

# Classifying of Maize Inbred Lines into Heterotic Groups using Diallel Analysis

Mozhgan Ziaie Bidhendi, Rajab Choukan, Farokh Darvish, Khodadad Mostafavi, Eslam Majidi

**Abstract**—The selection of parents and breeding strategies for the successful maize hybrid production will be facilitated by heterotic groupings of parental lines and determination of combining abilities of them. Fourteen maize inbred lines, used in maize breeding programs in Iran, were crossed in a diallel mating design. The 91 F1 hybrids and the 14 parental lines were studied during two years at four locations of Iran for investigation of combining ability of genotypes for grain yield and to determine heterotic patterns among germplasm sources, using both, the Griffing's method and the biplot approach for diallel analysis. The graphical representation offered by biplot analysis allowed a rapid and effective overview of general combining ability (GCA) and specific combining ability (SCA) effects of the inbred lines, their performance in crosses, as well as grouping patterns of similar genotypes. GCA and SCA effects were significant for grain yield (GY). Based on significant positive GCA effects, the lines derived from LSC could be used as parent in crosses to increase GY. The maximum best-parent heterosis values and highest SCA effects resulted from crosses B73 × MO17 and A679 × MO17 for GY. The best heterotic patterns were LSC × RYD, which would be potentially useful in maize breeding programs to obtain high-yielding hybrids in the same climate of Iran.

**Keywords**—biplot, diallel, Griffing, Heterotic pattern

## I. INTRODUCTION

MAIZE (*Zea mays* L.) is currently one of the most important crops in Iran. Although the average yield of maize has dramatically increased in the last decade in Iran, an additional increase in yield will be required to meet future needs of the country. To achieve this goal will require an efficient breeding program with well-established germplasm which would provide potential exploitation of heterosis in maize germplasm developed for Iran. Different maize hybrids have been introduced from other countries into Iran, and inbred lines were extracted from these germplasm in order to produce successful hybrid combinations. Information on germplasm diversity is also fundamentally important for hybrid breeding and population improvement programs, characterizing the maize germplasm and assigning them into different heterotic groups [20].

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After development of inbred lines from known or unknown sources, breeders need to make thousands of crosses and evaluate grain yield in resulting F1 plants in replicated field experiments. Assigning lines to heterotic groups would avoid the development and evaluation of crosses that should be discarded, allowing maximum heterosis to be exploited by crossing inbred lines belonging to different heterotic groups [23]. Combining ability analyses are widely used in maize breeding programs to determine GCA and SCA information of maize germplasm for identification of nature of genes action involved in the expression of quantitative traits, genetic diversity evaluation, suitable parental lines selection for hybridization, heterotic pattern classification, heterosis estimation, and hybrids development [2]-[3]-[8]-[9]-[11]-[17]-[18]-[22]-[23]. The hybrids performance that greatly depends on the level of heterosis expressed in them has been used to extrapolate information about the relationships among the inbred lines, but there have been few reports about heterotic groups and patterns of maize lines in Iran. This necessitated a study of heterotic relationships among Iranian maize germplasm. Choukan *et al.* (2006), using cluster analysis from genetic distance based on SSR markers to evaluate Iranian maize inbred lines reported that the lines could be classified into four preliminary heterotic groups [7]. Heterotic groups and patterns among inbred lines and the best hybrid combinations can be identified using information from several methodologies, including: field crosses (mainly using diallel or topcrosses to testers [14]-[16]-[23]); pedigree information; morphological traits; and molecular markers [21]. Diallel mating models developed by Griffing (1956), Gardner and Eberhart (1966), are the major models used in combining ability analyses [13]-[15]. Biplots can also be used for analyzing diallel crosses [4]-[25]. The graphical representation offered by biplot analysis allowed a rapid and effective overview of GCA and SCA effects of the inbred lines, their performance in crosses, as well as grouping patterns of similar genotypes. The objectives of the present study were to: (1) survey the combining abilities of 14 Iranian maize inbred lines for grain yield (GY) to promote their use by breeders and to (2) suggest potential heterotic groups and patterns for GY trait among the lines included in the study using diallel data analysis and biplot interpretation.

## II. MATERIALS AND METHODS

### A. Plant material

Fourteen maize inbred lines were introduced from maize breeding programs in Iran. The inbreds consisted of five lines from two well-known USA heterotic groups [B73 and A679

related to Reid Yellow Dent (RYD) and Mo17 related to Lancaster Sure Crop (LCS)] as well as two derived lines from Mo17 (K18 and K19/1 which are selected in Iran), five lines were extracted from CIMMYT germplasm, and four lines from a late synthetic (SYN L.) population originating from Iran. All lines and their pedigree sources/origin (if known) are listed in Table I. Ninety-one crosses were made by hand pollination among the 14 maize lines according to Griffing's diallel method II [15] in 2005 at Seed and Plant Improvement Institute, Iran. A total of 105 genotypes (91 crosses and 14 parental inbreds) were evaluated in the same trial during two growing seasons (2006–2007 and 2007–2008) at four locations: Kermanshah, Esfahan, Shiraz and Miandoab in the west, center, south and north- west of Iran, respectively. The 105 genotypes were arranged in a randomized complete blocks design with three replications at each location. Experimental maize lines and their F<sub>1</sub> crosses were each planted in single-row plots that were 7.40 m long, spaced 75 cm apart, each row consisted of 20 hills spaced 35 cm apart. Plots were over-planted at 80 seeds per row, and then thinned to a density equivalent to 76140 plants ha<sup>-1</sup> at the five-leaf stage. Standard agronomic farming practices were followed at each experimental site. Ten plants from the middle of each row were sampled randomly and the grain yield trait (shelled grain weight per plot adjusted to 14% grain moisture and converted to ton per hectare) was recorded for each genotype at each location.

TABLE I  
SOURCE AND PEDIGREE OF THE 14 IRANIAN MAIZE INBRED LINES INVOLVED IN THE DIALLEL CROSSES

Name	Inbred line	Source/Origin	Pedigree
A	MO17	Lancaster Sure Crop (LSC)	(CL.187-2 x C103)
B	K18	Lancaster Sure Crop (LSC)	Derived from MO17 changes
C	K19/1	Lancaster Sure Crop (LSC)	Derived from K19 changes
D	B73	Reid Yellow Dent (RYD)	BSSS C5 (Iowa Stiff Stalk Synthetic)
E	A679	Reid Yellow Dent (RYD)	(A662 x B73)
F	K3615/2	Late synthetic (SYN L.)	SYN-Late
G	K3640/5	Late synthetic (SYN L.)	SYN-Late
H	K3653/2	Late synthetic (SYN L.)	SYN-Late
I	K3651/1	Late synthetic (SYN L.)	SYN-Late
J	K166B	CIMMYT	Unknown
K	K3547/5	CIMMYT	Srinagar8848
L	K3544/1	CIMMYT	Unknown
M	K3545/6	CIMMYT	Tlaltizapan-8946
N	K3493/1	CIMMYT	Unknown from EVT 16A

### B. Statistical and biplot analysis

Data were subjected to an ANOVA analysis using Griffing's method 2 [15], using DIALLEL-SAS [26]. Significances of genotypes, GCA, and SCA mean squares were estimated with *F* tests, using their interaction with the

environment as an error term. The combining ability by environment interactions was tested against the error term. Best parent heterosis was calculated as described by Fehr (1987) [12]. The percentage of heterosis was considered significant when the difference of means between the cross and best parent exceeded the least significant difference (LSD). Estimates of GCA and SCA effects were calculated and their significance determined by *t* tests. Finally, a biplot analysis of diallel data was conducted using GGEbiplot software [24]. Although GGE biplot methodology was developed for multi-environment trial (MET) data analysis, it could be applied to all types of two-way data that assume an entry by tester structure [25].

### III. RESULTS AND DISCUSSION

The ANOVA layout of Griffing's method 2 of diallel crosses for grain yield trait is presented in Table II. The analysis of variance revealed the differences among the genotypes (inbred lines and crosses) were significant ( $P \leq 0.01$ ). The genetic variability among the genotypes was broken down to that due to the GCA of inbred lines and SCA of crosses. The genotypes performed significantly different ( $P \leq 0.01$ ) over the environments. This implies that the genotypes can be deployed in the development of varieties adapted to different environments [1]. The variations due to GCA, SCA and their interaction with environment were significant ( $p \leq 0.01$ ) (Table II).

TABLE II  
ANALYSIS OF VARIANCE FOR GRAIN YIELD 14 MAIZE INBRED LINES AND 91 SINGEL- CROSSES ALONG GRIFFING'S METHOD 2 EVALUATED ACROSS FOUR LOCATIONS DURING TWO YEARS

Source of variation	df	MS	F value
Environments	7	2252.50	
Rep within environments	16	56.96	
Genotypes	104	52.43	7.94**
GCA	13	90.42	7.42**
SCA	91	47	8.09**
Genotypes x environments	728	6.61	1.90**
GCA x environments	91	12.19	3.50**
SCA x environments	637	5.81	1.67**
Error	1664	3.48	

\*\* Significant at 0.01 probability level

The lines B and C had significantly positive GCA effects, whereas the lines with significant negative GCA effects were I, L, M and N for grain yield (Table III), which was consistent with Fig. 1. These observations indicate high potential of LSC lines to increase the GY for use as breeding materials in commercial maize hybrid development programs that have been proved by other studies [5] [19]. According to the assumption that SCA of two lines from different heterotic groups is greater than those from the same group, the 14 lines were divided into diverse heterotic groups. D × K (2.03) and A × H (2.02) had the highest significant SCA effects (Table III), reflecting high hybrid performance for yield, both of them belonging to intergroup crosses (Fig.1).

TABLE III

SPECIFIC COMBINING ABILITY EFFECTS, ABOVE DIAGONAL; GENERAL COMBINING ABILITY EFFECTS AND PARENTS MEAN (IN PARENTHESIS), ON DIAGONAL; PERCENTAGE OF BEST- PARENT HETEROISIS AND HEBRID MEANS (IN PARENTHESIS), BELOW DIAGONAL. PARENT CODES IN TABLE II

	A	B	C	D	E	F	G	H	I	J	K	L	M	N
A	0.28 (7.92)	-1.98**	-1.31*	0.47	1.46*	0.10	1.89**	2.02**	1.04	0.14	1.66**	-1.03	1.25	0.25
B	-3.68 (10.74)	0.79** (11.15)	-2.71**	1.30*	0.23	0.14	1.77**	1.46*	1.06	0.07	0.62	0.20	1.42	0.58
C	22.76** (14.08)	-7.85 (10.57)	0.83** (11.47)	0.62	1.58*	1.94**	1.50*	-0.26	0.57	0.31	0.29	1.47*	0.28	0.68
D	56.27** (12.58)	24.84** (13.92)	15.78** (13.28)	0.17 (8.05)	-3.46**	1.32*	1.60*	0.14	1.66*	-0.79	2.03**	0.98	1.53*	0.47
E	54.24** (13.28)	12.65** (12.56)	21.62** (13.95)	-4.30 (8.24)	-0.12 (8.61)	0.28	0.41	0.28	0.74	0.22	0.67	1.48*	0.15	1.55*
F	3.24 (12.11)	7.93 (12.66)	-9.55* (10.61)	12.70** (13.22)	1.28 (11.88)	0.07 (11.73)	-0.50	-1.27*	-0.96	0.43	0.57	-0.91	0.29	0.01
G	42.09** (13.74)	26.82** (14.14)	21.19** (13.90)	37.95** (13.34)	22.75** (11.87)	-5.03 (11.14)	-0.08 (9.67)	-0.54	-0.99	0.50	-0.52	-0.29	-0.80	-0.40
H	38.96** (14.16)	26.46** (14.10)	8.28 (12.42)	19.43** (12.17)	17.96** (12.02)	12.53** (13.20)	10.21 (11.23)	0.19 (10.19)	-0.67	0.69	-0.57	0.77	-0.75	-0.13
I	36.17** (12.46)	16.50** (12.99)	9.33* (12.54)	41.75** (12.97)	28.63** (11.77)	-12.62** (10.25)	4.14 (10.07)	4.71 (10.67)	-0.51** (9.15)	0.40	-0.86	0.42	0.13	0.39
J	17.59** (12.37)	14.89** (12.81)	14.12** (13.09)	7.70 (11.33)	14.54** (12.05)	6.14 (12.45)	17.59** (12.37)	21.96** (12.83)	12.55* (11.84)	0.29 (10.52)	0.18	1.00	-0.07	0.36
K	39.25** (13.41)	15.43** (12.87)	9.76* (12.59)	41.95** (13.67)	24.82** (12.02)	3.24 (12.11)	12.31* (10.86)	8.73 (11.08)	4.78 (10.09)	13.40** (11.93)	-0.19 (9.63)	-0.07	-1.69**	0.94
L	20.71** (10.49)	9.78* (12.24)	18.13** (13.55)	42.69** (12.40)	45.11** (12.61)	-11.34* (10.40)	12.41* (10.87)	19.82** (12.21)	21.97** (11.16)	19.20** (12.54)	14.02** (10.98)	-0.41* (8.69)	0.81	-0.55
M	45.83** (12.25)	15.87** (12.92)	3.05 (11.82)	47.74** (12.41)	24.85** (10.75)	-5.54 (11.08)	1.65 (9.83)	-0.29 (10.16)	12.90* (10.33)	3.90 (10.93)	-8.31 (8.83)	27.96** (11.12)	-0.94** (8.40)	0.19
N	35.05** (11.83)	13.63** (12.67)	11.68* (12.81)	36.30** (11.94)	45.32** (12.73)	-2.98 (11.38)	11.79* (10.81)	11.48* (11.36)	22.19** (11.18)	13.59** (11.95)	25.03** (12.04)	17.92** (10.33)	20.43** (10.55)	-0.36* (8.76)

\*, \*\* significant at the 0.05 and 0.01 probability level respectively. For mean values comparisons, LSD at 5% (1.05), and at 1% (1.39).

Since crosses D × E (-3.46), B × C (-2.71), A × B (-1.98), K × M (-1.69), A × C (-1.31) and F × H (-1.27) had the lowest significant SCA effects (Table III), all of them corresponding to crosses intra-groups (Fig. 1). These results agree with Choukan (2001); Fan *et al.* (2004); Choukan (2006) and Mostafavi *et al.* (2009), who reported high performances for crosses between maize populations [5]-[6]-[10]-[19].

The classification of inbreds into heterotic groups facilitates the exploitation of heterosis in maize, which can contribute to hybrid performance. According to the biplot approach of diallel data [25], a clear interaction can be seen between testers and entries, they are much closer each other, falling in the same side of the average tester coordinate (ATC). The first two principal components together explained 68% of the total variation for GY. Two heterotic groups can be observed, D to E as one group and A to B to L as the other (Fig. 1).

Inbreds A, D, E, M and L, which are located furthest from the origin, defined a polygon that was divided into four sectors (Fig. 1). Testers d, e, g, h, i, j, k and m fell in Sector A, indicating that their crosses with A generated good hybrid combinations. In sector D-E, the entries D and E showed a high positive response in hybrids with testers a, b, c, f, i, k, l and n. Sector M contained three adequate hybrid combinations: a × M, b × M, and d × M. In sector L, the best hybrid combination was the vertex entry L × tester d.

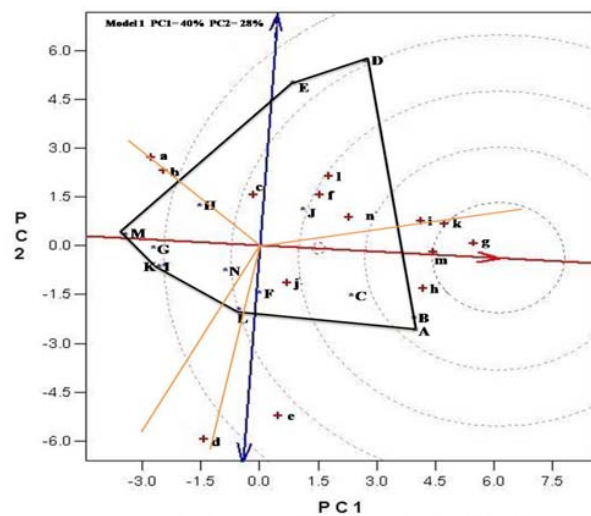


Fig. 1 Biplot based on diallel data of 14 maize inbred lines for grain yield. Genotypes are labeled with uppercase letters when viewed as entries and with lowercase letters when viewed as testers, The circle indicates the average tester

Interestingly, in Sector A, A was predicted to be the best mating partner for d and e, and in sector D-E, D and E were predicted to be the best partners for a. Therefore crosses A × D and A × E must be the best of all possible combinations for GY. High values of best-parent heterosis for these crosses were observed in Table III (56.20 and 54.24%, respectively).

The results showed that heterotic pattern of LSC × RYD would be potentially useful in maize breeding programs to obtain high-yielding hybrids in the same climate of Iran. It was consistent with Choukan (2006), who evaluated 45 maize inbred lines for grain yield by line × tester analysis and reported LSC × RYD as promising heterotic pattern [6]. In contrast, Mostafavi *et al.* (2009) using the estimates of specific combining ability from diallel crosses with several maize inbred lines suggested RYD × CIMMYT heterotic pattern for grain yield [19]. Furthermore, MO17 line derived from LSC can be used for grain yield improvement of other lines adapted to Iran growing conditions. Choukan *et al.* (2006) using SSR marker to evaluate lines from maize breeding programs in Iran also showed that the best heterotic pattern include lines from group RYD crossed to lines from group LSC and this pattern has produced some of the highest yielding hybrids in Iran [7]. Limited research, however, has been reported on established heterotic patterns in Iran, and much of the breeding material has unknown pedigrees and sources. Therefore, we propose that the biplot analysis of diallel data information presented in this study could be used to determine heterotic groups and patterns as well as best hybrid combinations, and hybrid maize breeding efforts may be greatly boosted by use of this information.

## REFERENCES

- [1] R. W. Allard, Principles of plant breeding, 2nd edition, John Wiley and Sons, Inc. USA, 1999.
- [2] C. Barata, and M. Carena, "Classification of North Dakota maize inbred lines into heterotic groups based on molecular and testcross data," *Euphytica*, vol. 151, pp. 339-349, 2006
- [3] O. B. Bello, and G. Olaoye, "Combining ability for maize grain yield and other agronomic characters in a typical southern guinea savanna ecology of Nigeria," *African Journal of Biotechnology*, vol. 8, pp. 2518-2522, 2009.
- [4] S. Bhatnagar, F. J. Betran, and L. W. Rooney, "Combining ability of quality protein maize inbreds," *Crop Science*, vol. 44, pp. 1997-2005, 2004.
- [5] R. Choukan, "Evaluation of grain yield and yield components in maize using diallel crosses," *Iranian Journal of Crop Science*, vol. 3, pp. 1-8, 2001.
- [6] R. Choukan, "Heterotic grouping of maize inbred lines based on specific combining ability with tester lines," *Seed and Plant*, vol. 22, pp. 399-409, 2006.
- [7] R. Choukan, A. Hossainzadeh, M. R. Ghannadha, M. L. Warburton, A. R. Talei and S. A. Mohammadi, "Use of SSR data to determine relationships and potential heterotic groupings within medium to late maturing Iranian maize inbred lines," *Field Crops Research*, vol. 95, pp. 212-222, 2006.
- [8] D. S. Falconer, Introduction to quantitative genetics, 3rd edition, Longman grp., Essex, 1979.
- [9] X. M. Fan, H. M. Chen, J. Tan, C. X. Xu, Y. D. Zhang, L. M. Luo, Y. X. Huang and M. S. Kang, "Combining abilities for yield and yield components in maize," *Maydica*, vol. 53, pp. 39-46, 2008.
- [10] X. M. Fan, J. Tan, J. Y. Yang and H. M. Chen, "Combining ability and heterotic grouping of ten temperate, subtropical and tropical quality protein maize inbreds," *Maydica*, vol. 49, pp. 267-272, 2004.
- [11] X. M. Fan, J. Tan, J. Y. Yang, F. Liu, B. H. Huang and Y. X. Huang, "Study on combining ability for yield and genetic relationship between exotic tropical, subtropical maize inbreds and domestic temperate maize inbreds," *Scientia Agriculture Sinica*, vol. 35, pp. 743-749, 2002.
- [12] W. R. Fehr, Principles of cultivar development, McGraw-Hill Inc., New York, 1987.
- [13] C. O. Gardner, and S. A. Eberhart, "Analysis and interpretation of the variety cross diallel and related populations," *Biometrics*, vol. 22, pp. 439-452, 1966.
- [14] S. Gonzalez, H. Cordova, S. Rodrigue, H. de Leon and V. M. Serrato, "Determinacion de un patron heterotico a partir de la evaluacion de un dialelo de diez lineas de maiz subtropical," *Agron. Mesoamericana*, vol. 8, pp. 1-7, 1997.
- [15] B. Griffing, "Concept of general and specific combining ability in relation to diallel crossing systems," *Australian Journal of Biological Science*, vol. 9, pp. 463-493, 1956.
- [16] G. C. Han, S. K. Vasal, D. L. Beck and E. Elis, "Combining ability of inbred lines derived from CIMMYT maize (*Zea mays* L.) germplasm," *Maydica*, vol. 36, pp. 57-64, 1991.
- [17] K. D. Kauffman, C. W. Crum and M. F. Lindsey, "Exotic germplasm in a corn breeding program," *Illinois Corn Breeding School*, vol. 18, pp. 6-39, 1982.
- [18] M. D. Melani, and M. J. Carena, "Alternative maize heterotic pattern for the Northern Corn Belt," *Crop Science*, vol. 45, pp. 2186-2194, 2005.
- [19] K. H. Mostafavi, R. Choukan, M. Taeb, M. R. Bihamta and E. Majidi Heravan, "Study of the gene action in controlling agronomic traits in maize (*Zea mays* L.) using diallel crossing design," *Iranian Journal of Crop Science*, vol. 10, pp. 331-348, 2009.
- [20] J. C. Reif, A. E. Melchinger, X. C. Xia, M. L. Warburton, D. A. Hoisington, S. K. Vasal, D. Beck, M. Bohn and M. Frisch, "Use of SSRs for establishing heterotic groups in subtropical maize," *Theoretical Applied Genetics*, vol. 107, pp. 947-957, 2003.
- [21] J. S. C. Smith, and O. S. Smith, "Fingerprinting crop varieties," *Advances in Agronomy*, vol. 47, pp. 85-129, 1992.
- [22] J. R. Sughroue, and A. R. Hallauer, "Analysis of the diallel mating design for maize inbred line," *Crop Science*, vol. 37, pp. 400-405, 1997.
- [23] A. Terron, E. Preciado, H. Cordova, H. Mickelson and R. Lopez, "Determinacion del patron heterotico de 30 lineas de maiz derivadas de la poblacion 43SR del CIMMYT," *Agron. Mesoamericana*, vol. 8, pp. 26-34, 1997.
- [24] W. Yan, "GGEBiplot – a Windows application for graphical analysis of multi-environment trial data and other types of two way data," *Agronomy Journal*, vol. 93, pp. 1111-1118, 2001.
- [25] W. Yan, and A. Hunt, "Biplot analysis of diallel data," *Crop Science*, vol. 42, pp. 21-30, 2002.
- [26] Y. Zhang, M. S. Kang and K. R. Lamkey, "DIALLEL- SAS05: A Comprehensive Program for Griffing's and Gardner- Eberhart Analysis," *Agronomy Journal*, vol. 97, pp. 1097-1106, 2005.