# Diallel analysis of maize inbred lines for agronomic traits in nitrogen stress and optimal conditions

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## ABSTRACT

Increasing demand, soil cultivation pressure and adverse climate change effects necessitated maize production in nitrogen stress soils. This study examined the general combining ability (GCA) of 12 maize inbreds and specific combining ability of their crosses for agronomic traits under varied nitrogen conditions. GCA accounted for 53% of the variation for grain yield (GY) under stress and 40% under optimal condition. GCA contributed over 59% for days to anthesis (DTA) and days to silking (DTS), anthesis-silking-interval (ASI) and ear aspect (EASP) under both conditions. BD74-165 and BD74-161 had positive significant GCA for GY under stress with TZEI12 under optimal and BD74-222 under both conditions. TZEI13 and TZEI16 had positive significant GCA for DTA and ASI under stress, and TZEI12, TZEI11 and BD74-161 under optimal. Additive genes control DTA, DTS and PH; non-additive genes were responsible for ASI, PASP and EASP while both additive and non-additive genes governed inheritance of GY, EH and leaf-senescence (SEN) under stress. Inheritance of GY, ASI, PH, PASP and EASP were due to non-additive genes; DTA and DTS to additive genes while additive and non-additive genes control EH and SEN under optimal condition. Inbreds with significant GCA can be parents for GY improvement under respective conditions.

**Keywords:** Combining ability; diallel; low nitrogen; maize lines; stress tolerance
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## Introduction

Maize is well adapted to West and Central Africa (Ajala *et al*., 2007), but its mean grain yields range between 1.0 and 2.0 t ha$^{-1}$ compared to about 8.6 t ha$^{-1}$ in the temperate countries (FAOSTAT, 2015). The low grain yield in Africa is partly ascribed to poor soil fertility status and management (Azeez & Adetunji, 2007). Nutrients leaching and low level of soil organic matter have made N insufficiency a major constraint to sustainable smallholder maize production in Africa (Badu-Apraku *et al*., 2010; Ismaila *et al*., 2010). The annual loss of maize yield due to N stress varies from 10 to 50% (Wolfe *et al*., 1988). Maize performance varies within varieties across soil fertility levels from location to location and N use efficiency (Liang *et al*., 2005), because variation exists in gene expression of maize for low N tolerance. Nitrogen is a major nutrient responsible for

high yields in maize. Availability of sufficient quantity of N during the growing period strongly influences the productivity of maize in respect of leaf production, stay green and high grain yield (Thomason *et al*., 2002). Moreover, poor adoption of fertilizers by African farmers due to the scarcity and high costs of the inorganic fertilizers had been a major constraint to manage soil N (Pinstrup-Andersen *et al*., 1999). CTA (2015) reported that fertilizers are applied at a rate of 10 kg ha$^{-1}$ in Africa compared to 100 kg ha$^{-1}$ worldwide, and 200 kg ha$^{-1}$ in Asia. Besides, inorganic fertilizers are harmful to the environment. In Africa, where fertilizer use is minimal, genetic approaches to yield maintenance at reduced rates of N are crucial. Expected high maize yields will be achieved at a reduced cost despite the poor soil fertility status or the hazardous effects of fertilizers.

Genetic analysis of the inheritance of important traits of a crop helps to reveal the combining ability which leads to the identification of the best parent combinations that result in superior performing hybrids (Bänziger & Cooper, 2001). Combining ability of inbred lines, in turn, helps in determining the potential value of the variety development programme (Legesse *et al*., 2007) and reveals the nature of gene actions involved in the expression of traits. General combining ability is the mean performance of a line in all its crosses, expressed as a deviation from the mean of all crosses (Hallauer & Miranda, 1988). General combining ability (GCA) is the main effect and indicate additive gene action while the SCA is associated with non-additive genetic effects controlling traits. The effects of SCA are used to identify the best cross combinations for hybrid production (Izge, 2007).

The diallel mating system is used for pre-selection of parents for developing hybrid varieties especially when there are no clear heterotic patterns among the parent lines. It involves crossing a certain number of inbred lines from the same gene pool to each other. Two major types of diallel system, namely full or half diallel, exist. It is full diallel or reciprocal when the lines are used as both female and male plants, but half or non-reciprocal cross when they are used either as male or female plants. The diallel mating system can also be used to estimate the GCA and SCA, determine the mode of inheritance of traits, genes that control the trait and gene effects. The mating system has been used extensively in genetic research to study the inheritance of important traits for stress tolerance among selected maize lines and to identify superior parents for hybrids or cultivar development (Olakojo & Olaoye, 2005; Dhliwayo *et al*., 2009; Yallou *et al*., 2009; Badu-Apraku *et al*., 2011**).**

The objective of the study was to evaluate the combining ability and determine genes actions controlling the expression of grain yield and low N tolerance in crosses of local (IITA) and exotic (CIMMYT) maize inbred lines under N stress and optimal conditions using diallel analysis

**Materials and Methods**

12 yellow kernel inbred lines representing nine lines obtained from IITA, Nigeria and three elite lines originating from CIMMYT, Kenya were crossed in a non-reciprocal diallel mating system (Griffing, 1956) to generate 66 single cross hybrid maize. Description of the inbred lines used is shown in Table 1. The 66 hybrids were evaluated along with four check hybrids under soil N stress and optimal conditions in Ibadan, Nigeria in 2014 and 2015. The experimental soil was purposely depleted of its native N by continuously planting maize at a very high population density without application of fertilizer, uprooting and removing the biomass completely after each cropping. This depletion

procedure was repeated until the soil N was completely removed. Soil analysis was carried out to confirm the N status after each depletion process. The soil was depleted to about zero levels of N.

The trial was laid out in $10 \times 7$ lattice design with three replicates. Plots consisted of two rows of 5 m long and 0.75 m apart, where plants were spaced 0.5 m in a row. Three seeds were sown and later thinned at two weeks after planting (WAP) to two stands per hill to attain plant population density of 53,333 plants ha$^{-1}$. Two N concentrations, namely 30 and 90 kg N ha$^{-1}$ were applied to denote N stress and optimal conditions, respectively. The fertilizer was applied in the form of NPK 15:15:15 at 30 kg ha$^{-1}$ to each plot at three WAP. The optimal N plots received 60 kg N ha$^{-1}$ in the form of urea to bring the available N to 90 kg ha$^{-1}$ two weeks later. All the plots received 60 kg P ha$^{-1}$ as single super phosphate ($P_2O_5$) and 60 kg K ha$^{-1}$ as muriate of potash ($K_2O$). Standard cultural practices were applied for field maintenance and harvesting practices according to IAR&T (2010) package. This package included plowing and harrowing of land before planting, keeping the field weed-free by applying herbicides and hoeing. The crops were protected by hand picking and destruction of pests and roguing diseased plants as required and harvesting when the maize was dry.

*Data collection*
Data were collected on 10 plants from each plot under both N stress and optimal conditions in each year. Days to anthesis (DTA) and days to silking (DTS) were number of days from planting to the day 50% of the plants in a plot shed pollen, and had emerged silk, respectively. Anthesis-silking-interval (ASI) was calculated as the difference between DTS and DTA. Plant

height (PH) and ear height (EH) were recorded as the height of the maize from ground level to the base of its tassel, and the base of the uppermost ear, respectively. Stay green (SG) was scored three times at eight days interval during the latter part of grain filling, according to Bänziger *et al*. (2000) on a scale of 0 to 10, by dividing the percentage of total leaf area that was green by 10. Scale 1 = 10% of leaves are green, 2 = 20%, 3 = 30%, 4 = 40 %, 5 = 50%, 6 = 60%, 7 = 70%, 8 = 80%, 9 = 90% and 10 = 100% of the leaves were green.

Plant aspect (PASP) was visually assessed on plot basis before harvest, after flowering (at brown silk stage) when plants were still green and ears fully developed on scale 1 to 5 where 1 was excellent; 5 was very poor. The PASP was the general appeal of the whole plants, based on the plant and ear heights, uniformity of the plant stands, reaction to diseases and insects as well as lodging. Ear aspect (EASP) was also visually scored on a scale of 1 to 5 where 1 was excellent; 5 was very poor. The score was taken on the pile of harvested ears of each plot when spread out and the general look of the ears was taken into account. Ear size, uniformity of colour and texture, grain fill, disease and insect damage were also considered for this score. Grain yield (GY), measured in kg ha$^{-1}$ adjusted to 15% moisture content was estimated from grain weight and percent moisture following Badu-Aparaku *et al*. (2012) as:

$$GY \ (kg \ ha^{-1}) = \frac{GWT \ (kg)}{7.5 \ m^2} \times \frac{(100 - MC)}{(100 - 15\%)} \times 10{,}000 \ m^2$$

where GWT = Grain weight, MC = grain moisture content at harvest, seed moisture content at weighing = 15%, plot area = 7.5 m$^2$ and 1 ha = 10,000 m$^2$.

## Data analysis

Analysis of variance was performed on the data collected for the 66 hybrids. Variation among hybrids was partitioned into that due to male and female parents and interaction between female and male parents. Main effects due to male and female were independent estimates of GCA whereas effects due to male × female were the SCA for each trait considered, for each N condition. Significance of effects of GCA of the parents and SCA of the hybrids were estimated for the traits from their mean square values adjusted for block effects for individual and across environments following Griffing's method 4 model 1 (fixed model) (Griffing, 1956) and DIALLEL SAS programme developed by Zhang *et al*. (2005) adapted to SAS software with PROC GLM in SAS (SAS Institute, 2009) using a RANDOM statement with TEST option. The high strength of GCA effects for a particular trait implies that additive genetic effects are more important for the inheritance of that trait, while non-additive genetic effects were more important for a trait for which SCA effects are predominant. The statistical model for the combined diallel analysis was as follows:

$$Y_{ijkl} = \mu + E_e + g_i + g_j + s_{ij} + {}_gE_{eg} + {}_sE_{es} + \varepsilon_{ijk}$$

where $Y_{ijk}$ is the observed measurement for the $ij^{th}$ cross grown in the $k^{th}$ environment; $\mu$ is the grand mean; $g_i$ and $g_j$ are the GCA effects; $s_{ij}$ is the SCA effects; ${}_gE_{eg}$ is the interaction effect between GCA and the environment; ${}_sE_{es}$ is the interaction effect between SCA and the environment, and $\varepsilon_{ijk}$ is the error term associated with the $ij^{th}$ cross evaluated in the $k^{th}$ replication and $E_e$ environment (Hallauer *et al*., 2010). On the combining ability effects: $\Sigma g_i = 0$, and $\Sigma s_{ij} = 0$ for each j.

## Results

### Analysis of variance of the agronomic traits of the hybrids under varied N conditions

Environmental effects were highly significant for all the traits (*P<0.01*) except SG and PASP under N stress (Table 2). Effects of genotype, G × E, female, male and female × male were highly significant for GY and other traits considered. However, effects of environment × female (E × F) and environment × male (E × M) were significant (*P<0.05*) for DTA, DTS and PH only. Analysis of data in optimal condition showed that environmental effects were significant for GY and other traits while the effects of genotype, G × E, female, male as well as female × male were significant (*P<0.001*) for all the traits. The E × F and E × M had non-significant effects for all the traits under optimal condition.

### Combining ability for the traits of the inbred lines under varied N conditions GCAs of the maize inbred lines

Significant and non-significant GCAs existed for the 12 inbred lines used in generating the hybrids as evaluated under N stress and optimal conditions for five agronomic traits considered as indices of low N tolerance in maize (Table 3). Significant GCAs for GY ranged from -1001.0 in TZEI11 to 491.4 in BD74-161 under N stress. Inbred lines TZEI11 and TZEI124 had negative and significant GCA while the GCA of BD74-165 (327.10**), BD74-161 (491.10**) and BD74-222 (199.20**) were positive and significant for GY. Inbred lines TZEI13 and TZEI16 had positive and significant GCA for DTS and ASI while TZEI146 and BD74-165 had negative GCA for the traits. BD74-161 and BD74-222 had positive and significant GCA for GY and DTS while only BD74-165 had significant GCA for all the traits under the N stress.

On the other hand, GCA for GY under optimal condition ranged from -591.9 for TZEI11 to 572.0 for TZEI12 (Table 3). Only inbred lines TZEI12 and BD74-222 had GCAs as positive and significant for GY while GCAs of TZEI10, TZEI11 and TZEI8 for the GY were negative and significant. TZEI12, TZEI11 and BD74-161 also had positive GCA for DTS and ASI while BD74-222 had positive GCA for GY and DTS (0.736[**]) only. The significance of GCAs of TZEI13, TZEI12, TZEI11 and TZEI8 for SEN were positive while TZEI10, TZEI11 and TZEI8 had positive significant GCAs for PASP under the optimal N condition.

*SCAs of the maize inbred lines in selected hybrids*

The SCA effects for GY and some traits of the 12 maize inbred lines as components of selected hybrids are shown in Table 4. The selected hybrids were those that had significant SCAs for GY, at least under stress or optimal condition. Positive and negative significant SCAs existed for GY, ASI, SG and PASP for the hybrids. The SCA for GY ranged from -1328 for TZEI146 × BD74-222 to 1275 for TZEI128 × BD74-161 under N stress. Hybrids TZEI128 × BD74161, TZEI11 × TZEI124, TZEI12 × BD74161, TZEI11 × TZEI16, TZEI8 × TZEI124 and TZEI13 × TZEI46 were prominent among those that had positive and significant SCA for GY. Hybrids TZEI13 × TZEI11, TZEI13 × TZEI146, TZEI124 × BD74165 and TZEI124 × BD74161 had negative significant SCAs for GY but significance of their SCA effects for SG and PASP were positive under N stress. Only TZEI146 × BD74222 had significant GCA for all the traits under the N stress condition.

The SCA for GY was from least (-1566) for TZEI13 × TZEI11 to highest (1561) for TZEI13 × TZEI12 under optimal N. TZEI13 × TZEI12, TZEI10 × BD74-165 and TZEI8 × TZEI16 had the highest positive and significant SCA while TZEI124 × BD74-165 had significant SCA for at least four out of the five traits under the N condition. It was also found that hybrids TZEI13 × TZEI128, TZEI11 × BD74161 and TZEI124 × BD74-222 had positive and significant SCA for GY under both N stress and optimal conditions.

*Contributions of female and male GCA effects of inbred lines in varied N conditions*

Percentages sums of squares of GCA effects for GY and other traits of the hybrids evaluated under N stress and optimal conditions are shown in Table 5. The GCA explained greater than 50% of the variation for DTA, DTS and PH; about 40% for EASP and only 25% for ASI, while the GCA effect was almost similar to that of SCA for GY, EH, SG and PASP under N stress condition. The sum of squares of $GCA_f$ was less than that of $GCA_m$ for SG, PASP and EASP but the two parameters were similar for DTS and ASI, while the effects of $GCA_f$ were greater than that of $GCA_m$ under N stress.

Under optimal N, the GCA accounted for about 40% of the variation for GY, PH and PASP (Table 5). The GCA accounted for higher variation for DTA and DTS, but the effect was relatively similarly with SCA for EH and SG. The SCA effects were greater than 70% for ASI and EASP. However, the effects of $GCA_f$ were less than that of $GCA_m$ for DTA and DTS only and vice versa for GY, EH, SG, PASP and EASP under optimal condition. Sum of squares for $GCA_f$ was similar to that of $GCA_m$ for PH and ASI under the optimal condition.

## Discussion

Significant variations due to genotypes, environments and G × E observed for the GY and other traits under both N conditions indicate the presence of variability in the response of the hybrids to changes in N conditions. Maize performance has been reported to vary within varieties across soil fertility from location to location and N use efficiency (Liang *et al.*, 2005). Hence, selection for promising hybrids under either of the N conditions is imperative. Moreover, the significant effects of male, female and female × male necessitated the estimation of combining the ability of the inbred lines for GY and other traits. The presence of non-significant main effect (GCA) × environment under each N condition suggests that the performance of both male and female were stable across the years. Therefore, the selection of parent lines for hybrids can be done under various N conditions in any of the two years with respect to the traits.

The significant interaction effects such as GCA × environment for DTA, DTS and PH under N stress underlines the sensitivity of maize to N thereby exhibiting differential responses in different years. This was buttressed by the fact that the GCA × environments were not significant for all the traits under an optimal condition where a higher amount of N was available. According to the results of this study, the selection of parents based on the flowering traits under N stress is important for each year. Flowering traits have been suggested as selection indices for low N tolerance (Bänziger *et al.*, 2000). This may be responsible for the variability due to the GCA × environments for the flowering traits under the N stress.

The GCAs of the inbred lines varied widely, from low to high and from significant to not significant in the crosses they were involved under different N conditions. The significant effects of GCA indicate that at least one of the maize lines differs in the content of favorable genes with additive effects. High GCA effects for GY and other traits in many of the parents indicates good general combination under the two N conditions and presence of heterotic response for the traits. However, the variation was wider under N stress than optimal condition, hence N stress provided a greater prospect for the expression and combination of genes in crosses. Three inbred lines namely, BD74-165, BD74-161 and BD74-222 exhibited high positive GCA effects for GY under N stress while TZEI12 and BD74-222 had high GCA effects under optimal. Hence, the inbred lines are suitable as parents for yield improvement in the respective N conditions. It has earlier been reported that lines derived from the same population may have either good or poor general combining ability (Sharma & Garg, 2002; Lubadde *et al.*, 2016). On the other hand, the high GCA obtained for exotic lines evaluated in this study emphasized the importance of novel alleles in CIMMYT (exotic) lines in maize improvement in Nigeria under both N conditions.

Highly positive and significant GCA effects of inbred lines TZEI13 and TZEI16 for DTA and ASI classified the lines in same maturity class under N stress while TZEI12, TZEI11 and BD74-161 were grouped together under optimal condition. Their combining ability will enhance synchronization during pollination. This indicates the presence of source from which genes can be drawn and manipulated for better pollination especially in areas where rainfall is not reliable. Bello and Olaoye (2009) also reported positive and significance of GCA effects for DTS in maize improvement. Incidentally, none of the two inbred lines (TZEI13 and TZEI146) that had significant GCA for flowering had significant GCA for GY under N stress. Inbred lines BD74165 and BD74222

also had opposite significance for GY and flowering traits under the condition. Likewise, TZEI11 had negative significant GCA for GY but positive GCA for all other traits under optimal condition. Hybridization can therefore be made among selected inbred lines for desired traits. For instance, lines TZEI11, TZEI124, TZEI10 and TZEI8 had negative significant GCAs for GY, DTS or ASI, but could be useful in hybridization with other inbred lines that have high GY attribute. These inbred lines could be crossed with other promising ones to generate early maturity and high yielding hybrids.

The GCA effect was higher than that of SCA for DTA, DTS and PH confirming the predominance of additive genes actions for the inheritance of the traits but non-additive for ASI, PASP and EASP while both additive and non-additive genes govern inheritance of the GY, EH and SG under N stress. Derera *et al*. (2008); Senthil and Bharthi (2009) in their studies observed additive and non-additive effects were important for DTA, DTS and ASI of maize under stress. Nass *et al*. (2000) have also reported that both additive and non-additive effects are important for identifying promising parental lines and productive hybrids. The sum of squares of SCA for GY, ASI, PH, PASP and EASP were higher than those of GCA indicating non-additive genes control the traits while additive gene actions were responsible for the inheritance of DTA and DTS under optimal condition. The EH and SG had GCA effects similar to the SCA, hence the traits are governed by both additive and non-additive genes under the N condition. The SCA effects under both conditions were high especially, for GY, ASI and PASP indicating good specific combination for the trait. This also suggests that non-additive gene effects also played a major

role in the expression of GY among crosses in respective N conditions. Non-additive gene action in inheritance of low N tolerance is exhibited by hybrids TZEI8 × TZEI124, TZEI11 × TZEI124, TZEI11 × TZEI16, TZEI128 × BD74161, TZEI12 × BD74161 and TZEI13 × TZEI46 because of their significant SCA under N stress condition. Therefore, they could be utilized as sources of inbred line extraction for the development of high yielding varieties for cultivation in N deficient soils. The TZEI13 × TZEI12, TZEI10 × BD74165 and TZEI8 × TZEI16 top the list of good combiners for GY for their significant SCA under optimal N while hybrids TZEI13×TZEI128, TZEI11×BD74161 and TZEI124×BD74-222 are suitable for both N stress and optimal conditions. Betran *et al*. (2003) had earlier emphasized the importance of non-additive genes actions among maize inbred lines and hybrids in low N environments. Maize GY and flowering traits have been reported to be under the control of non-additive genes actions (Aminu *et al*., 2014; Pádua *et al*., 2016).

The sum of squares for $GCA_f$ was less than $GCA_m$ for all the traits except SG, PASP and EASP under N stress and DTA and DTS under optimal N indicating large genetic variability of female than the male contribution for the traits in the respective N conditions. The $GCA_f$ and $GCA_m$ had similar effects for DTS and ASI under N stress, as well as ASI and PH under optimal N condition. This means that both male and female contributed almost equally to the genetic variability among the hybrids for the traits. Contributions of female were more important in gene expression for yield, growth and flowering of the hybrid maize under low N, but for all the traits except flowering under optimal condition. This may imply that maternal effects influence the expression of these traits.

Derera *et al*. (2008) had earlier reported higher female GCA effects compared to male GCA effects for ASI.

## Conclusion

Inbred lines BD74-165 and BD74-161 were best combiners under N stress, TZEI12 under optimal condition while BD74222 is promising under both N conditions. Hence, these lines can be used as parents for yield improvement in respective N conditions. Additive gene actions control DTA, DTS and PH; non-additive gene effects were responsible for ASI and EASP while both additive and non-additive gene actions were responsible for the inheritance of GY, EH and SG under N stress. Inheritance of GY, ASI, PH, PASP and EASP was due to non-additive gene actions; those for DTA and DTS was due to additive genes actions while additive and non-additive genes control EH and SG under optimal condition. Hence, these lines are suitable for the development of commercial hybrids for cultivation in respective N conditions. Females contributed more than males in the genes expression for GY and most other traits in the two N conditions. Exotic inbred lines are useful in maize improvement programmes for low N tolerance.

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TABLE 1

*Description and sources of the maize inbred lines used for hybrids development using diallel mating system*

| No. | Inbred line | Pedigree | Source | Low-N |
|---|---|---|---|---|
| 1 | TZEI8 | TZE-Y Pop STR $C_0$ $S_6$ Inbred 62-3-3 | IITA | Tolerant |
| 2 | TZEI10 | TZE-Y Pop STR $C_0$ $S_6$ Inbred 152 | IITA | Tolerant |
| 3 | TZEI11 | TZE Comp5-Y $C_6$ $S_6$ Inbred 8 | IITA | Tolerant |
| 4 | TZEI12 | TZE Comp5-Y $C_6$ $S_6$ Inbred 8 | IITA | Tolerant |
| 5 | TZEI13 | TZE Comp5-Y $C_6$ $S_6$ Inbred 12 | IITA | Tolerant |
| 6 | TZEI16 | TZE Comp5-Y C6 S6 Inbred 31 | IITA | Tolerant |
| 7 | TZEI124 | TZE-Y Pop STR $C_0$ $S_6$ Inbred 3-1-3 | IITA | Tolerant |
| 8 | TZEI128 | TZE-Y Pop STR $C_0$ $S_6$ Inbred 10-4-4 | IITA | Tolerant |
| 9 | TZEI146 | TZE-Y Pop STR $C_0$ $S_6$ Inbred 21-1-3 | IITA | Tolerant |
| 10 | BD74-165 | (DTPYC9-F74-3-4-1-3-B)-B | CIMMYT | Tolerant |
| 11 | BD74-161 | (DTPYC9-F46-3-9-1-1-B)-B | CIMMYT | Susceptible |
| 12 | BD74-222 | (CLYN262)-B | CIMMYT | Susceptible |

IITA, CIMMYT indicate International Institute of Tropical Agriculture, International Maize and Wheat Improvement Centre, respectively

TABLE 2
*Mean squares of traits from combined analyses of variance
for the hybrids under N stress and optimal conditions in 2014 and 2015*

| Source of variation | df | Grain yield | DTA | DTS | ASI | Plant height | Ear height | SG | PASP | EASP |
|---|---|---|---|---|---|---|---|---|---|---|
| N stress condition (30 kg N ha$^{-1}$) | | | | | | | | | | |
| Environment (Env.) | 1 | 23322062.28*** | 49.30*** | 50.79*** | 0.011*** | 998.12** | 429.53** | 0.16ns | 0.00 ns | 4.52*** |
| Replicate (Env.) | 4 | 43422.62ns | 1.06ns | 1.11ns | 0.032 ns | 540.05** | 316.98*** | 0.75* | 0.07 ns | 0.20ns |
| Block (Env.×replicate) | 36 | 120486.98ns | 1.12ns | 1.06ns | 0.273 ns | 212.28** | 49.05** | 0.34ns | 0.10 ns | 0.10ns |
| Genotype | 69 | 1047433.3*** | 6.36*** | 8.34*** | 0.88*** | 898.56*** | 178.10*** | 0.92*** | 0.30*** | 0.64*** |
| Genotype × Env. | 69 | 1146459.1*** | 4.13*** | 5.35*** | 1.08*** | 509.15*** | 119.24** | 0.55*** | 0.20* | 0.48*** |
| Female | 10 | 7662155.00** | 23.50** | 22.17* | 1.04** | 4484.35*** | 615.65*** | 2.34*** | 1.18* | 1.25** |
| Male | 10 | 2802340.00* | 20.32* | 22.04* | 1.17*** | 1983.94* | 366.59* | 4.30*** | 1.76** | 2.57* |
| Female × Male | 45 | 2020071.00*** | 4.08*** | 5.91*** | 1.49*** | 825.03*** | 256.40*** | 1.35*** | 0.73*** | 1.35* |
| Env. × Female | 10 | 1208384.00ns | 4.03* | 5.58* | 0.21ns | 412.03* | 52.45ns | 0.16ns | 0.21ns | 0.18ns |
| Env. × Male | 10 | 824286.00 ns | 6.70* | 6.57* | 0.41ns | 450.50* | 105.24ns | 0.10ns | 0.25ns | 0.70ns |
| Env. × Female × Male | 45 | 552995.00*** | 1.31ns | 1.51* | 0.26ns | 192.64* | 79.86** | 0.24ns | 0.16** | 0.42*** |
| Pooled error | 224 | 102919.10 | 1.02 | 0.90 | 0.21 | 144.21 | 47.14 | 0.28 | 0.90 | 0.12 |
| Optimal condition (90 kg N ha$^{-1}$) | | | | | | | | | | |
| Environment (Env.) | 1 | 26790246.3*** | 69.96*** | 87.56*** | 0.91* | 212.42** | 684.78*** | 2.56*** | 0.78*** | 3.36*** |
| Replicate (Env.) | 4 | 255879.5ns | 12.00*** | 10.39*** | 0.30ns | 690.60*** | 460.14*** | 0.93*** | 0.45*** | 0.04ns |
| Block (Env.×replicate) | 36 | 170945.3ns | 1.17ns | 1.12ns | 0.29* | 106.73ns | 36.69ns | 0.20ns | 0.11ns | 0.07ns |
| Genotype | 69 | 1899724.8*** | 5.90*** | 8.77*** | 1.21*** | 905.44*** | 222.77*** | 0.87*** | 0.32*** | 0.69*** |
| Genotype × Env. | 69 | 1643032.5*** | 3.41*** | 5.23*** | 0.70*** | 467.45*** | 115.09** | 0.46*** | 0.25*** | 0.51*** |
| Female | 10 | 6683783.0** | 14.45* | 18.44* | 1.46** | 1331.42*** | 534.19*** | 2.68** | 3.06*** | 3.30* |
| Male | 10 | 2258967.0* | 22.48** | 27.45*** | 1.25** | 1268.88** | 347.14** | 1.66** | 1.08** | 1.52** |
| Female × Male | 45 | 3023169.0*** | 4.90** | 7.07*** | 1.59*** | 928.66*** | 233.42** | 0.93*** | 1.38*** | 2.65*** |
| Env. × Female | 10 | 1298058.0ns | 3.16ns | 3.90ns | 0.20ns | 101.00ns | 45.04ns | 0.42ns | 0.31ns | 0.77ns |
| Env. × Male | 10 | 471267.0ns | 3.19ns | 2.86ns | 0.20ns | 241.52ns | 69.50ns | 0.26ns | 0.19ns | 0.26ns |
| Env. × Female × Male | 45 | 882175.0*** | 2.07*** | 1.91*** | 0.18ns | 166.91ns | 92.76** | 0.27* | 0.28*** | 0.36*** |
| Pooled error | 224 | 203684.5 | 0.98 | 0.96 | 0.20 | 142.98 | 50.12 | 0.17 | 0.01 | 0.11 |

ns, *, **, *** and df are not significant, significant at 5%, 1%, 0.1% degrees of freedom, respectively. DTA, DTS, ASI, SG, PASP and EASP are days to anthesis, days to silking, anthesis-silking-interval, stay green, plant aspect and ear aspect, respectively.

TABLE 3
*General combining ability of agronomic traits of the maize inbred line used in the diallel
analysis for low N tolerance under N stress and optimal conditions*

| Inbred line | N stress condition | | | | | Optimal condition | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | GY | DTS | ASI | SG | PASP | GY | DTS | ASI | SG | PASP |
| TZEI13 | 90.43 | 1.097** | 0.292** | 0.131 | 0.156** | -38.65 | 1.019* | -0.094 | 0.200* | 0.035 |
| TZEI146 | 37.38 | -0.686** | -0.142* | -0.156* | 0.031 | 44.46 | -1.314 | -0.311 | -0.164 | -0.099 |
| TZEI10 | -111.70 | -0.586** | -0.075 | 0.017 | 0.006 | -289.40* | -0.281 | 0.006 | 0.017 | 0.143* |
| TZEI12 | 140.80 | 0.881** | 0.008 | -0.15* | 0.089* | 572.00** | 1.186* | 0.306* | 0.136* | -0.407 |
| TZEI128 | 13.95 | -0.769** | 0.142* | -0.081 | 0.131* | 12.19 | -0.597 | -0.011 | 0.006 | -0.024 |
| TZEI11 | -1001.00** | 0.497* | -0.008 | 0.058 | 0.331** | -591.90** | 0.536* | 0.172* | 0.342* | 0.460* |
| TZEI8 | -89.35 | -1.419** | 0.192** | 0.658** | 0.047 | -380.60** | -1.247 | 0.006 | 0.319* | 0.185* |
| TZEI16 | 124.20 | 0.681** | 0.258** | -0.100 | -0.094* | 139.90 | 0.419* | -0.161 | -0.147 | 0.060 |
| TZEI124 | -222.10* | -0.253 | -0.092 | 0.242** | -0.086* | 102.30 | -0.464 | -0.078 | -0.072 | -0.090 |
| BD74-165 | 327.10** | -0.703** | -0.292** | -0.336** | -0.236** | 125.80 | -0.481 | -0.078 | -0.092 | 0.035 |
| BD74-161 | 491.40** | 0.664** | -0.092 | -0.275** | -0.078 | 110.30 | 0.486* | 0.272* | -0.461 | -0.065 |
| BD74-222 | 199.20* | 0.597** | -0.192** | -0.008 | -0.294** | 204.70* | 0.736* | -0.028 | -0.49 | -0.232 |

* and ** are significant at 5% and 1% respectively.
GY, DTS, ASI, SG and PASP indicate grain yield, days to silking, anthesis-silking-interval, stay green and plant aspect, respectively.

TABLE 4
*Specific combining ability for some traits in the hybrid maize under*
*varied N conditions in 2014 and 2015*

| Hybrid | N stress condition | | | | | Optimal condition | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | GY | DTS | ASI | SG | PASP | GY | DTS | ASI | SG | PASP |
| TZEI13×TZEI146 | 744.8** | 0.80 | 0.37* | -0.22 | -0.73 | 347.9 | 0.43 | 0.29 | 0.00 | -0.05 |
| TZEI13×TZEI10 | -150.7 | -0.47 | -0.19 | -0.67** | -0.20 | -1165.0** | 1.23* | 0.31 | -0.15 | 0.63* |
| TZEI13×TZEI12 | -330.0 | -0.77 | -0.44* | 1.33** | 0.05 | 1561.0** | 0.27 | 0.34* | 0.67* | -0.57 |
| TZEI13×TZEI128 | 987.2** | 0.22 | -0.24 | -0.24 | -0.58 | 1037.0** | 0.38 | 0.16 | 0.28 | -0.87 |
| TZEI13×TZEI11 | -664.4* | -0.22 | -0.09 | 0.43* | 0.89* | -1566.0** | 0.42 | 0.64* | -0.11 | 1.14* |
| TZEI13×TZEI8 | 363.4 | -0.47 | 0.54* | 0.08 | -0.49 | 540.1* | 0.03 | -0.36 | -0.23 | -0.58 |
| TZEI13×TZEI16 | -1317.0** | -0.07 | -0.53* | 0.78** | 0.73* | -2021.0** | 0.53 | -0.19 | -0.35 | 0.96* |
| TZEI13×TZEI124 | 420.3 | 0.87 | 0.82** | -0.23 | -0.03 | 583.0* | -0.75 | -0.28 | -0.06 | 0.03 |
| TZEI146×TZEI10 | -266.4 | 1.15* | 0.41* | 0.64** | 0.59* | -908.8* | 0.73 | 0.02 | 0.19 | 0.68* |
| TZEI146×TZEI128 | 103.3 | -1.00 | 1.19** | 0.63** | -0.12 | 707.9* | 0.55 | 0.37 | 0.70 | -0.32 |
| TZEI146×TZEI11 | -487.6* | 0.90 | 0.34 | -0.68** | 0.02 | 916.1** | 0.75 | 1.02* | -0.47 | -0.64 |
| TZEI146×TZEI8 | 145.1 | -0.02 | -0.69** | -0.86** | -0.45 | -562.3* | 0.03 | 0.36* | 0.22 | 0.55* |
| TZEI146×BD74165 | 564.6* | -1.07 | -0.38* | -0.39 | 0.42* | 311.6 | 0.27 | -0.06 | -0.01 | -0.63 |
| TZEI146×BD74222 | -1328** | 1.97* | 0.69** | 0.92** | 0.39* | -1312.0** | 0.72 | 0.06 | 0.33 | 1.05* |
| TZEI10×BD74-165 | -76.0 | -0.50 | -0.28 | 0.49* | 0.11 | 1017.0** | 0.23 | -0.54 | 0.00 | -0.12 |
| TZEI10×BD74-161 | -116.8 | 0.47 | 0.19 | -0.15 | 0.12 | -770.7* | 1.93* | -0.23 | 0.20 | 0.73* |
| TZEI10×BD74222 | 482.3* | -1.30 | -0.04 | -0.61* | 0.25* | 152.8 | -0.65 | 0.41* | 2.41 | -0.02 |
| TZEI12×TZEI11 | -731.7* | 1.17* | 0.19 | 0.07 | 0.12 | -623.3* | 2.08* | 0.24 | 0.00 | 0.58* |
| TZEI12×TZEI16 | -451.1 | 1.48* | 0.76** | -0.44* | 0.55 | -983.9* | 0.70 | 0.24 | -0.84 | 0.32* |
| TZEI12×BD74165 | -114.8 | -0.30 | 0.31 | -0.34 | 0.11 | -563.0* | 0.10 | 0.49* | 0.27 | -0.16 |
| TZEI12×BD74161 | 1250.0** | 0.17 | 0.27 | -0.38 | 0.20 | 260.1 | 0.80 | 0.14 | -0.58 | -0.31 |
| TZEI128×TZEI8 | -841.4* | -0.10 | 0.02 | -0.13 | 0.95* | -37.2 | 0.15 | 0.56* | 0.02 | 0.06 |
| TZEI128×TZEI124 | -1287.0** | 1.23* | -0.03 | -0.32 | 0.33* | -1014.0** | 1.53* | 0.14 | -0.34 | 0.75* |
| TZEI128×BD74161 | 1275.0** | -0.18 | -0.19 | -0.42* | -0.09 | -898.1 | 0.25 | 0.79* | -0.12 | -0.02 |
| TZEI11×TZEI16 | 955.1** | -0.47 | -0.06 | 0.32 | -0.03 | -233.7 | 0.68 | 0.54* | 0.01 | 0.37* |
| TZEI11×TZEI124 | 1243.0** | -3.20 | -0.88** | 0.18 | -0.37* | 316.9 | -0.93 | -0.38 | 0.21 | -0.40 |
| TZEI11×BD74161 | 930.6** | -0.62 | -0.04 | -0.67** | -0.13 | 881.3** | 0.28 | -0.06 | -0.20 | -0.34 |
| TZEI11×BD74222 | 807.8** | -1.72 | -0.61** | 0.57* | 0.09 | 920.4 | -1.97 | -0.26 | -1.55 | -0.26 |
| TZEI8×TZEI16 | 349.4 | -0.38 | -0.09 | 0.22 | 0.17 | 1110.0** | -0.87 | -0.96 | 0.48 | -0.44 |
| TZEI8×TZEI124 | 799.6** | 0.88 | 0.09 | -0.51* | -0.08 | -258.8 | -0.32 | 0.46* | 0.24 | -0.29 |
| TZEI8×BD74165 | -885.9* | -2.83 | -0.38* | 0.66** | -0.02 | -821.0* | 0.03 | 0.46* | 0.17 | 0.50* |
| TZEI124×BD74165 | -986.9* | 0.17 | -0.93** | 0.79** | 0.70* | -1293.0** | 0.92 | 0.54* | 1.06* | 0.94* |
| TZEI124×BD74161 | -1259.0* | 1.13* | 0.21 | 0.76** | 0.71* | -934.8* | 1.28* | -0.14 | -0.12 | 0.38* |
| TZEI124×BD74222 | 689.9* | 0.53 | 0.47* | 0.33 | -0.41 | 910.9* | -0.13 | -0.51 | -3.03 | -0.46 |
| BD74165×BD74161 | 511.5* | 0.75 | -0.09 | 0.06 | -0.56 | 422.5 | -0.87 | -0.14 | -0.85 | 0.08 |
| BD74165×BD74222 | 424.0 | 0.98* | 0.01 | -0.43* | -0.34 | -662.0* | 2.38* | 0.49* | 2.91 | 0.08 |

*, and **, GY, DTS, ASI, SG and PASP are significant (*P<0.05*) and (*P<0.01*), grain yield, days to silking, anthesis-silking-interval, stay green and plant aspect, respectively

TABLE 5
*Percentages of cross sums of squares contributed by GCA effects from female and male parents for selected traits of the maize evaluated in N stress and optimal conditions in 2014 and 2015*

| *Trait* | *N condition* | $GCA_f$ | $GCA_m$ | SCA |
|---|---|---|---|---|
| | | *Percentages of cross sums of squares* | | |
| Grain yield | Stress | 39 | 14 | 47 |
| | Optimal | 30 | 10 | 60 |
| Days to anthesis | Stress | 38 | 33 | 30 |
| | Optimal | 25 | 38 | 37 |
| Days to silking | Stress | 31 | 31 | 38 |
| | Optimal | 24 | 35 | 41 |
| Anthesis silking interval | Stress | 12 | 13 | 75 |
| | Optimal | 15 | 13 | 72 |
| Plant height | Stress | 44 | 20 | 36 |
| | Optimal | 20 | 19 | 61 |
| Ear height | Stress | 29 | 17 | 54 |
| | Optimal | 28 | 18 | 54 |
| Stay green | Stress | 18 | 34 | 48 |
| | Optimal | 32 | 19 | 49 |
| Plant aspect | Stress | 19 | 28 | 53 |
| | Optimal | 30 | 10 | 60 |
| Ear aspect | Stress | 12 | 26 | 61 |
| | Optimal | 20 | 9 | 71 |

GCA$f$ , GCA$_m$ and SCA indicate general combining ability for female, general combining ability for male and specific combining ability, respectively