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# ROLE OF GENETIC DIVERSITY AND COMBINING ABILITY AND HETEROSIS IN MAIZE (*ZEA MAYS* L.) HYBRIDS UNDER DROUGHT STRESS

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*Key words:* Genetic diversity, Combining ability, Heterosis, Maize, Mahalanobis distance, Drought stress

**Abstract**

Water stress during flowering stage is a key factor effecting maize productivity in India. Mahalanobis $D^2$ method was used to group one hundred and thirty-three inbred lines based on their phenotypic performance under waters stress condition. Fifteen selected lines were crossed in half diallel fashion to produce 105 direct $F_1$ crosses which were then evaluated under water stress and non-stress condition. Negative correlation was observed between parents mahalanobis distance and grain yield suggesting parents with diverse phenotypic expression need not always produce heterotic hybrids. Mid-parent heterosis showed linear relationship with grain yield and none of the combining ability effects showed significant association with grain yield. Thus, *per se* performance of inbred lines across the traits under water stress situation has to be considered in selection of parents for production of heterotic hybrids.

## Introduction

Maize (*Zea mays* L.) is one of the important cereal crops in the world agricultural economy as food (Morris *et al*. 1999) for humans, fuel for animals and as a crop of industrial utilization (White and Johnson 2003). Abiotic stress is a major constraint in increasing of productivity of cereal crops. Yield losses of 63–87 % due to moisture stress and 42% due to heat stress have been recorded (Kamara *et al*. 2003) in maize (*Zea mays* L.). For every degree of an increase in global mean temperature, average maize yields are projected to decrease by 7.4% (Tigchelaar *et al*. 2018). India ranks $4^{th}$in area but is $7^{th}$ in production of maize, this gap in ranking is attributed to low productivity of 29.46 q/ha when that compared to 57.50 q/ha of world (FAOSTAT, 2018). Over 83% of maize is grown in India during kharif season and of that 70% of the area is under rainfed condition (DAC and FW 2020). Maize is widely regarded to be susceptible to drought stress during flowering stage than other cereal crops due to its monoecious type of flowers where both male and female flowers are born on different parts of plants.

Heterosis has been successfully exploited in maize with exponential increase in yield. Various hypothesis such as dominance, over dominance and epistasis have been suggested to explain the feature among crops. To estimate the quantum of heterosis in $F_1$ generation Falconer and Mackay (1996) gave formula $H_{F1} = \sum dy^2$, as per which genetic distance ($y$) and gene action ($d$) are two key factors in order to obtain a heterotic hybrid. Mahalanobis $D^2$ method is widely used statistical method used to analyse the genetic diversity among genotypes using phenotypic traits. The concept of general combing ability and specific combining ability (Sprague and Tatum 1942) which are representative of additive and non-additive gene action respectively is employed by breeders to select parental lines and crosses in heterosis breeding.

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In maize appreciable percentage of combining ability and heterosis for yield and other traits were studied by several workers. Aminu *et al*. (2014) reported significant difference of GCA effects of parents and that of SCA effects of hybrids for grain yield and other agronomic traits and that both additive gene effects & non-additive gene effects controlled most traits but non-additive genetic effects were most prevalent. Thus the present study was carried out to assess the genetic diversity, combining ability and gene action in selection of parents to obtain heterotic hybrids.

**Materials and Methods**

One hundred and thirty-three $S_5$ generation inbred lines derived from various populations, synthetics and public sector hybrids through pedigree method were evaluated under two regimes namely irrigated non-stress and water stress condition at two locations *viz.,* Botanical Garden, College of Agriculture, Dharwad Karnataka and Maize Research Center and Seed Farm (MRC&SF), Devihosur (Haveri) Karnataka during summer 2018-19 in randomized complete block design (RCBD) with two replications.

Water stress was induced by withholding irrigation one week before flowering such that water stress is built up during flowering stage and crop has to rely on remaining soil moisture to complete its crop cycle. The normal and water stress plots were separated by a gap of 5 meter to avoid seepage of water below the soil. Twelve traits namely days to anthesis, days to silking, days to maturity, plant height (cm), ear height (cm), cob length (cm), cob girth (cm), number of kernel rows, number of kernels per row, hundred seed weight (g), shelling per cent (%) and grain yield (kg/ha) were recorded. Drought susceptibility index was calculated by Fisher and Maurer (1978).

To assess the genetic diversity among the inbred lines using phenotypic data, the multivariate statistical method Mahalanobis $D^2$ method was followed. Two datasets *i.e.,* non-stress data and water stress data were formed by pooling (average) the data for each of the twelve traits across both the locations Dharwad and Devihosur. Assuming multivariate gaussian distribution of variables mahalanobis distance is calculated as follows:

$$D_i^2 = (x_i - \mu)' \Sigma^{-1} (x_i - \mu)$$

where, $D_i^2$ = D square value of $i^{th}$ observation, $x_i$ = $i^{th}$ observation, $\mu$ = overall mean across the variables, $\Sigma^{-1}$ = inverse of variance-covariance matrix. The $D^2$ value is a measure of how far an observation (x) is from the center of distribution taking into account all the variables and their covariances (Mahalanobis, 1936). The mahalanobis distance and cluster grouping were carried out using '*mahalanobis'* function and '*biotools'* package of R program (R core team 2020).

Fifteen inbred lines selected based on the *per se* performance, drought susceptibility index (DSI) and mahalanobis distance (Table 1) were crossed in diallel mating design 2 (half Diallel) to produce 105 direct single cross hybrids. These hybrids along with their 15 parents and three checks were evaluated in irrigated non-stress and water stress condition of four meter plot containing two rows (60 x 20 cm) with two replications at MRC&SF, Devihosur (Haveri), Karnataka in RCBD during *rabi* 2019-20. Combining ability effects and variances were estimated by Griffing (1956) using "*gpbStat*" package of R program (Patil and Gangavati 2021).

**Results and Discussion**

Grain yield per se performance of inbred lines was reduced by 42.02% due to water stress from 3,959 kg/ha (non-stress) to 2,295 kg/ha (water stress). Flowering traits such as days to anthesis, days to silking and days to maturity saw a minimum mean reduction of 2.65%, 3.38%

and 6.88% respectively. Mahalanobis cluster analysis identified seven clusters under non-stress condition and eleven clusters under water stress condition. More number of clusters were formed under water stress conditions indicating varied phenotypic expression of lines under water stress. The 105 hybrids were classified into two types i.e., intra, if both the parents belong to same cluster (water stress) and inter if both the parents belong to different clusters (water stress). Thirty-seven hybrids were of intra type (parents in same cluster) with a mean grain yield of 8,452 kg/ha and sixty-eight hybrids were of inter type (parents in different cluster) with a mean of 8,236 kg/ha. Results of independent t-test (unequal variance) showed no significant ($p = 0.43$) difference exists between the mean of the two groups. Mahalanobis distance ($D^2$) of inbred lines represents divergence from the centroid. Under water stress condition highest $D^2$ values were shown by lines DIL-55 (46.82), DIL-131 (27.79), DIL-44 (22.92), DIL-86 (22.07), DIL101 (21.40), DIL-106 (21.25), DIL-28(21.07). $D_{ij}$ of a single cross hybrid is the absolute difference in the mahalanobis distance of both the parents (under water stress). The magnitude of $D_{ij}$ represents the sense of how distant the parents are from each other. Hybrids with highest $D_{ij}$ values is presented in Table 2. Hybrids DIL-44 x DIL-60 (16.25), DIL-12 x DIL-44 (15.80), DIL-14 x DIL-44 (15.47) and DIL-10 x DIL-60 (14.13) showed highest $D_{ij}$ values indicating distinct nature of their parents. Spearman's rank correlation coefficient between $D_{ij}$ and grain yield was -0.25 ($p \leq 0.01$) indicating negative association between two variables. Larger genetic distance between parents need not always be manifested into higher heterosis. Thus, careful selection of parents which harbor useful alleles is critical in achieving maximum heterosis. Prasad and Singh (1986) observed that parents with moderate (intermediate) parental diversity produced higher heterosis than the extreme ones.

Average grain yield of 105 direct crosses decreased from 8,312 kg/ha in non-stress situation to 3,587 kg/ha under water stress situation with a 56.60 % reduction. Diallel analysis revealed significant gca and sca variance for grain yield indicating presence of ample variation among both additive and non-additive genes among the hybrids. The gca effects of both parents (water stress) were added to calculate sum of gca and combining ability effect was calculated by adding sum of gca and sca effects (water stress). Top three hybrids with highest sum of gca effects, sca and combining ability effects is presented in Table 3. Hybrids DIL-12 x DIL-14 (636.30), DIL-14 x DIL-23 (635.75) and DIL-14 x DIL-58 (624.25) recorded highest sum of parental gca effects whereas hybrids DIL-58 x DIL-60 (2,049.09), DIL-60 x DIL-78 (1,834.19) and DIL-58 x DIL-80 (1,812.87) showed highest sca effects. Similarly, DIL-1 x DIL-14 (1,928.40), DIL-12 x DIL-51 (1,898.75) and DIL-1 x DIL-58 (1,889.84) recorded highest combining ability effects. Best performing hybrids for grain yield (Table 4) under water stress condition were DIL-12 x DIL-23 (5,465 kg/ha), DIL-58 x DIL-60 (5,442 kg/ha), DIL-44 x DIL-64 (5,390 kg/ha) and DIL-78 x DIL-107 (5,374 kg/ha).

Spearman correlation was calculated between the ranks of sum of gca, sca, combining ability, mid-parent heterosis and grain yield. Significant correlation of 0.29 ($p \leq 0.01$) was observed between sum of gca and combining ability similarly significant association of 0.96 ($p \leq 0.01$) was detected between sca and combining ability. As none of the gene action related variables such as gca, sca and combining ability showed significant correlation with grain yield, a pattern can’t be established between gene action and grain yield. Non-additive gene actions *viz.*, dominance, over dominance and epistasis played a prominent role in the development of plants under water stress condition. Recent studies have indicated the role of epigenetics in regulation of heterosis among various crops (Groszmann *et al.* 2013). Mechanisms such as DNA methylation, histone modification, and small RNAs influence the expression of heterotic hybrids (He *et al.* 2013). Whereas, grain yield and mid-parent heterosis showed near perfect correlation 0.99 ($p \leq 0.01$) indicating linear relationship between the two variables further indicating parental lines with high

**Table 1. Mahalanobis distance, Cluster group and Drought susceptibility index of 15 parental inbred lines under non-stress and water stress conditions.**

| Parent | Cluster ID under non-stress | Cluster ID under Stress | $D^2$ value under non-stress | $D^2$ value under water stress | Grain yield under non-stress (kg/ha) | Grain yield under water stress (kg/ha) | Drought susceptibility index (DSI) |
|---|---|---|---|---|---|---|---|
| DIL-1 | 3 | 8 | 8.02 | 12.01 | 4,661 | 3,427 | 0.46 |
| DIL-6 | 6 | 5 | 16.49 | 16.42 | 5,967 | 2,995 | 0.86 |
| DIL-10 | 2 | 8 | 9.76 | 20.80* | 4,405 | 3,490 | 0.36 |
| DIL-12 | 2 | 5 | 6.77 | 7.12 | 4,598 | 3,067 | 0.57 |
| DIL-14 | 2 | 9 | 6.11 | 7.45 | 4,015 | 3,178 | 0.36 |
| DIL-23 | 2 | 5 | 12.77 | 10.41 | 4,166 | 3,077 | 0.45 |
| DIL-39 | 2 | 5 | 12.47 | 13.87 | 4,332 | 3,006 | 0.53 |
| DIL-44 | 2 | 8 | 17.3 | 22.92* | 4,023 | 3,332 | 0.30 |
| DIL-51 | 2 | 5 | 10.32 | 10.43 | 4,020 | 3,036 | 0.42 |
| DIL-58 | 5 | 8 | 10.91 | 14.35 | 5,523 | 3,279 | 0.70 |
| DIL-60 | 2 | 5 | 9.16 | 6.67 | 3,930 | 3,022 | 0.40 |
| DIL-64 | 3 | 5 | 14.06 | 10.91 | 5,030 | 2,980 | 0.70 |
| DIL-78 | 2 | 8 | 9.55 | 16.73 | 4,188 | 3,446 | 0.31 |
| DIL-80 | 2 | 9 | 12.22 | 13.12 | 4,341 | 3,118 | 0.49 |
| DIL-107 | 2 | 8 | 6.89 | 11.49 | 3,801 | 3,249 | 0.25 |

* Significant at $p \leq 0.05$.

**Table 2. Top 10 single cross hybrids with highest $D_{ij}$ values under water stress condition.**

| Hybrid | Parent 1 mahalanobis distance# | Parent 2 mahalanobis distance# | $D_{ij}$ | $D_{ij}$ rank | Type | Grain yield under# (kg/ha) | Grain yield rank | Mid-parent heterosis# |
|---|---|---|---|---|---|---|---|---|
| DIL-44 x DIL-60 | 22.92 | 6.67 | 16.25 | 1 | Inter | 5,137 | 105 | 5.11 |
| DIL-12 x DIL-44 | 7.12 | 22.92 | 15.80 | 2 | Inter | 8,388 | 54 | -17.43 |
| DIL-14 x DIL-44 | 7.45 | 22.92 | 15.47 | 3 | Inter | 7,963 | 67 | -17.91 |
| DIL-10 x DIL-60 | 20.80 | 6.67 | 14.13 | 4 | Inter | 7,701 | 77 | -10.15 |
| DIL-10 x DIL-12 | 20.80 | 7.12 | 13.68 | 5 | Intra | 6,408 | 95 | -2.23 |
| DIL-10 x DIL-14 | 20.80 | 7.45 | 13.35 | 6 | Inter | 7,721 | 76 | 55.98* |
| DIL-23 x DIL-44 | 10.41 | 22.92 | 12.51 | 7 | Inter | 9,231 | 30 | -26.68 |
| DIL-44 x DIL-51 | 22.92 | 10.43 | 12.49 | 8 | Inter | 8,769 | 41 | -27.45 |
| DIL-44 x DIL-64 | 22.92 | 10.91 | 12.02 | 9 | Inter | 5,605 | 103 | 34.06 |
| DIL-44 x DIL-107 | 22.92 | 11.49 | 11.43 | 10 | Intra | 9,999 | 10 | -12.49 |
| DIL-1 x DIL-44 | 12.01 | 22.92 | 10.91 | 11 | Intra | 8,465 | 51 | -13.48 |
| DIL-10 x DIL-23 | 20.80 | 10.41 | 10.39 | 12 | Inter | 7,491 | 79 | -16.08 |
| DIL-10 x DIL-51 | 20.80 | 10.43 | 10.37 | 13 | Intra | 6,012 | 98 | 20.92 |
| DIL-60 x DIL-78 | 6.67 | 16.73 | 10.07 | 14 | Inter | 8,771 | 40 | -65.27* |
| DIL-10 x DIL-64 | 20.80 | 10.91 | 9.90 | 15 | Inter | 10,322 | 6 | 19.85 |

* Significant at $p \leq 0.05$, # under water stress condition.

**Table 3. Top three single cross hybrids with highest sum of gca, sca and combining ability along with grain yield under water stress condition.**

| Hybrid | Parent 1 gca# | Parent 2 gca# | Sum of gca# | gca rank | sca# | sca rank | Combining ability# | Combining ability rank | Grain yield# (kg/ha) | Grain yield rank | Mid-parent heterosis# |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Sum of gca | | | | | | |
| DIL-12 x DIL-14 | 171.72* | 464.58* | 636.3 | 1 | -153.10 | 57 | 483.20 | 31 | 2,192 | 95 | -44.39* |
| DIL-14 x DIL-23 | 464.58* | 171.20* | 635.78 | 2 | 104.63 | 42 | 740.41 | 25 | 4,984 | 12 | 26.98 |
| DIL-14 x DIL-58 | 464.58* | 159.67* | 624.25 | 3 | -189.32 | 60 | 434.93 | 33 | 3,623 | 52 | -9.99 |
| | | | | | sca | | | | | | |
| DIL-58 x DIL-60 | 159.67* | -409.19* | -249.52 | 84 | 2,049.09* | 1 | 1,799.57 | 4 | 5,442 | 2 | 37.77* |
| DIL-60 x DIL-78 | -409.19* | -97.66 | -506.85 | 102 | 1,834.19* | 2 | 1,327.34 | 11 | 1,347 | 105 | -65.27* |
| DIL-58 x DIL-80 | 159.67* | -199* | -39.33 | 56 | 1,812.87* | 3 | 1,773.54 | 5 | 3,477 | 58 | -15.22 |
| | | | | | Combining ability | | | | | | |
| DIL-1 x DIL-14 | 137.65* | 464.58* | 602.23 | 4 | 1,326.17* | 11 | 1,928.40 | 1 | 5,268 | 6 | 48.09* |
| DIL-12 x DIL-51 | 171.72* | 5.78 | 177.5 | 27 | 1,721.25* | 4 | 1,898.75 | 2 | 4,817 | 18 | 22.85 |
| DIL-1 x DIL-58 | 137.65* | 159.67* | 297.32 | 16 | 1,592.52* | 6 | 1,889.84 | 3 | 3,762 | 45 | -3.62 |

* Significant at $p \leq 0.05$, # under water stress condition.

**Table 4. Top 10 single cross hybrids with highest grain yield under water stress condition.**

| Hybrid | Parent 1 gca# | Parent 2 gca# | Sum of gca# | gca rank | sca# | sca rank | Combining ability# | Combining ability rank | Grain yield# (kg/ha) | Grain yield rank | Mid-parent heterosis# |
|---|---|---|---|---|---|---|---|---|---|---|---|
| DIL-12 x DIL-23 | 171.72* | 171.2* | 342.92 | 11 | -449.51* | 71 | -106.59 | 57 | 5,465 | 1 | 55.24* |
| DIL-58 x DIL-60 | 159.67* | -409.19* | -249.52 | 84 | 2,049.09* | 1 | 1799.57 | 4 | 5,442 | 2 | 37.77* |
| DIL-44 x DIL-64 | -60.23 | 26.04 | -34.19 | 54 | -1,653.53* | 102 | -1687.72 | 100 | 5,390 | 3 | 34.06 |
| DIL-78 x DIL-107 | -97.66 | -236.49* | -334.15 | 93 | -573.23* | 78 | -907.38 | 83 | 5,374 | 4 | 34.12 |
| DIL-10 x DIL-14 | -213.6* | 464.58* | 250.98 | 22 | -372.35 | 70 | -121.37 | 58 | 5,291 | 5 | 55.98* |
| DIL-1 x DIL-14 | 137.65* | 464.58* | 602.23 | 4 | 1,326.17* | 11 | 1928.4 | 1 | 5,268 | 6 | 48.09* |
| DIL-1 x DIL-60 | 137.65* | -409.19* | -271.54 | 87 | 955.20* | 19 | 683.66 | 27 | 5,258 | 7 | 37.69 |
| DIL-1 x DIL-12 | 137.65* | 171.72* | 309.37 | 14 | -320.35 | 66 | -10.98 | 53 | 5,257 | 8 | 32.14 |
| DIL-14 x DIL-39 | 464.58 | 123.9 | 588.48 | 5 | -539.84* | 73 | 48.64 | 51 | 5,213 | 9 | 43.83* |
| DIL-64 x DIL-78 | 26.04 | -97.66 | -71.62 | 64 | -1,595.65* | 100 | -1667.27 | 99 | 5,091 | 10 | 35.00 |

* Significant at $p \leq 0.05$, # under water stress conditions.

*per se* performance produced heterotic hybrids under water stress condition. Various methodologies have been employed in prediction of hybrid yield such as using co-ancestry among lines (Charcosset *et al*. 1998), combining ability effects (Melchinger *et al*. 1987), molecular markers (Bernardo 1994) and genomic prediction (Zhang *et al*. 2021) under optimum management conditions. Crops exhibit complex genetic mechanism when exposed to abiotic stress situation, thus prediction of hybrid performance can be improved by including *per se* performance of parental lines under abiotic stress.

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