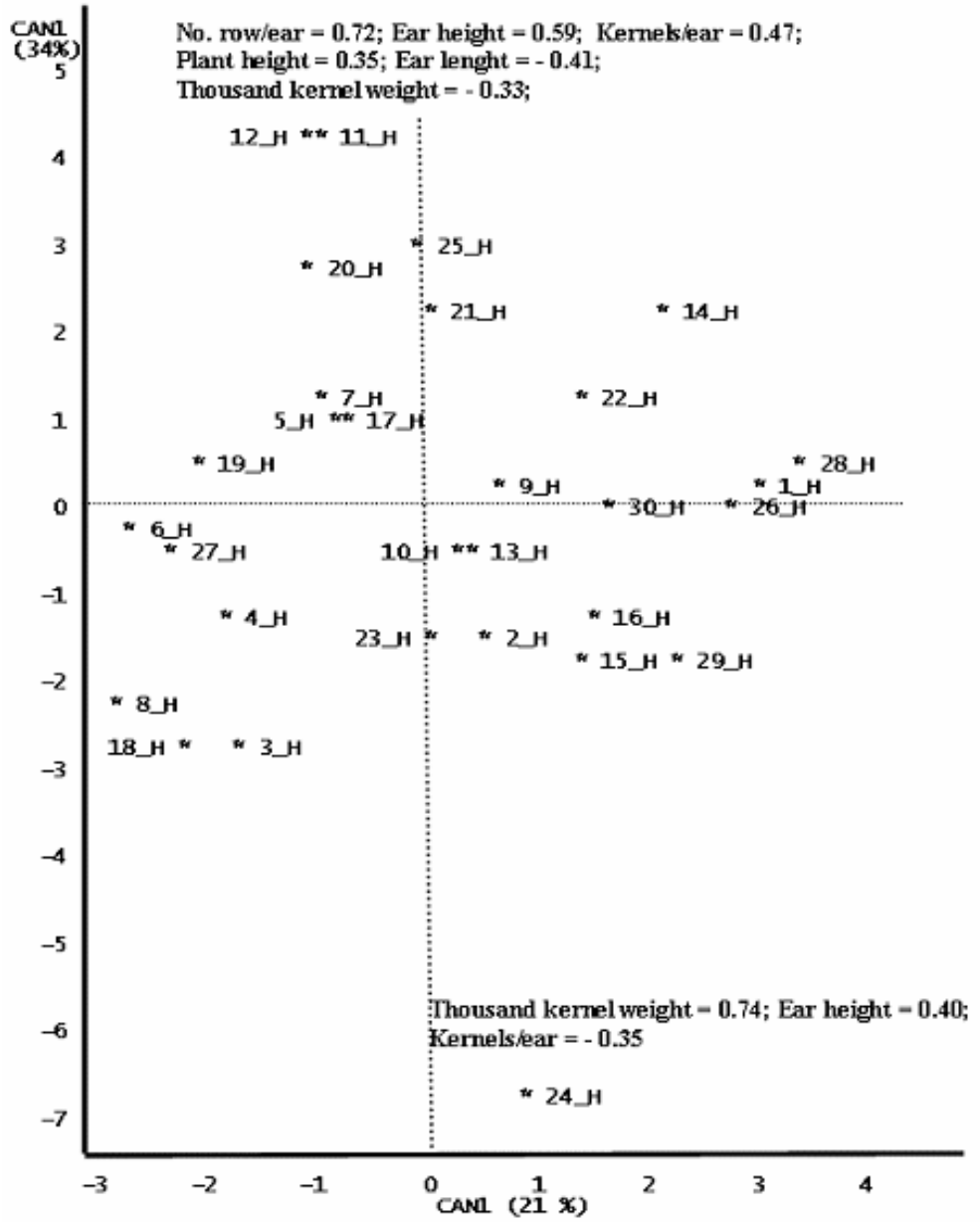

Figure 3. Scatter plot of first and second canonical variables showing discrimination among F_1 hybrids based on nine quantitative traits.

DISCUSSION

Heterosis, measured as hybrid average significantly better than the average of the best parent, was evident for yield, single ear yield, number of kernel per ear, ear height and plant height. For several traits, hybrids were also more stable across two environments. Inbred lines with the exception of inbreds 6, 13, 19 and 24 were all able to combine with the tester Fr Mo 17 (line 31) in order to significantly increase yield. For ear length and tasseling time none of the F_1s were heterotic, probably because of the low level of combining ability of the tester line as shown also by the low level of heritability within hybrids (Table 3 and 4). Heterosis for yield traits is not just the result of genetic distance for the studied traits of breeding interest between the tester line and inbred lines. For example, the best performing hybrids in terms of yield and yield components (7, 12, 15, 16, and 18) on average are not the result of crosses between genetically the most distant lines for these traits, with the exception of the crosses12 X 31 and 7 X 31 (two genotypes quite distant relatively to both first and second canonical variable, Fig. 4). In contrast, heterosis for yield seems to be a result of the combination of the best parents for the trait of interest (Table 3). The inbred lines Akpinar55 and Yildiz32 will be used in maize genetics and breeding program due to their high yielding performance and high level of combining ability.

Our data support the hypothesis that not just genetic distances are responsible for heterosis, but especially for quantitative traits such as yield, plant height, ear size and number and weight of kernels per spike, desirable F_1s are *in primis* the result of the combination of good parental genes [14]. Also using molecular

data (Restriction Fragment Length Polymorphisms) Godshalk et al. [16] found no relationship between genetic distances and hybrid performance. In addition, most of the traits analyzed in this experiment were all strongly correlated with yield and thus given that different correlated traits can share the same genes (pleiotropy). Also, phenotypic distances are not representative of the whole genome. It is possible to conclude that inbred lines not very distant for the analyzed traits can be genetically distant at genomic level.

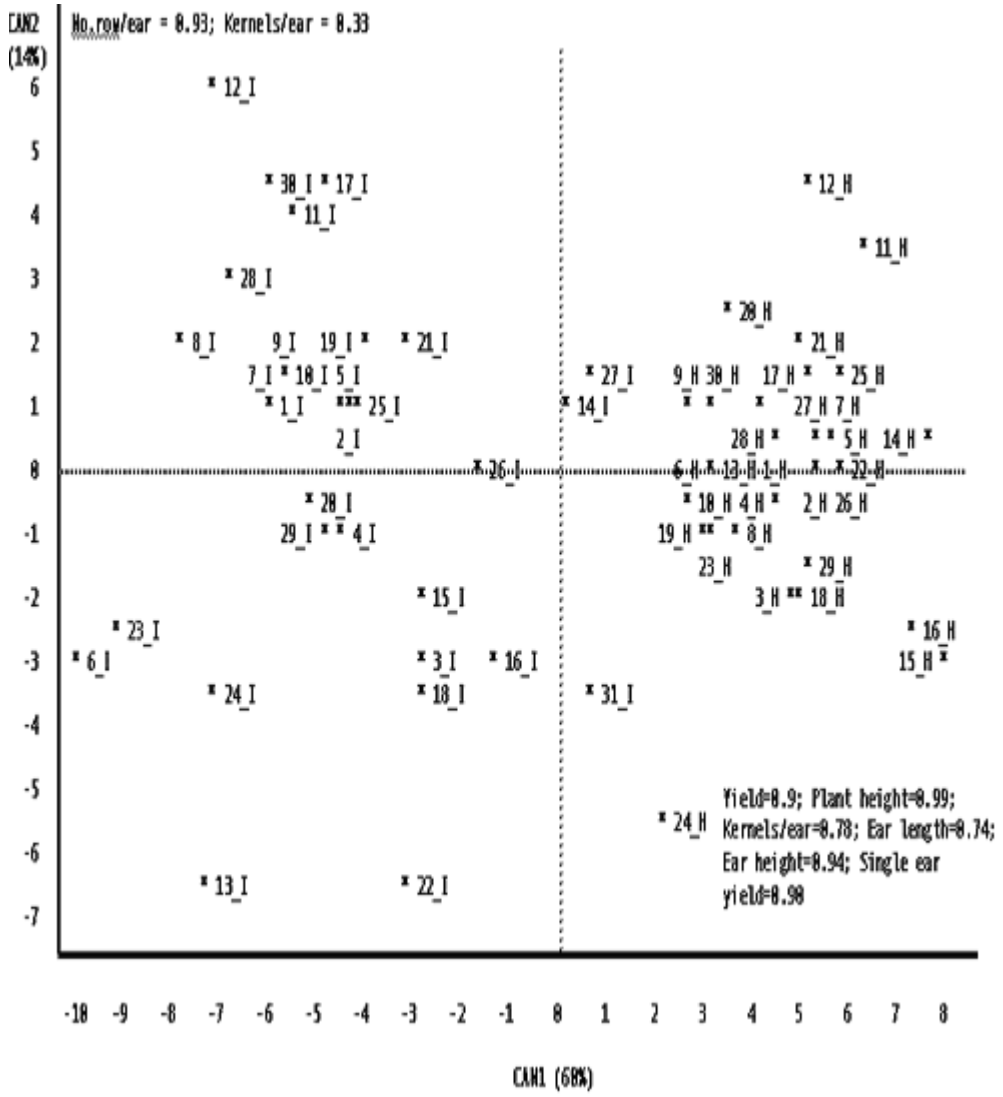

Figure 4. Scatter plot of first and second canonical variables showing discrimination among both inbred lines (I) and F_1 hybrids (H) based on nine quantitative traits.

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