COMBINING ABILITY IN MAIZE SINGLE CROSS HYBRIDS FOR GRAIN YIELD: A GRAPHICAL ANALYSIS

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ABSTRACT

Combining ability estimates are important genetic attributes to maize breeders in anticipating improvement via hybridization and selection. This study sought single cross maize hybrids (SC) that could be used for the production of double cross maize hybrids (DC) for improved grain yield. Five commercial SC hybrids were crossed following a complete diallel fashion. All the DC hybrids along with self progenies were evaluated for combining abilities and to determine heterotic effects among the SC hybrids, using biplot diallel analysis at the KPK Agricultural University, Peshawar, Pakistan during 2007. General combining ability (GCA) and specific combining ability (SCA) effects were significant for the studied trait. Genotype hyb-4 was identified to be the best general combiner for grain yield, while high SCA effects were observed for crosses hyb-4 × HYB-3, hyb-4 × HYB-1 and hyb-5 × HYB-2. The graphical demonstration proposed by the biplot analysis provided an effective overview of GCA and SCA effects, mean performance in crosses, as well as grouping of similar genotypes on the basis of heterosis. Moreover, the biplot also provided an opportunity for assessing the interrelationship among the genotypes.

INTRODUCTION

Maize (*Zea mays* L.) is the most important crop after wheat in Pakistan's Khyber Pukhtunkhuwa (KPK) where it was grown on about 0.51 million hectares with a total production of 0.96 million tons and average yield of 1.88 ton ha^{-1} (Anonymous, 2009). Maize is the primary crop in majority of the farming systems and also staple food of the rural population and poor resource farmers in KPK (Khan *et al.,* 2003). The maize demand is considerably increasing due to poultry and livestock feed industries. Regardless of pronounced developments in maize research, the average maize grain yield in KPK (1.88 ton ha^{-1}) is far below than national average yield (3.4 ton ha^{-1}). The use of outdated open pollinated varieties is a major factor responsible for low grain yield of maize in KPK. The poor resource farmers can not afford the high cost single cross maize hybrid, and its use is almost negligible. It is confirmed that single cross hybrids are more productive than double cross hybrids, but keeping in view the low socio-economic status of the farming community, the use of double cross hybrids will be much better rather than use of low yielding open pollinated varieties. The double cross hybrids will obviously increase the per unit area production, which is also the primary objective in maize breeding programs. Maize breeding programs mostly involve hybridization, evaluation and selection of desirable genotype(s). In addition, the assessment of combining ability and heterotic effects are also elementary tools for selection of desirable genotype. The concept of combining ability was introduced by Sprague and Tatum (1942). Combining ability has a prime importance in plant breeding since it provides information for the selection of parents and also provides information regarding the nature and magnitude of involved gene action. The knowledge of genetic structure and mode of inheritance of different characters helps breeders to employ suitable breeding methodology for their improvement (Kiani *et al.,* 2007).

Diallel analysis is primary method and has been widely used to determine combining ability, heterotic responses and patterns in maize populations (Bertoia *et al.,* 2006; Hallauer and Miranda Filho,

1981). Conventional diallel analysis (Griffing 1956) limited to partitioning total variation into GCA of the parents and SCA of the crosses. GCA is average performance of a parent in a series of crosses and SCA designates those cases in which certain combinations perform relatively better or worse than would be expected on the basis of average performance of lines involved. The GCA includes additive and additive × additive variances, while SCA responsible for non-additive genetic variances.

Diallel data is usually analyzed through Griffing (1965) however, in near past Yan and Hunt (2002) suggested the application of GGE biplot techniques for graphical analysis and presentation of diallels. Although the GGE biplot methodology was developed for multi-environment trial (MET) data analysis, but they stated that it should be applicable to all types of two-way data that assume an entry-by-tester data structure. In MET data, genotypes are entries and environments are testers. In diallel data, each genotype is both an entry and a tester (Yan and Hunt, 2002). The GGE biplot technique has also been used for diallel combining ability analysis of forage maize landraces (Bertoia *et al.,* 2006). Thus keeping in view the importance of diallel analysis and the aforesaid problems of poor farmers, the present study was undertaken to identify superior double cross maize hybrid(s) for general cultivation, genetic potential of maize hybrids which may be used in future maize breeding program and heterotic groups among single cross hybrids by principal component biplot analysis.

MATERIALS AND METHODS

Five single cross (SC) maize hybrids from different seed companies were used in this study. These SC hybrids were crossed following a complete diallel fashion to produce double cross (DC) hybrids during spring season during 2007. All the DC hybrids (including reciprocals) and self progenies were evaluated along with parents per se (parental single cross hybrids) in randomized complete block design with three replications in the following summer season (July to October, 2007) at KPK Agricultural University, Peshawar, Pakistan. Each plot consisted of four 5 meter rows and 0.75 m apart. Two seed per hill were planted, which were later thinned to have a population density of 53,333 plants ha^{-1} at 4-5 leaf stage. Standard cultural practices were applied uniformly at all experimental units. At maturity central two rows from each plot were separately harvested and the fresh ear weight was measured in each plot. Grains were shelled from five randomly selected cobs to observe the percent grain moisture at harvest for each plot. Grain yield (kg ha^{-1}) was estimated for each plot from the data of fresh ear weight.

Table I   *Analysis of variance for grain yield of five maize hybrids, and their crosses*

Source	df	Sum of squares	Mean squares	F-values	Probability
Replication	2	2214775.66	1107387.83	2.8645	0.0668
Female parent (F)	4	12509509.86	3127377.46	8.0897	0.0000
Male parent (M)	4	2663431.96	665857.99	1.7224	0.1605
Crosses (F×M)	16	39184834.39	2449052.15	6.3351	0.0000
Error	48	18556098.21	386585.379		
Total	74	75128650.08			
CV = 14.29 %					

Statistical Analysis

The grain yield data was subjected to analysis of variance. Since, crosses effect was significant (Table I) then the biplot analysis was performed following the method of Yan and Hunt, (2002) and Bertoia *et al.,* (2006). Applying GGE biplot methods to diallel data, the terms "average yield" and "stability" of the genotypes correspond to GCA and SCA, respectively. The mean values for hybrids and parental populations across environments are used to form a symmetrical diallel data matrix from which the first two principal components (PC1 and PC2) were extracted. In this matrix, each population corresponds to one row and one column of data, where the row is considered an "entry" and the column a "tester" (Yan and Hunt, 2002). Thus, each population can be considered both an entry and a tester. Means of each column are calculated and a new, adjusted (nonsymmetrical) data matrix is obtained by subtracting the column (tester) mean from each cell. After obtaining the first two principal components of the adjusted data matrix, the biplot model can be written as:

$$Y_{ij} - \hat{a}_j = \ddot{e}_1 \hat{i}_{i1} \, \varsigma_{j1} + \ddot{e}_2 \hat{i}_{j2} \, \varsigma_{j2} + \mathring{a}_{ij}$$

Where Y_{ij} is the genotypic value of the cross between entry *i* and tester *j* for the trait of interest; $â_j$ is the mean of all crosses involving tester *j*; $ë_1$ and $ë_2$ are the singular values for PC1 and PC2 respectfully; $î_{i1}$ and $î_{i2}$ are the PC1 and PC2 eigenvectors, respectively, for entry *i*; $ç_{j1}$ and $ç_{j2}$ are the PC1 and PC2 eigenvectors, respectively, for tester *j*; and $å_{ij}$ is the residual of the model associated with the combination of entry *i* and tester *j*. The eigenvectors (PC1 & PC2) and biplot axis scores (X-axis & Y-axis) for entries and testers are presented in Table II. Since in diallel data each genotype is both an entry and a tester, *i* and *j* can refer to the same or different genotypes. When $i = j$, the combination is a pureline (self progeny) rather than a hybrid. Principal components scores for entries and testers were scaled symmetrically (Yan and Hunt, 2002; Bertoia *et al.,* 2006). The analyses reported in this study were conducted using the GGE-biplot software, a windows application that fully automates biplot of two-way data (Yan, 2001). As mentioned earlier that in GGE biplot each genotype is both an entry and a tester, therefore, all the biplots presented in this study and in the results and discussion section the parental lines (single cross hybrids) are termed as "entry" and the crosses (double cross hybrid) are termed as "testers".

Table II *Biplot axis scores (X-axis and Y-axis) and eigenvectors (PC1 and PC2) for the entries and testers*

	Axis scores		Eigenvectors	
Name	**X-axis**	**Y-axis**	**PC1**	**PC2**
Entries				
Hyb-1	-14.794	1.022	0.226	0.024
Hyb-2	21.523	-34.161	-0.328	-0.805
Hyb-3	-44.949	-2.229	0.686	-0.053
Hyb-4	39.893	17.067	-0.609	0.402
Hyb-5	-1.674	18.301	0.026	0.431
Testers				
HYB-1	5.983	13.197	-0.129	0.284
HYB-2	-8.235	43.993	0.177	0.945
HYB-3	44.577	6.184	-0.958	0.133
HYB-4	2.848	-3.746	-0.061	-0.08
HYB-5	8.238	2.227	-0.177	0.048

RESULTS AND DISCUSSION

General and Specific Combining Ability

The Biplot for grain yield data explained 76.6% (54 and 22.6% by PC1 and PC2, respectively) of the total variation (Fig. 1). The general combining ability (GCA) and specific combining ability (SCA) effects of the single crosses (entries) were examined by drawing an average tester coordinate (ATC) view for entries. The GCA effect of the entries was approximated by the projection of their markers to the ATC X-axis (the single arrowed line; the direction indicated the positive end) while the SCA of the entries was approximated by the projection of their markers to the ATC Y-axis (double arrowed line) (Yan and Hunt, 2002; Bertoia *et al.,* 2006). As shown in Fig. 1a, entry hyb-4 has a largest projection onto ATC X-axis exhibiting the highest and positive GCA effect for grain yield. Similarly, hyb-5 also showed higher and positive GCA effects. Whereas, the entries hyb-3, hyb-2 and hyb-1 were located on the left side of the ATC Y-axis (in the opposite direction of ATC X-axis) indicating the lowest and negative GCA effects. The Biplot also displayed the SCA effects of the entries, the vectors length help in ranking the entries as shown in Fig. 1a. Since, the entries hyb-2 and hyb-3 have the largest projections on to the ATC Y-axis exhibiting that they have the highest SCA effects for grain yield. Similarly hyb-4 also showed highest and positive SCA effects. Whereas, entries hyb-1 and hyb-5 showed smallest SCA effects (small projections on to ATC Y-axis). Based on heterosis, two different groups were suggested (Fig. 1a). First group contained hyb-2 and hyb-4 while the second group has hyb-1, hyb-3 and hyb-5. Therefore, six crosses i.e. [hyb-4, hyb-2] × [hyb-1, hyb-3, hyb-5] are expected to be heterotic (better than both parents).

Best Double Cross Hybrid Combinations

The biplot (Fig. 1b) provided the best way to demonstrate the interaction patterns between entries and testers as mentioned by Yan et al., (2000) and Yan and Hunt (2002). A polygon view is shown in the biplot, such that four entries are on the vertices while one is inside the polygon. Since the vertex entries have the largest distances from the origin, they are most responsive to the change of testers relative to other entries within respective groups. According to Yan and Hun (2002), vertex entries are either the best or the

poorest mating partners with some or all of the testers. The polygon view also provides a way to group the testers based on their best mating partners. In such away that testers which fall in the same sector share the same best mating partner (i.e. entry at the vertex in that sector) and those fall in different sectors have different best mating partners.
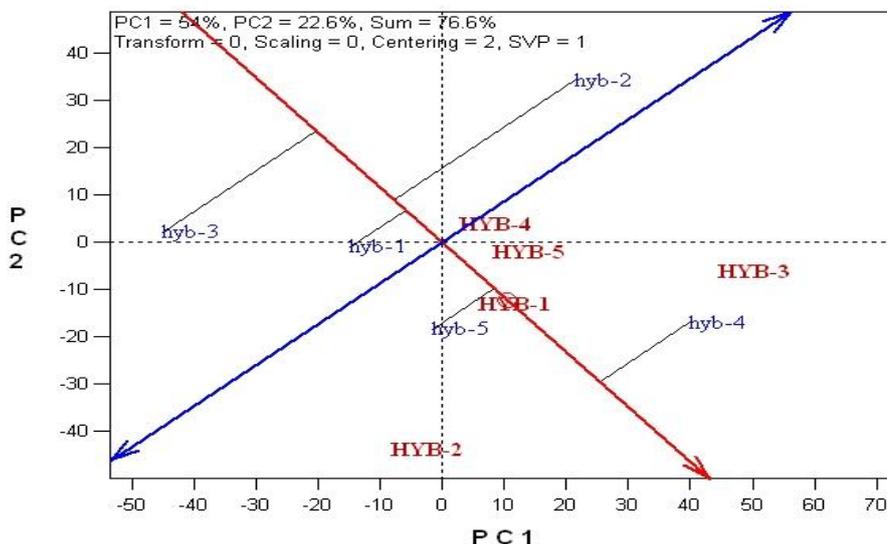

Fig. 1.   Biplot based on diallel data of five maize hybrids for grain yield. (A) Average tester ordination view; (B) Polygon view. Codes for genotypes are: HYB-1 = ICI-974, HYB-2 = Opener, HYB-3 = CS-201, HYB-4 = CS-222, HYB-5 = Pioneer 3025. Genotypes are labeled with uppercase letters when viewed as testers (parents, single crosses) and with lowercase letters when viewed as entries (double crosses)
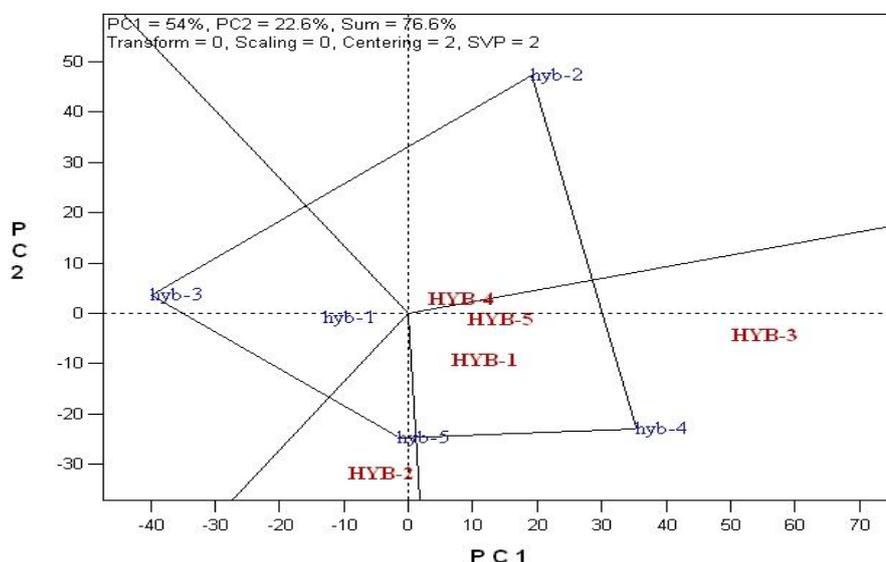
The biplot was divided into four sectors with entries hyb-4, hyb-5, hyb-3 and hyb-2 as the vertex entries, and are referred as 1st, 2nd, 3rd and 4th sectors, respectively (Fig 1b). No tester fell in the 3rd and 4th sectors, depicting that the vertex entries (hyb-3 and hyb-2 respectively) of the said sectors were not the best mating partner, with any of the tester, and produced worst combinations. In the 1st sector where hyb-4 is a vertex entry, produced best hybrids with tester HYB-3, HYB-5 and HYB-1. These combinations hyb-4 × HYB-3, hyb-4 × HYB-5 and hyb-4 × HYB-1 produced a grain yield of 6108, 5294 and 5202 kg ha⁻¹,

respectively (Table III). Moreover, tester HYB-4 is not fallen in the same sector, which may due to heterotic responses (Yan and Hunt, 2002). A single tester HYB-2 fell in 2nd sector, meaning that entry hyb-5 (vertex entry) was the best mating partner for HYB-2. The tester HYB-5 was not found in the same sector, therefore the cross hyb-5 × HYB-2 (4993 kg ha^{-1}) was heterotic. Based on original data (Table III), there were other best combinations such as hyb-2 × HYB-3 (5204 kg ha^{-1}) and hyb-1 × HYB-4 (5278 kg ha^{-1}) but were not predicted to be heterotic by the biplot. The possible reason may be that these crosses were apparently inferior to those that were identified to be superior based on Fig 1b. As mentioned earlier that the biplot explained only 76.6% of the total variation rather than 100%, therefore such contradictions are expected to be raised (Yan and Hunt, 2002).

Table III *Mean grain yield of double cross progenies (including reciprocals), and self progenies (diagonal) of five maize single cross hybrids.*

	Testers					
Entries	**HYB-1**	**HYB-2**	**HYB-3**	**HYB-4**	**HYB-5**	**Entries Mean**
	-------------------------------------Kg ha^{-1}------------------------- --------					
Hyb-1	<u>3540</u>	4453	3731	5278	4820	4364 b
Hyb-2	3953	<u>2379</u>	5204	4790	4197	4105 bc
Hyb-3	4617	4103	<u>2249</u>	4039	4209	3843 c
Hyb-4	5202	4314	6108	<u>4425</u>	5294	5068 a
Hyb-5	4191	4993	4692	4643	<u>3361</u>	4376 b
Testers Mean	4300	4048	4397	4635	4376	
Parent per se (check)	5187	4392	5496	3253	6637	

Means with different letters are significantly different at 5% level of probability.
$LSD_{0.05}$ value for entry means = 456.5

Best Tester for GCA

The average tester coordinate view for testers (Fig. 2a), demonstrates the discriminating ability and representativeness of the testers. Yan and Hunt (2002) suggested that the tester(s) with high GCA effects must have the ability to distinguish the entries and be highly interpreter of all testers. Therefore, the discriminating ability of the testers can be approximated by the projection of their marker to the ATC X-axis (longer the projection the more discriminating will be the tester) and representativeness of the tester is approximated by the projection of their markers to the ATC Y-axis (smaller the projection the more representative will be the tester). Thus, tester HYB-3 exhibited good GCA effects and supposed to be best tester. Following the hypothesis of Yan and Hunt (2002), the GCA effects of the entries should be reasonably assessed by the performance of their hybrid with best tester. The results further revealed that the entries were in the order of hyb-4 > hyb-2 > hyb-5 > hyb-1 > hyb-3, based on the actual values of the hybrids with tester HYB-3 (Table III), which roughly were in agreement with the order of hyb-4 > hyb-5 > hyb-1 > hyb-2 > hyb-3, based on their GCA effects (Fig 1a).
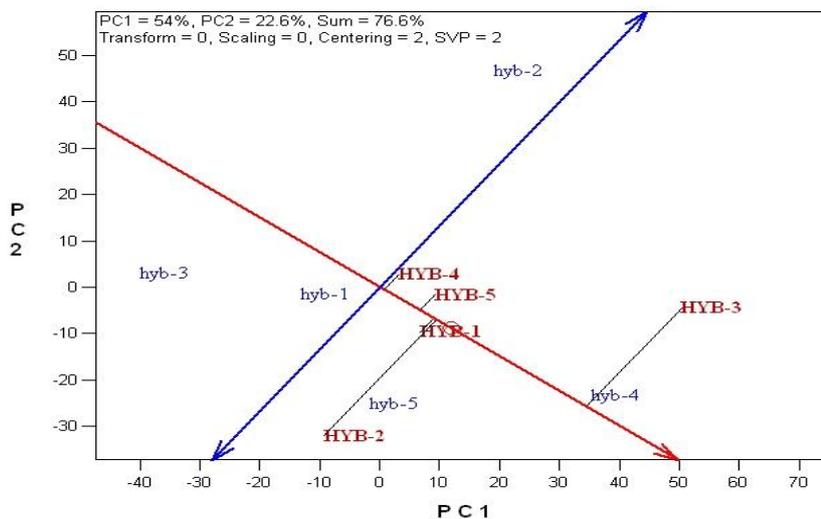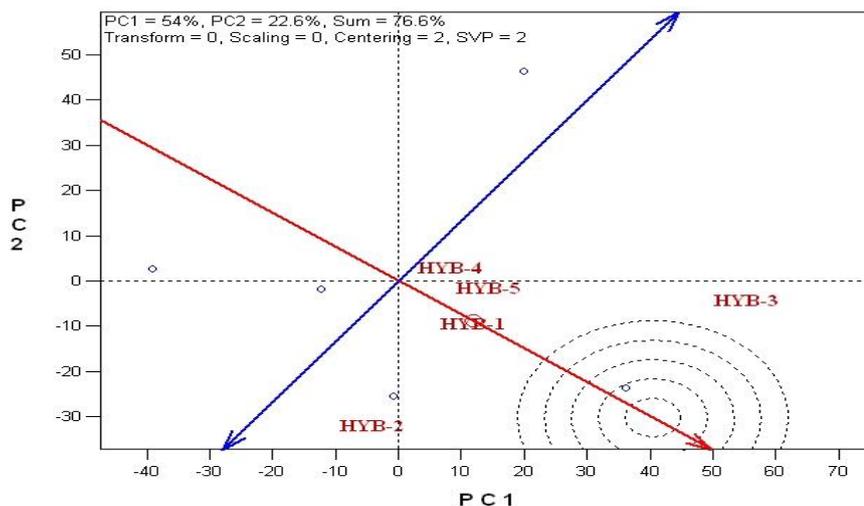
Fig. 2.   Biplot for general combining ability (GCA) of the testers. (A) Average tester ordination view for GCA of testers. (B) Comparison of testers with "ideal" tester

Relationship among Genotypes

The interrelationship among genotypes is visualized in Fig. 3. The lines that connect the biplot origin and the markers of the genotypes are known as vectors. The angle between vectors of two genotypes relates to the correlation coefficient between them, and the linear map to the right of the graph (in degrees) help in explaining the relationship among them (Yan and Kang, 2003 and Fan, *et al.* 2007). For positive relationship between two genotypes the angle between their vectors must be smaller than 90° (Yan 2002). Thus, based on Fig 3a, entries hyb-1 and hyb-3 showed a strong and positive relationship between them, and were too close to each other (almost 0° angle). The entry hyb-5 is also lying close to both hyb-1 and hyb-3 (angle < 90°), and predicted a positive relationship among them. Similarly, the angles between hyb-5 and hyb-4, hyb-4 and hyb-2 were also smaller which also exhibited the positive relationship among them. However, the negative relationship was observed between two groups i.e (hyb-1, hyb-3) and (hyb-4, hyb-2), meaning that these genotypes were apparently different from one another. Tester HYB-3 and HYB-5 showed positive and strong relati*onshi*p (Fig. 3b). Moreover, the testers (HYB-3 and HYB-5) showed an equal and positive relationship with HYB-4 and HYB-1, since they are located in-between their vectors, but tester HYB-1 and HYB-4 were negatively correlated (angle > 90°). Similarly, HYB-2 and HYB-4 were located in opposite directions (angle > 140°) exhibiting negative relationship.
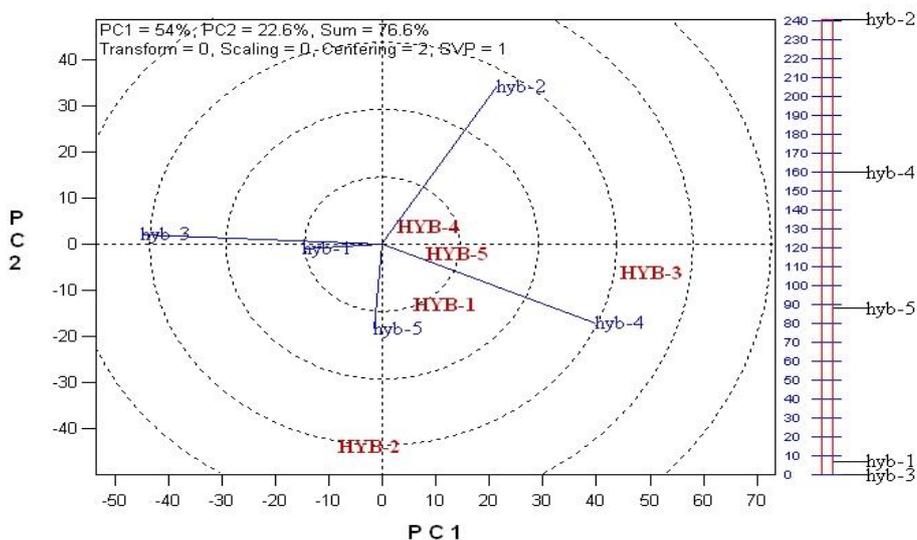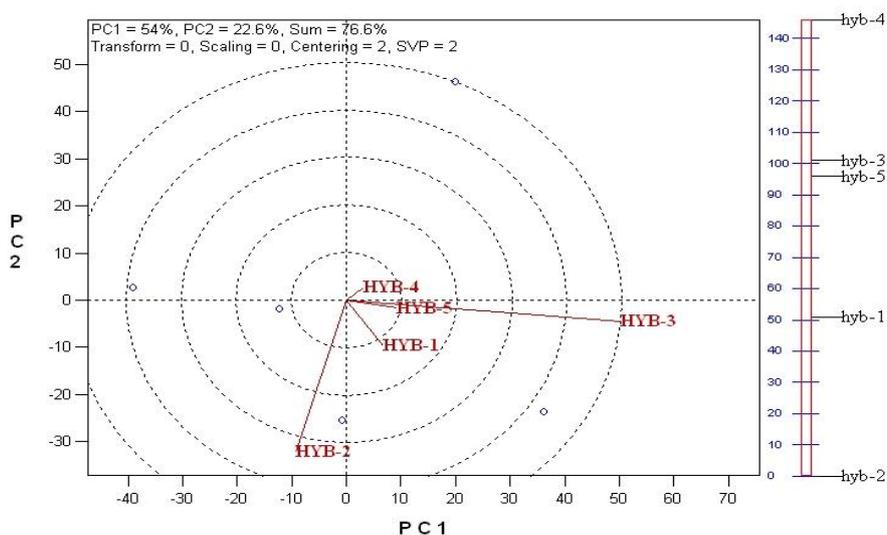
Fig 3.   *Biplot showing relationship among genotypes. (A) Relationship among entries (progenies); (B) Relationship among testers (parents)*

CONCLUSION AND RECOMMENDATIONS

The significance of GCA and SCA effects suggested the importance of both additive and non-additive gene actions for grain yield. The higher GCA effects of hyb-4 indicated that this parent may be preferred for hybridization and selection programs. The SCA effects revealed that for production of double cross maize hybrid.

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