Screening of Maize Doubled Haploid Derived Hybrids at Flowering Stress and Optimal Condition

B. V. Ananda Kumar1*, S. R. Venkatachalam2, R. Ravikesavan3, P. Kathirvelan1, S. Nackeeran1 and Venkatesh Selvarangam4

1Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
2Tapioca and Castor Research station, Tamil Nadu Agricultural University, Yethapur, Tamil Nadu, India
3Department of Millets, Department of Pathology, Centre for Plant Breeding and Genetics, Tamil Nadu Agricultural University, Coimbatore, Tamil Nadu, India
4Pioneer Hi-Bred Pvt. Ltd., Multi Crop Research Center, Wargal, Hyderabad, Telangana, India

*Corresponding author

ABSTRACT

Two field experiments were conducted at Hyderabad and Aurangabad in India under water stress at flowering and well watered condition during the rabi season (2018). The objective of the study was to assess the drought tolerance traits in maize DHL’s x Tester (Top cross hybrids). Three hundred top crosses developed from crossing between 150 DHL’s and tester from heterotic group A similarly another 150 DHL’s and tester from heterotic group B along with four checks were evaluated under two watering treatments, i.e. well watering (WW) and water stress at flowering (WSF) using augmented complete block design. The effect due to genotypes x location was highly significant for all drought tolerance parameters studied. The drought stress at flowering stages of maize results in a drastic reduction in grain yield by 41.34 percent when compared to optimal condition. DSI had a significant and negative correlation coefficient with grain yield (-0.852), chlorophyll content (-0.622), ear height (-0.348) and plant height (-0.253) under WSF condition. Moreover, DSI had bigger in-magnitude, but highly significant and positive correlation coefficient with days ASI (0.745) tassel sterility (0.785) and tassel blast (0.771). Therefore, based on drought susceptible index, the top 34 hybrids have been shortlisted. The secondary drought traits play an important role which could be used for selection and improving grain yield and facilitate further efforts in maize breeding programs.

Keywords
Correlation coefficient, Drought susceptible index, Drought, Top crosses, Maize (Zea mays L.).

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**Introduction**

Drought is one of the most detrimental abiotic stresses across the world which is seriously hampering the productivity of agricultural crops. Water scarcity is the most important environmental limiting factor for maize productivity in tropical and subtropical regions (Messmer et al., 2011). Maize is among the leading cereal crops in the world, but it is sensitive to drought. Maize is affected by drought at different growth stages in different regions. Drought stress at seedling and flowering stages of maize has been estimated to cause annual yield losses of about 13% in the tropics (Edmeades et al., 1993). When drought stress occurs before or during flowering in maize, a delay in silk emergence is observed, resulting in an increase in anthesis-silking interval (ASI) (Hall et al., 1982).

Moisture stress affects crop yield by reducing the plant stand, leaf area and photosynthesis rate during pre-flowering period, ear and kernel set during flowering and by inducing early leaf senescence during grain filling stage. Additional yield reduction may be due to increased energy and nutrient consumption of drought adaptive responses, such as enhanced root growth (Zhan et al., 2015). In India, though breeding work on maize drought resistance is being operative since many years, the present day hybrids available in public and private sector need to be improved for drought tolerance with stable yield levels.

It has been found that, a complex physiogenetic mechanism highly influenced by environment exists for drought response in maize. In this regard, there is a need to develop maize hybrids with enhanced tolerance to drought situation with stable yield levels across different environments. The main objective of this study was to screen maize hybrids for tolerance to moisture stress at flowering at two maize growing areas of south India where water scarcity occurs frequently.

**Materials and Methods**

**Population development**

Based on preliminary germplasm evaluation study of CIMMYT lines (from IMIC-Asia) carried out by Ananda Kumar B V (Research Scientist Pioneer Hi-Bred Private limited) (Unpublished) Six inbred lines viz., ZL113812, ZL135133, ZL135154, ZL113908, ZL135137 and ZL135158 with relatively drought tolerant lines were identified based on the phenotyping data. During Rainy 2016 the three inbreds ZL113812, ZL135133 and ZL135154 were crossed to known tester CML479 (Neutral for drought) and ZL113908, ZL135137 and ZL135158 were crossed to known tester CML451 (Neutral for drought) to develop breeding crosses within heterotic group.

The developed six single crosses ZL113812 X CML459, ZL135133 X CML459, ZL135154 X CML479, ZL113908 X CML451, ZL135137 X CML451 and ZL135158 X CML451 were subjected to production of double haploid lines at Pioneer Hi-Bred private limited facility Bangalore and developed 50 DHL’s from each population. During Rainy 2018 all the DHL’s (300) were crossed to opposite heterotic group testers (CML 451 and CML 478) to get 300 hybrids.

**Evaluation of hybrids**

Three hundred hybrids along with competitive and relatively stress tolerant check hybrids viz., P3401, P3550, DKPDKC 9133, EUAS6668 were planted under normal well-watered (WW) and water stress at flowering condition (WSF) was considered to screen for
moisture stress tolerance by following Augmented complete block design during PR 2018. The experimental material was grown in two separate sets in two different locations viz, Hyderabad, Telangana (lat. 17°0.46 N, long. 78°0.46 E) and Aurangabad, Maharashtra (lat. 19°0.72 N, long. 75°0.20 E) which are under the control of Pioneer Hi-Bred Pvt Ltd. One set was sown under moisture stress and another set under normal conditions by following 60 × 30 cm spacing and 100:50:25 kg ha⁻¹ N:P:K.

Irrigation was given to both the sets up to forty days after sowing with a regular interval of seven to ten days. Moisture stress was induced by withholding the irrigation between 55–75 DAS (i.e. during anthesis). To avoid barren cobs and ensure optimum plant stand, a protective irrigation was given at 75 days after sowing whereas, normal field received irrigation at an interval of seven to ten days, till physiological maturity.

**Field data recording**

Data were recorded from each plot in both WSF and WW blocks at Hyderabad and Aurangabad in 2018. Days to 50 % anthesis (DTA) and days to 50 % silking (DTS) were recorded as the number of days from planting to when 50 % of plants in a plot sheded pollen, and had emerged silks, respectively. Anthesis silking interval was computed as the difference between DTS and DTA. Tassel sterility was scored on a scale of 1–9 at peak flowering stage, where 1 = all tassel branches in the plot are fertile and 9 = all the tassel branches are sterile.

Tassel blast was scored on a scale of 1–9 at peak flowering stage, where 1 = all the tasses on all plants in the plot are fertile and 9 = all the tasses on all the plants in the plot are dried up. Grain yield, measured in t ha⁻¹ adjusted to 15 % moisture content was calculated from grain weight and percent moisture. During seed setting stage the plant height was measured from the base of the randomly selected three plants at ground level to the tip of the tassel, averaged and expressed in centimeters (cm). The ear height was measured from the base of the randomly selected three plants at ground level to the Base of the ear, averaged and expressed in centimeters (cm). The relative chlorophyll content of the third leaf from the top was measured at 70 days after sowing (DAS) on three randomly selected competitive plants using chlorophyll meter (SPAD-502, Konica Minolta make). The SPAD values were recorded as the average value of chlorophyll content at lower, upper and middle portion of the leaf from each entry in both the treatments. Drought susceptibility index was computed as suggested by Fisher and Maurer (1978) by considering the data of grain yield under moisture stress and normal condition.

\[
\text{DSI} = \frac{1 - (Y_s / Y_i)}{D}
\]

Where, \(Y_s\) - Grain yield of a genotype under moisture stress environment \(Y_i\) - Grain yield of a genotype under normal environment

\[
D = \frac{\text{Mean yield of all genotypes under moisture stress condition} (\bar{Y_s})}{\text{Mean yield of all genotypes under normal environment} (\bar{Y_1})}
\]

**Statistical analysis**

Analysis of variance (ANOVA) was performed for each character using the computer system WINDOSTAT (version 9.3 from Indostat), to reveal significant effects among the genotypes and environment. Analysis of variance was carried out for all studied characters in each location separately by using augmented design. Furthermore, combined analysis of variance is given.
Results and Discussion

Mean performance of the hybrids

Combined analysis (Table 1) shows the variability of the different drought tolerance parameters. Highly significant differences between genotypes (P ≤ 0.01) were recorded for all the traits except Anthesis silking interval and Tassel sterility under WW condition and significant differences between genotypes (P ≤ 0.01) were recorded for all the traits under WSF (Table 1).

The effect due to genotypes x location's interaction showed highly significant differences for all the traits under WSF. Highly significant differences observed for all the traits except tassel sterility and tassel blast for genotypes x location interaction under WW condition. The performance of genotypes was variable according to the difference in time of incidence of drought stress (Figure 1).

The highest grain yield under WSF condition (7.82 t/ha) was achieved by the hybrid (DHL’s x tester) H148, while the lowest grain yield (0.49 kg/ha) was obtained by the hybrid H205 (Table 2). When there was no stress induced during the (WW) the highest grain yield (11.32 t/ha) was produced by hybrid H64 and the lowest grain yield (4.27 t/ha) was obtained by genotype H201.

The drought susceptibility index (DSI) shows that the most tolerant hybrids are H36 and H79 (0.48) and the most susceptible hybrid is H205 (1.4). The numbers of topmost tolerant hybrids based on DSI (Table 2) were 34 out of 300 hybrids tested across locations when compared to mean of check hybrids. Overall the reduction of 41.34 percent yield and reduced ASI for 5.27 days was also observed in WSF when compared to WW condition (Table 3).

Phenotypic correlation between the drought tolerance traits

The phenotypic correlations between the studied drought tolerance traits under WSF were exhibited in Table 4. The correlation for grain yield was highly significant and negative for DSI, ASI, Tassel sterility, Tassel blast, while highly significant and positive with chlorophyll content, ear height and plant height. The correlation for DSI was highly significant and positive with ASI, tassel sterility, and tassel blast, while it was highly significant and negative with grain yield, plant height, ear height, and chlorophyll content. There was positive and significant correlation for ASI with tassel sterility and tassel blast, but it was negative and significant with chlorophyll content, ear height, plant height, and grain yield. However, highly significant negative correlation was found for chlorophyll content with tassel sterility and tassel blast (Table 4). The correlation between ear height and plant height was significant and positive whereas highly significant and positive between tassel sterility and tassel blast. Significant negative correlation was found for plant height with tassel sterility and tassel blast (Table 4).

Drought stresses affect's maize grain yield to some degree at almost all growth stages (Grant et al., 1989, Ahmed, 2002). Flowering stage has been considered the most sensitive stage accompanying with reduce pollen production, pollen viability, tassel blasting and prolong anthesis– silking interval (ASI) under moisture stress (Kumar et al., 2015). In this study, there were reductions in the estimate of genetic variability of traits under the water-stress treatments depending on the severity of drought. The reduction of grain yield was observed by 41.34 percent when compared to optimal condition and ASI is considered to be most important trait for drought tolerance has reduced by 5.27 days.
which is also observed by Almeida et al., (2013) and Abuali et al., (2014). The effect due to genotypes x location was highly significant for all traits under study and significant for grain yield and chlorophyll content under WSF indicating that the genetic variance in stress environment was more than non-stress conditions (Hohls, 2001).

The highest yield was recorded for the hybrid H148 under WSF and the top yielding hybrid under WW was H64 similarly the least values of DSI for hybrids H79 and H36 indicates that the hybrids which are top yielding under WSF are not same in WW condition and response of genotypes to drought differs according to their genetic structure and adaptability. Wenzel (1999) reported that some genotypes yielded more under moisture stress than under near-ideal moisture conditions. Johnson and Geadelmann (1989) reported that a low genetic correlation was often observed to yield in high-and low-productivity environments, indicating that unusual sets of genes may be important, indicating the yield in different environments.

Thirunavukkarasu et al., (2014) concluded that phenotypic correlation coefficient showed that grain yield and the traits contributing to it were positively and significantly correlated with each other, and ASI was negatively but significantly correlated with other agronomic traits similar trend has been observed in the study. The grain yield was positively correlated with chlorophyll content, plant height and ear height, indicating the importance of these traits in selection for yield and grain yield was negatively correlated with ASI and DSI.

| Table 1 | Mean squares from the analysis of variance due to genotypes (G) and their interaction with locations (GxL) between 304 maize genotypes for all the traits studied under WSF and WW condition |
|-----------------|-----------------|-----------------|
| **Drought Tolerance** | **Genotypes** | **G X L** |
| DF | 303 | 303 |
| Anthesis silking interval WW | 0.98 | 89.29** |
| Anthesis silking interval WSF | 10.5** | 588.16** |
| Chlorophyll content WW | 74.57** | 528.95** |
| Chlorophyll content WSF | 44.96** | 20.04* |
| Ear height WW | 173.42** | 373.75** |
| Ear height WSF | 124.19** | 213.7** |
| Plant height WW | 275.82** | 817.5** |
| Plant height WSF | 342.95** | 1339.6** |
| Tassel sterility WW | 0.03 | 0.02 |
| Tassel sterility WSF | 7.41** | 43.69** |
| Tassel blast WW | 0.04** | 0.006 |
| Tassel blast WSF | 7.29** | 127.11** |
| Grain Yield WW | 3.88** | 4.46* |
| Grain Yield WSF | 3.54** | 3.85** |
| Drought susceptibility index | 0.048** | 0.64** |

*Significant at 5%  ** Significant at 1%
Table 2: Means of drought tolerance traits of top 34 maize top cross hybrids evaluated at two water treatments across two locations (Hyderabad and Aurangabad) during Rabi 2018

| Hybrid | DSI | Grain Yield (t/ha) | ASI | Chlorophyll content (SPAD values) | Ear height (cm) | Plant height | Tassel sterility (score) | Tassel blast (score) |
|---|---|---|---|---|---|---|---|---|
| | WW | WSF | WW | WSF | WW | WSF | WW | WSF |
| H79 | 0.481 | 10.22 | 7.29 | 1.67 | 4.21 | 37.10 | 25.66 | 123.87 | 108.63 |
| H36 | 0.485 | 9.12 | 6.19 | 1.54 | 3.58 | 36.90 | 32.37 | 125.13 | 105.00 |
| H12 | 0.490 | 9.02 | 6.09 | 2.54 | 3.08 | 44.90 | 26.92 | 126.13 | 99.49 |
| H214 | 0.498 | 8.87 | 5.94 | 2.04 | 5.58 | 31.30 | 27.32 | 117.43 | 106.98 |
| H29 | 0.501 | 8.87 | 5.94 | 2.04 | 4.58 | 49.45 | 27.97 | 119.87 | 102.24 |
| H81 | 0.504 | 9.77 | 6.84 | 2.17 | 5.71 | 43.70 | 25.96 | 112.14 | 101.87 |
| H63 | 0.508 | 9.77 | 6.84 | 1.17 | 3.71 | 41.75 | 25.91 | 115.89 | 109.13 |
| H148 | 0.510 | 10.75 | 7.82 | 2.92 | 4.21 | 40.06 | 28.97 | 106.79 | 100.64 |
| H77 | 0.510 | 9.62 | 6.69 | 1.17 | 3.21 | 52.20 | 28.01 | 110.89 | 93.87 |
| H175 | 0.510 | 9.62 | 6.69 | 3.04 | 4.71 | 29.45 | 26.41 | 101.02 | 94.87 |
| H180 | 0.512 | 9.67 | 6.74 | 1.54 | 4.21 | 39.80 | 28.31 | 107.27 | 96.37 |
| H211 | 0.517 | 8.57 | 5.64 | 3.04 | 4.58 | 51.95 | 25.87 | 101.43 | 95.50 |
| H94 | 0.521 | 9.42 | 6.49 | 2.17 | 4.71 | 34.15 | 29.36 | 122.88 | 95.38 |
| H160 | 0.521 | 9.47 | 6.54 | 1.54 | 4.21 | 38.45 | 29.06 | 108.52 | 105.37 |
| H14 | 0.522 | 8.47 | 5.54 | 1.54 | 3.58 | 39.50 | 33.17 | 116.62 | 116.49 |
| H41 | 0.522 | 8.47 | 5.54 | 2.04 | 4.08 | 30.10 | 26.37 | 113.63 | 112.74 |
| H245 | 0.523 | 8.47 | 5.54 | 1.54 | 4.58 | 53.10 | 31.32 | 113.44 | 103.74 |
| H87 | 0.530 | 9.27 | 6.34 | 1.67 | 5.21 | 44.20 | 27.41 | 123.63 | 105.62 |
| H16 | 0.531 | 8.42 | 5.49 | 1.04 | 3.58 | 36.30 | 24.77 | 121.62 | 99.25 |
| H23 | 0.535 | 8.27 | 5.34 | 1.54 | 5.08 | 51.00 | 34.27 | 107.12 | 102.74 |
| H190 | 0.541 | 9.07 | 6.14 | 2.04 | 4.71 | 37.00 | 22.06 | 99.02 | 95.02 |
| H165 | 0.544 | 9.02 | 6.09 | 1.04 | 5.71 | 31.85 | 21.51 | 96.27 | 88.86 |
| H70 | 0.551 | 11.00 | 7.34 | 1.67 | 3.71 | 35.90 | 26.81 | 121.38 | 111.88 |
| H168 | 0.551 | 8.92 | 5.99 | 2.04 | 5.71 | 31.50 | 23.56 | 109.76 | 98.38 |
| Traits                           | WW    | WSF   | Difference | Reduction under stress (%) | Increase under stress (%) |
|---------------------------------|-------|-------|------------|-----------------------------|---------------------------|
| Grain yield (t/ha)              | 7.27  | 4.26  | 3.00       | **41.34**                   | -                         |
| Anthesis silking interval       | 2.26  | 7.53  | -5.27      | -                           | **232.85**                |
| Chlorophyll content (SPAD Value)| 36.37 | 21.70 | 14.67      | **40.34**                   | -                         |
| Ear height (cm)                 | 110.83| 98.79 | 12.04      | **10.86**                   | -                         |
| Plant height (cm)               | 206.23| 182.66| 23.57      | **11.43**                   | -                         |
| Tassel sterility (score)        | 1.03  | 4.16  | -3.13      | -                           | **302.77**                |
| Tassel blast (score)            | 1.04  | 3.84  | -2.80      | -                           | **268.82**                |

Table 3: Effect of moisture stress on yield, its components traits and traits associated with moisture stress tolerance in maize hybrids.
Table 4 Phenotypic coefficient of correlations between the different drought tolerance traits for maize genotypes under two locations under WSF during Rabi 2018

| Traits                          | DSI       | ASI   | Chlorophyll content (SPAD) | Ear height (cm) | Plant height (cm) | Tassel sterility (score) | Tassel blast (Score) | Grain Yield (t/ha) |
|--------------------------------|-----------|-------|---------------------------|-----------------|------------------|-------------------------|---------------------|-------------------|
| DSI                            | 1         |       |                          |                 |                  |                         |                     |                   |
| ASI                            | 0.745**   | 1     |                          |                 |                  |                         |                     |                   |
| Chlorophyll content (SPAD)     | -0.622**  | -0.599** | 1                        |                |                  |                         |                     |                   |
| Ear height (cm)                | -0.348**  | -0.241** | 0.361**                  | 0.241**        |                  |                         |                     |                   |
| Plant height (cm)              | -0.253**  | -0.179** | 0.298**                  | 0.471**        | 1                |                         |                     |                   |
| Tassel sterility (score)       | 0.785**   | 0.631** | -0.585**                 | -0.363**       | -0.192**         | 1                       |                     |                   |
| Tassel blast (Score)           | 0.771**   | 0.605** | -0.604**                 | -0.350**       | -0.165**         | 0.906**                 |                     |                   |
| Grain Yield (t/ha)             | -0.852**  | -0.636** | 0.612**                  | 0.424**        | 0.252**          | -0.901**                | -0.912**            | 1                 |

*Significant at 5%  ** Significant at 1%

Fig. 1 Mean Grain Yield (t/ha) for 304 maize hybrids evaluated under two water treatments at two locations (Hyderabad and Aurangabad)

These observations are in conformity with the findings of Kumar et al., (2006), Pavan et al., (2011), Dar et al., (2015), Sabiel et al., (2015) and Jakhar et al., (2017a). On studying association and interrelationships among the traits other than grain yield which might aid in understanding an idea of plant type it was revealed that plant height had highly significant positive correlation with ear height and grain yield. Similar observations were reported by Bhole and Patil (1984) and Jakhar et al., (2017b).

Based on the findings in this study, we concluded that the water stress at flowering reduced grain yield significantly and the single hybrid has not performed across treatment hence the drought related secondary traits plays an important role in selection of best hybrids which would be used for selecting the best genotypes. The traits drought susceptible index, Anthesis silking interval, chlorophyll content, plant height, ear height, tassel blast and tassel sterility were highly correlated with grain yield and need to
be considered for selection. The conclusions revealed that there is scope for simultaneous improvement of these traits through selection. The highest positive and direct effects of chlorophyll content similarly highest negative and indirect effects of anthesis silking interval, DSI, tassel sterility and tassel blast were revealed on grain yield. These traits contributed maximum to higher grain yield compared to other characters, thus, selection for these characters helps in identifying the superior cross combinations for improvement of yield.

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