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Physiological and Biochemical Evaluation of Maize Hybrid Germplasm Lines for Drought Tolerance under Receding Soil Moisture Conditions

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A B S T R A C T

A field experiment was conducted during *Rabi* 2015 in a Randomized block design (RBD) with three replications to screen 12 hybrid maize germplasm lines viz., Z630-1, Z630-2, Z630-3, Z630-4, Z637-1, Z637-2, Z695-1, Z695-2, Z695-3, Z638-1, Z638-2, Z638-3 along with three hybrid checks NK6240, P1O3396 and 900MGold. This study was carried out to screen the hybrid germplasm lines exposed to water deficit stress during the reproductive growth stage. The analysis of variance revealed significant differences among the test germplasm line for all the parameters recorded. The direct measurement of RWC and LWP which are considered as consensus estimates of plant water status revealed that Z695-3 (92.32%, -1.84 Mpa) and Z638-2 (89.33%, -1.79 Mpa) are drought tolerant lines along with high yield potential. The biochemical traits include Proline accumulation, epi-cuticular wax content, calcium content and the chlorophyll content, the calcium content is negatively associated with the yield, the leaf wax content and the Proline at 15 DASi observed to be recorded in high yielding germplasm line. The proline content, RWC, LWP and CSI are the most reliable parameters for the phenotypic drought tolerant screening.

Introduction

Maize, (*Zea mays* L.) is the third most important cereal food crop consumed by the world's population after wheat and rice. It is a versatile crop grown under different agroclimatic conditions.

Owing to its genetic yield potential maize is referred as "queen of cereals". It is grown throughout the year in all the seasons in India but predominantly grown as a kharif crop and accounts approximately 9 percent of the total food grain production of the country. Maize plays an important role in Indian economy, besides being a potential food source, it is also used as a fodder crop, cattle feed, poultry feed and industrial purposes such as glucose and starch production.

Water is the elixir of life, and is the most important input in agriculture. When the soil water is insufficient and results in lack of crop growth and production is referred to as drought. Drought is the major stress that compromises the yield around the world

(Reddy *et al.,* 2004). Drought, being the most important environmental abiotic stress, severely impairs plant growth and development, limits plant performances and productivity, more than any other environmental factor.

Plants adapt to drought stress by different mechanisms, including morpho-physiological and biochemical process. Under water stress in the field, genotypes with low crown root number (CN) having 13% greater leaf relative water content and 57% greater yield than genotypes with high CN where reduced CN improves water acquisition under water deficit stress in maize (Yingzhi and Jonathan, 2016). Maintaining well water status in plant is crucial to perform optimal physiological functioning and growth under stressed conditions some studies have suggested that high RWC is closely related to drought tolerance (Chen *et al.,* 2016). Analysis of trait – trait and trait –yield associations indicated significant positive correlations amongst the water relations traits of relative water content (RWC), leaf water potential and osmotic potential as well as of RWC with grain yield underwater stressed condition (Maheswari *et al.,* 2017).

Drought stress significantly reduces chlorophyll a and b contents of leaf in maize genotypes (Ahmad *et al.,* 2017). Under water stressed conditions the genotype with highest spad chlorophyll meter reading (SCMR) value given higher yield (Maheswari *et al.,* 2017). The presence of epicuticular waxes protects plants from water loss and other environmental stresses the plants with lesser epicuticular wax content were more susceptible to drought (Li *et al.,* 2018).

Under drought stress, calcium acts as a second messenger, which is employed to regulate specific protein kinase activity and downstream gene expression and may be involved in plant tolerance to heat stress by regulating antioxidant metabolism or/and water relations (Jiang and Huang, 2001). Chlorophyll stability index, tolerant lines either resisted decrease in chlorophyll content during stress conditions or showed very little reduction, in contrast, the susceptible lines showed large reduction in the chlorophyll content under drought environments (Meena *et al.,* 2004). Biochemical analysis including mannitol, glycine betaine, trehalose and proline contents, have long been proposed to be useful as a complementary strategy for selection of drought tolerant genotypes in plant breeding (Mwadzingeni *et al.,* 2016).

In the present study twelve germplasm lines along with three checks were studied under receding soil moisture conditions based on the physiological and biochemical traits contributing for drought tolerance.

Materials and Methods

A field experiment was conducted on maize (*Zea mays*) during *Rabi* 2015 at EB-II section of the Department of Plant Breeding and Genetics, College of Agriculture, OUAT, Bhubaneswar. Geographically the field experimental site is located on 20º 25´ latitude, 82º 52´ longitude and at an altitude of 25.9 m above mean sea level and nearly 64 km west of Bay of Bengal. It falls in the humid sub-tropical climatic zone of the state.

The experiment was conducted by the collaboration CIMMYT, Mexico. The germplasm lines screened were Z630-1, Z630-2, Z630-3, Z630-4, Z637-1, Z637-2, Z695-1, Z695-2, Z695-3, Z638-1, Z638-2, Z638-3 along with checks NK6240, P1O3396, 900MGold. The stress period is given up to 4 weeks, 2 weeks before the tasselling and two weeks after tasselling. The materials used and the methods followed were briefly explained here.

Relative water content (RWC %)

Relative water content expresses the water in the original sample as a percentage of the water in the fully hydrated tissue. It was estimated by following the method of Barrs and Weatherly (1962) at 45, 55, 65, 75 and 90 DAS. Leaf discs of the third leaf from the top were collected (from five random plants) and weighed up to three decimals. This was taken as fresh weight of the tissue sample. The weighed leaf discs were kept immersed in Petri dish containing distilled water and allowed to take up water for 24 hours. After 24 hours, leaf discs were blotted gently and weighed to get a turgid weight. After recording turgid weight, the leaf discs were dried in an oven at 80° C for 48 hours to get the dry weight. Then, RWC was calculated by using the following formula:

RWC % = {Fresh weight (g) - Dry weight (g)} / {Turgid weight (g) - Dry weight (g)}

Chlorophyll stability index

Two clean glass test tubes were taken and 100 mg of representative leaf sample is placed in one of them with 50ml distilled water. The tube was then subjected to heat treatment in a water bath at 56°C + 1°C for exactly 30 minutes.

The leaves are then ground in a mortar for five minutes with 20ml of 80 per cent acetone. The slurry is then filtered with Whatman No.1 filter paper. This chlorophyll extract was immediately measured for light absorption with spectrophotometer at 652 nm. The other leaf sample kept control was also ground and the chlorophyll was extracted and the absorbance measured at 652 nm. (Murthy and Majumdar, 1962)

CSI (%) = total chlorophyll of treated/total chlorophyll of untreated * 100

Cell membrane stability

Leaf pieces of leaf tissue cut with scissors placed in standard glass vials that can accommodate a conductivity electrode. The total area of leaf material per vial is about 15 to 25 cm^2. The sample is then washed for 2-3 times with de-ionized water. The water is drained off but samples remain wet so that they would not desiccate. Five pairs of vials are taken from five different plants (replicates). For each pair, one vial is designated as treatment (T) and the other as control (C). The treatment vials are subjected to the heat stress treatment in vitro. They are placed in racks and covered (not stoppered) with 'Saran' wrap so as to avoid drying the samples. Racks are placed in thermostated water bath so that the leaf samples will be completely below the water surface level. Temperature is set to a predetermined stress (treatment) temperature and the samples remain in the bath for 1h. The control vials are placed in a rack, covered with Saran wrap and placed at room temperature. The treatment temperature is 56ºC. After treatment 20cc of deionized water is added to each vial making certain that all leaf materials are submerged. All vials are then placed for incubation at about 10^0C for 24h. After incubation the samples are equilibrated for 1h at room temperature and the conductivity of the medium is measured by inserting a conductivity electrode into each vial. All vials covered with Saran wrap or plastic sheet are placed in an autoclave for 15 min to kill all tissues. Conductivity of all samples is measured after samples are equilibrated to room temperature (Sullivan 1979).

$CMS\% = \{[1-(T_1/T_2)]/[1-C_1/C_2)]\}*100$

Where T1 and T2 are treatment conductivities before and after autoclaving and C1 and C2 are the respective control conductivities. Calculated results are often better when each

T value is calculated against the average of all C values for the given accession.

Leaf water potential

Leaf samples of 10 to 20 cm^2 area were cut from the third leaf of the plant and those are kept in a polythene bags and wrapped in a wet cloth to conserve the moisture and those were brought to lab and there the samples were made into circular discs of size that could cover the area of the potentiometer (WP4C Dewpoint Potentiometer) sample slot and the water potential is measured with the help of potentiometer.

Estimation of chlorophyll content

Total chlorophyll content in the leaves were determined by using the method stated by Arnon (1949). The leaf samples collected from the field were immediately kept in moist polythene bags to keep them fresh. 100 mg of fresh leaf was taken from the middle portion of the leaf and were cut into small pieces. The leaf discs were then put in 80 % v/v acetone solution and kept in dark for 24 hours. Then they were filtered by Whatman No.1 filter paper and the filtrate was used to record the absorbance (OD) at 645 nm and 663 nm. The respective chlorophyll content was calculated using the following formula and expressed as mg g^{-1} FW leaf.

$$\text{Chlorophyll a}=(12.7*OD_{663}-2.69*OD_{645})\frac{V}{1000*W}$$

$$\text{Chlorophyll b}=(22.9*OD_{645}-4.68*OD_{663})\frac{V}{1000*W}$$

$$\text{Total Chlorophyll}=(20.2*OD_{645}-8.02*OD_{663})\frac{V}{1000*W}$$

Where, OD_{645} = OD value at 645 nm, OD_{663} = OD value at 663 nm, V = Total volume of extract (ml), W = Fresh weight of leaf (g)

SCMR (Spad chlorophyll meter reading)

The SCMR value was recorded from a randomly selected leaf at 45, 55, 65, 75 and 90 DAS on ten random plants using chlorophyll meter (Hanstech model CL01).

Carotenoid content

Carotenoid content in the leaves were determined by using the method stated by Nayek *et al.,* (2014). The 3rd leaf from the top was sampled for the purpose. The leaf samples were immediately kept in moist polythene bags to keep them fresh. 100 grams of fresh leaf was taken from the middle portion of the leaf and were cut into small pieces. The leaf discs were then put in 80 % v/v acetone solution and kept in dark for 24 hours. Then they were filtered by Whatman No.1 filter paper and the filterate was used to record the absorbance (OD) at 470nm. The respective chlorophyll content was calculated using the following formula and expressed as mg g^{-1} FW leaf (Sumanta *et al.*, 2014).

Carotenoids (mg g^{-1}) = (1000 OD_{470} – 1.82Chl a –85.02Chl b)/198

Proline content

Leaf sample of 100 mg was taken in mortar and homogenised with 10 ml of 3% aqueous sulfosalicylic acid and filtered through Whatman No. 2 filter paper. The extracted filtrates were added 2ml each of glacial acetic acid and acid ninhydrin and mixed. Then the test tubes are incubated in boiling water bath at 100 ºC for 1 hour and the test tubes were kept in ice bath to terminate the reaction then add 4 ml toluene and shake vigorously the colour developed during the incubation was being transferred to toluene, the top clear layer was separated with a micropipette and the OD was measured at 520nm against the blank prepared. The value got from standard curve is

µg of proline/ml, the µ moles of proline/g tissue was calculated from the fallowing formula **(**Bates *et al.,* 1973).

$$\mu \text{ moles of proline/g tissue} = \frac{\mu g \text{ proline / ml x ml toluene}}{115.5} x \frac{5}{\text{g sample}}$$

Leaf wax content

The individual sample consisted of 10 leaf discs having a total area (both surfaces) of known value. Each sample was immersed in 15 ml redistilled chloroform for 15 secs. The extract was filtered and evaporated on a bailing water bath, until the smell of Chloroform could not be detected. After adding 5 ml of reagent, samples are placed in boiling water for 30 min. After cooling, 12 ml of deionized water is added. The samples were allowed for colour development and cooling and then the OD of the sample was recorded at 590 nm. Standard wax solutions were prepared from caruanba wax (Ebercon *et al.,* 1977).

Calcium content

A volume of 25 ml triple acid extract was taken in to a porcelain basin. 10% Sodium hydroxide was added drop by drop to neutralise the acidity (red litmus turns blue) and 5 ml excess to maintain the pH at 12. A pinch of muroxide indicator was added and titrated against 0.02 N EDTA till red color changed from pinkish red to purple or violet. The percentage calcium content of the sample was calculated from the following formula (Jackson 1973).

% of calcium in the given sample on moisture free basis = $0.0004(B * \frac{V}{25} * \frac{100}{W_g})$

Where, Weight of plant sample taken (Wg), Volume of triple acid extract prepared (Vml 100ml), Volume of triple acid extract taken for titration (25 ml), Volume of 0.01 N EDTA used (B ml), 1 ml of 0.02 N EDTA (0.0004 g of calcium)

Results and Discussion

The RWC value was recorded highest in Z630-2 (93.87%) at 90 DAS followed by Z695-3 (92.32%) and Z638-1 (91.98%) and are depicted in the table 1. These showed a percentage decrease of -2.61%, -4.22% and -4.63% respectively with the tolerant check PIO3396. The percentage decrease in the RWC from 45 DAS is of -1.04%, -3.06% and -3.13% respectively. Water stress significantly affected the water potential (Table 1). Increase in water stress caused substantial decline in leaf water potential. Highest leaf water potential was (-1.79 MPa) recorded at 45 DAS (before imposition of stress) followed by -1.97 MPa at 5 DASi. Lowest leaf water potential (-3.60 MPa) recorded at 15 DASi in the germplasm line Z638-3. Leaf water potential in all the germplasm lines was significantly different. Highest leaf water potential (-1.62 MPa) recorded in Z637-1 at 90 DAS that was significantly higher than all other germplasm lines followed by Z695-3 (-1.99 MPa). Lowest leaf water potential (-2.66 MPa) recorded in Z630-4. With increase in water stress, leaf water potential decreased significantly in all hybrid germplasm lines under study. Soil water potential was measured at 5 DASi and 15 DASi. The water potential of different soil samples at different depths were taken and are averaged and the mean values were depicted in the table 2. The soil water potential is observed to be more during 15 DASi and there is no significant difference among the plots of germplasm lines. The soil water potential is less negative during 5 DASi and a significant difference was observed

The changes in total chlorophyll content and SCMR were recorded and given in table 3. The result revealed that The SCMR value was recorded highest in Z630-2 (36.48) at 90 DAS

followed by Z638-2 (29.75) and Z638-3 (26.47) respectively. Initially the SCMR values increased up to 15 DASi and thereafter a declining trend was observed. The decrease was also visualised from that of the tolerant check PIO3396 with a tune 17.49%, -4.19% and -14.77% respectively. Statistically significant decrease from 15 DASi up to 90 DAS was observed. The total chlorophyll content found highest in Z630-3 (1.84 $mg.g^{-1}$ FW) at 90DAS followed by Z638-2 (1.81 $mg.g^{-1}$ FW) and Z695-1(1.80$mg.g^{-1}$ FW) (Table 3). These showed a percentage change of 15.09%, 13.28% and 12.54% with the tolerant check 900M Gold. There is a significant increase in the total chlorophyll value up to 15 DASi. Then there is a significant decrease from 15 DASi up to 90 DAS.

The chlorophyll a was recorded highest in Z695-1 (1.49$mg.g^{-1}$ FW) at 90 DAS followed by Z630-3 (1.39$mg.g^{-1}$ FW) and Z695-2 (1.39$mg.g^{-1}$ FW). That showed a percentage change of 9.92%, 2.51% and 2.91% with the tolerant check NK6240. There is a significant increase in the chlorophyll a content up to 15 DASi in all the germplasm lines. Then there is a significant decrease from 15 DASi up to 90 DAS. The chlorophyll b was recorded the same trend to that of chl a with highest in Z638-2 (0.5339 mg. g^{-1} FW) at 90 DAS followed by Z630-3 and Z638-1. These showed a percentage change of 92.85%, 65.05% and 34.27% with the tolerant check 900M Gold. There is a significant increase in the chlorophyll b content up to 15 DASi. Then there is a significant decrease from 15 DASi up to 90 DAS. The carotenoid content was recorded highest in Z630-3 (3.35 mg. g-1 FW) at 90 DAS followed by Z695-2 (3.09 mg. g^{-1} FW) and Z638-2 (3.06 mg. g^{-1} FW). These show a percentage change of 23.97%, 13.78% and 13.27% with the tolerant check 900M Gold. These showed a significant increase from 15 DASi with a percentage of 63.08%, 39.87% and 3.15% respectively. However, the carotenoid content increased from 65DAS to 90DAS in all the germplasm lines (Table 4).

The proline content was recorded and found highest in Z638-1(8.1mgg^{-1} FW) followed by Z630-2 (7.7mgg^{-1} FW) and Z695-2 (5.5 mgg^{-1} FW). These showed a percentage increase of 25.76%, 18.48% and 7.71% respectively with the tolerant check PIO3396. The percentage increase from 45 DAS is 34.78%, 111.37% and 120.17% respectively. There is a significant increase of proline content in all the germplasm lines after the recovery from stress. The epi-cuticular wax content was recorded highest in Z695-3 (3.70 mgg^{-1}) followed by Z695-2 (3.15 mgg^{-1}) and Z630-2 (2.63 mgg^{-1}). These showed a percentage change of 19.48%, 1.83% and -14.89% with the tolerant check PIO3396. Leaf wax content show significant difference among the germplasm lines. The calcium content was recorded highest in Z637-1, Z695-1, and Z695-2 (0.71 % DW). These showed a percentage change of 3.41%, 3.90% and 3.90% with the tolerant check 900M Gold. Calcium content shows a significant difference among the germplasm lines.

The CMS was recorded at 45 DAS, 5 DASi, 15 DASi, 75 DAS and 90 DAS and those values were depicted in the Table 5. The highest value of CMS at 90 DAS was recorded in the germplasm lineZ638-3 (91.77%) followed by Z630-1 (91.63%) and Z630-2 (88.74). these show a percentage decrease of 1.83%, 1.98% and 5.08% with respect to the tolerant check PIO3396. Z638-2 Z695-2 show constancy in CMS along with the tolerant check NK6240. The CSI was recorded at 45 DAS, 5 DASi, 15 DASi, 75 DAS and 90 DAS and those values were depicted in the Table 6. The highest value of CSI at 90 DAS was recorded in the germplasm lineZ637-2 (92.3%) followed by Z695-2 (89.8%) and Z638-2 (86%). these show a percentage

increase of 23.03%, 19.69% and 14.68% with respect to the tolerant check PIO3396.

The yield in tonnes/ha was recorded maximum in the germplasm line Z695-3 (2.65 t ha^{-1}) Followed by Z638-2 (2.61 t ha^{-1}) which are considered to be drought tolerant based on their Phiso-biochemical parameters. Proline content, RWC, LWP and CSI were suitable traits which can be used for the screening of maize lines for drought tolerance.

Direct measurement of leaf water potential by dew depression method and leaf RWC are consensus estimates of plant water status. RWC is considered as a preferred estimate in breeding work since it accounts for the effect of osmotic adjustment of leaf hydration. Highest leaf water potential observed in Z638-2 (-1.79 MPa) followed by Z695-3 (-1.84 MPa) at initial stage while lowest leaf water potential (-2.59 MPa) recorded in Z630-1 followed by Z637-3 (-1.95 MPa) at 15DASi. Overall data clearly showed the significant decline in leaf water potential under water deficit conditions. The similar findings of the maintenance of turgor by adjustments in osmotic potential in response to water stress was also observed by (Hanson and Hitz, 1982; Muhammad *et al.,* 2013). Further a significant decrease in the relative water content in the present investigation during the water stressed situation was observed at 50 DAS corresponding to 5 DASi and 15 DASi. The highest RWC was recorded in the germplasm line Z695-3 (92.32%) and Z638-1 (91.98%) but a significant increase was observed during the recovery at 75 DAS and 90 DAS. The tolerant hybrids showed more decrease in the relative water content. The above results were in line with the findings of (Jaberi *et al.,* 2014; Efeoğlu *et al.,* 2009).

The root to shoot ratio on dry weight basis was observed to increase from 5 DASi to 15 DASi. The maximum root: shoot ratio was recorded in Z637-2 (0.48) followed by 0.62 and 0.73 at 5 DASi, 15 DASi and 90 DAS respectively. The root: shoot ratio was observed to be increased in tolerant germplasm lines during the moisture stress period (Katerji *et al.,* 2009).

The chlorophyll content, in general, decreased in response to moisture stress to a tune of 12.7% (on an average) from control irrespective of growth stages and the germplasm lnes. Similar reduction in chlorophyll content in maize hybrid lines have been observed by (Revilla *et al.,* 2016; Shakeel 2008; Efeoğlu *et al.,* 2009).

The tolerant lines Z630-2 registered lowest decrease compared to susceptible line Z638-1. Total chlorophyll, chlorophyll-a and chlorophyll-b accumulated in all germplasm lines was significantly different. However, Chlorophyll "b" content slightly increased at first but thereafter decreased sharply.

A significant difference of chlorophyll was also observed in the SPAD value and the findinds was in the line work of Revilla *et al.,* 2016. Carotenoid pigments are responsible for scavenging of singlet oxygen hence comparatively high carotenoid levels in genotypes have been suggested to be a measure of their tolerance (Chandrasekar *et al.,* 2000).

The chlorophyll stability index (CSI) exhibited similar decrease with imposition of moisture stress. The decrease in CSI due to imposition of stress in different hybrid germplasm lines ranged from 53% to 95%. This corroborates with the results obtained by Roshni (2016). However, the decrease was minimum in case of tolerant lines.

Greater amount of free proline was found to accumulate when plants were subjected to stress irrespective of growth stages.

Table.1 Effect of water stress on Relative water content and water potential in different germplasm lines of maize

Treatment	Relative water content (%)					Water potential (Mpa)				
	45 DAS	5 DASi	15 DASi	75 DAS	90 DAS	45 DAS	5 DASi	15 DASi	75 DAS	90 DAS
Z630-1	94.02 (0.83)	88.61 (-4.73)	89.93 (-6.70)	93.42 (2.90)	87.30 (-2.60)	-2.59 (25.18)	-1.74 (-24.51)	-3.60 (16.88)	-2.55 (-15.84)	-2.37 (-12.89)
Z630-2	94.86 (1.73)	92.87 (-0.15)	93.87 (-2.61)	84.96 (-6.43)	91.88 (2.50)	-2.27 (9.69)	-1.66 (-27.98)	-3.25 (5.52)	-2.55 (-15.84)	-2.22 (-18.42)
Z630-3	93.96 (0.77)	90.90 (-2.27)	89.52 (-7.13)	94.50 (4.09)	92.28 (2.94)	-2.26 (9.44)	-1.53 (-33.84)	-2.76 (-10.39)	-2.33 (-23.27)	-2.00 (-26.34)
Z630-4	93.47 (0.24)	80.20 (-13.78)	76.16 (-20.98)	95.97 (5.71)	84.23 (-6.03)	-2.06 (-0.48)	-1.56 (-32.32)	-2.75 (-10.71)	-3.04 (0.17)	-2.66 (-2.03)
Z637-1	96.60 (3.60)	89.29 (-4.00)	88.13 (-8.57)	94.71 (4.31)	90.45 (0.91)	-2.45 (18.64)	-2.64 (14.53)	-3.42 (10.88)	-2.59 (-14.69)	-1.62 (-40.33)
Z637-2	93.31 (0.07)	91.66 (-1.45)	90.26 (-6.36)	93.20 (2.65)	93.06 (3.82)	-1.92 (-7.26)	-1.34 (-42.08)	-1.95 (-36.85)	-2.02 (-33.33)	-2.26 (-16.76)
Z695-1	95.85 (2.79)	89.38 (-3.90)	88.56 (-8.12)	99.24 (9.30)	90.20 (0.63)	-2.07 (0.00)	-1.93 (-16.27)	-2.21 (-28.25)	-3.23 (6.44)	-2.97 (9.39)
Z695-2	96.71 (3.71)	89.53 (-3.75)	86.62 (-10.13)	86.51 (-4.71)	92.43 (3.11)	-2.29 (10.90)	-1.39 (-39.91)	-3.16 (2.44)	-2.77 (-8.58)	-2.45 (-9.76)
Z695-3	95.23 (2.13)	93.98 (1.04)	92.32 (-4.22)	92.15 (1.49)	95.65 (6.70)	-1.84 (-11.14)	-1.92 (-16.92)	-2.28 (-25.97)	-2.63 (-13.37)	-1.99 (-26.70)
Z638-1	94.90 (1.78)	92.68 (-0.36)	91.93 (-4.63)	96.40 (6.17)	93.43 (4.23)	-2.51 (21.31)	-1.49 (-35.36)	-3.36 (8.93)	-3.00 (-1.16)	-2.60 (-4.24)
Z638-2	94.96 (1.83)	90.46 (-2.74)	89.33 (-7.32)	96.22 (5.98)	91.60 (2.19)	-2.37 (14.77)	-2.45 (6.29)	-3.04 (-1.46)	-2.99 (-1.49)	-2.42 (-11.05)
Z638-3	96.41 (3.40)	92.53 (-0.52)	90.78 (-5.81)	95.11 (4.76)	94.27 (5.17)	-1.79 (-13.32)	-1.97 (-14.53)	-2.74 (-11.20)	-2.58 (-15.02)	-2.39 (-12.15)
NK6240	92.32 (-0.99)	85.93 (-7.61)	84.28 (-12.56)	87.30 (-3.85)	87.58 (-2.29)	-2.11 (2.18)	-1.86 (-19.52)	-3.32 (7.63)	-2.12 (-30.20)	-1.90 (-30.02)
900M Gold	92.63 (-0.66)	76.15 (-18.13)	64.40 (-33.18)	93.30 (2.76)	87.89 (-1.95)	-2.75 (32.93)	-1.58 (-31.45)	-3.29 (6.82)	-2.98 (-1.82)	-2.76 (1.47)
PIO3396	93.24	93.01	96.39	90.79	89.64	-2.07	-2.31	-3.08	-3.03	-2.72
SE(m)±	0.33	2.12	4.12	1.60	1.54	0.07	0.31	0.03	0.19	0.17
CD(0.05)	0.81	5.25	10.22	3.97	3.81	0.17	0.76	0.06	0.46	0.42
CV(%)	0.60	4.11	8.16	2.99	2.93	-5.30	-29.20	-1.50	-11.98	-12.37

Figures in Parenthesis indicate % change over check

Table.2 Soil water potential

Soil water potential (Mpa)		
Treatment	**5 DASi**	**15 DASi**
Z630-1	-0.45	-0.82
Z630-2	-0.43	-0.79
Z630-3	-0.50	-0.81
Z630-4	-0.44	-0.80
Z637-1	-0.46	-0.79
Z637-2	-0.43	-0.80
Z695-1	-0.49	-0.81
Z695-2	0.51	-0.80
Z695-3	-0.44	-0.80
Z638-1	-0.51	-0.77
Z638-2	-0.46	-0.80
Z638-3	-0.43	-0.79
NK6240	-0.46	-0.80
900M Gold	-0.53	-0.81
PIO3396	-0.51	-0.81
SE(m)±	0.02	0.01
CD(0.05)	0.06	0.03
CV(%)	-8.50	-2.73

Table.3 Effect of water stress on SCMR value and total chlorophyll content in different germplasm lines

Treatment	SCMR					Total Cholrophyll				
	45 DAS	5 DASi	15 DASi	75 DAS	90 DAS	45 DAS	5 DASi	15 DASi	75 DAS	90 DAS
Z630-1	26.26	32.24	45.81	30.08	22.95	2.68	2.68	2.12	2.51	1.50
Z630-2	29.12	40.14	44.50	29.1	36.48	2.89	2.17	1.78	2.07	1.27
Z630-3	28.95	39.63	42.03	29.10	25.62	1.80	2.71	1.51	1.38	1.84
Z630-4	24.92	38.83	39.49	30.47	19.98	2.73	2.64	2.30	1.79	0.77
Z637-1	23.68	35.65	29.19	27.16	15.52	2.19	2.19	2.20	1.58	1.29
Z637-2	22.68	35.90	44.43	29.16	18.48	2.31	2.31	2.39	1.41	1.64
Z695-1	21.15	35.58	35.57	29.1	22.54	2.31	2.31	2.21	2.27	1.80
Z695-2	21.83	28.11	41.12	22.99	19.03	2.07	2.07	1.77	1.06	1.71
Z695-3	18.61	37.34	44.40	30.48	20.59	2.40	2.40	2.40	2.40	0.76
Z638-1	21.66	29.42	35.38	25.2	21.61	2.03	2.03	1.40	1.10	1.63
Z638-2	28.74	33.69	49.08	46.39	29.75	2.17	2.17	2.15	2.01	1.81
Z638-3	21.57	29.17	61.97	32.59	26.47	2.42	2.42	2.25	1.93	1.59
NK6240	22.85	26.26	32.03	22.28	16.43	2.27	2.07	2.11	1.96	1.01
900M Gold	22.69	28.74	42.5	36.85	31.05	2.21	2.21	2.01	2.32	1.60
PIO3396	33.77	44.05	57.67	27.31	23.22	2.20	2.20	2.53	2.36	1.40
SE(m)±	1.43	1.77	4.72	2.83	0.60	0.10	0.10	0.15	0.10	0.22
CD(0.05)	3.53	4.40	11.71	7.02	1.48	0.24	0.25	0.36	0.24	0.54
CV(%)	10.05	8.96	19.03	16.41	4.43	7.34	7.53	12.25	8.89	26.23

Table.4 Effect of water stress on chlorophyll a, b and carotenoids content in different germplasm lines of maize

Treatment	Chlorophyll a					Chlorophyll b					Carotenoids	
	45 DAS	5 DASi	15 DASi	75 DAS	90 DAS	45 DAS	5 DASi	15 DASi	75 DAS	90 DAS	15 DASi	90 DAS
Z630-1	1.92	1.98	1.52	1.98	1.16	0.76	0.76	0.60	0.53	0.33	2.75	3.25
Z630-2	2.09	1.63	1.29	1.59	0.99	0.80	0.60	0.49	0.48	0.27	1.98	1.98
Z630-3	1.22	1.89	1.02	1.11	1.39	0.58	0.88	0.49	0.27	0.45	3.35	2.05
Z630-4	1.99	1.99	1.68	1.46	0.60	0.74	0.72	0.62	0.33	0.16	1.25	2.01
Z637-1	1.60	1.66	1.61	1.31	1.02	0.58	0.58	0.59	0.27	0.27	2.51	3.25
Z637-2	1.78	1.84	1.84	1.17	1.33	0.53	0.53	0.55	0.24	0.31	2.28	2.80
Z695-1	1.82	1.88	1.74	1.78	1.49	0.49	0.49	0.47	0.49	0.31	2.68	2.12
Z695-2	1.59	1.65	1.36	0.95	1.39	0.48	0.48	0.41	0.11	0.32	3.07	2.20
Z695-3	1.94	2.00	1.95	1.84	0.64	0.46	0.46	0.46	0.56	0.12	1.43	3.25
Z638-1	1.44	1.50	0.99	0.89	1.26	0.59	0.59	0.41	0.21	0.37	2.24	1.54
Z638-2	1.50	1.56	1.49	1.60	1.29	0.67	0.67	0.66	0.41	0.53	3.06	2.97
Z638-3	1.99	2.05	1.86	1.50	1.35	0.42	0.42	0.39	0.43	0.24	2.59	2.09
NK6240	1.86	1.75	1.72	1.52	0.86	0.41	0.38	0.38	0.44	0.16	1.73	2.57
900MGold	1.81	1.87	1.64	1.79	1.35	0.41	0.41	0.37	0.53	0.25	2.70	2.93
PIO3396	1.67	1.73	1.92	1.68	1.13	0.53	0.53	0.61	0.68	0.27	2.66	3.73
SE(m)±	0.07	0.07	0.11	0.13	0.16	0.03	0.03	0.04	0.05	0.07	0.27	0.54
CD(0.05)	0.18	0.18	0.27	0.33	0.39	0.07	0.07	0.10	0.14	0.17	0.67	1.33
CV(%)	7.13	7.04	11.88	15.73	23.81	8.23	8.52	13.75	23.76	40.38	18.15	38.54

Table.5 Effect of water stress on proline content, epicuticular wax and calcium content in different germplasm lines of maize

Treatment	Proline(mgg^{-1})					Epicuticular wax		Calcium content
	45 DAS	5 DASi	15 DASi	75 DAS	90DAS	5 DASi	15 DASi	90 DAS
Z630-1	3.2 (27.8)	1.5 (56.5)	5.4 (-6.3)	1.2 (4.9)	3.1 (-86.1)	1.82	3.20	0.62
Z630-2	3.6 (18.1)	3.5 (-0.29)	7.7 (-51)	1.4 (-13.0)	1.8 (-9.0)	2.63	3.30	0.71
Z630-3	3.2 (28.7)	4.8 (-36.5)	7.0 (-38)	4.9 (-300.8)	2.5 (-47.6)	2.05	3.18	0.71
Z630-4	4.4 (0.7)	4.8 (-36.8)	5.0 (1.4)	1.6 (-26.8)	3.4 (-106)	1.42	2.89	0.71
Z637-1	3.5 (21.4)	2.2 (37.1)	5.8 (-15)	1.9 (-50.4)	2.7 (-60.8)	2.21	3.34	0.64
Z637-2	2.6 (42.0)	3.7 (-6.00)	4.6 (8.7)	2.4 (-95.9)	4.9 (-195)	2.47	3.46	0.62
Z695-1	5.6 (-26.6)	6.5 (-86.0)	4.1 (18.4)	2.4 (-94.3)	3.5 (-113)	2.5	3.27	0.67
Z695-2	5.0 (-12.2)	2.4 (31.7)	5.5 (-9.5)	1.6 (-26.0)	2.5 (-50.0)	3.15	3.67	0.67
Z695-3	4.7 (-5.4)	3.8 (-8.86)	2.7 (46.9)	3.1 (-148.0)	3.0 (-81.3)	3.7	3.37	0.55
Z638-1	6.0 (-36.1)	7.1 (-102)	8.1 (-61)	1.5 (-19.5)	2.6 (-59.0)	1.62	2.51	0.66
Z638-2	6.2 (-40.4)	4.9 (-39.4)	5.2 (-3.6)	3.4 (-178.9)	2.2 (-31.9)	1.51	3.25	0.57
Z638-3	3.3 (24.8)	4.3 (-22.2)	4.8 (5.1)	1.5 (-19.5)	3.3 (-99.4)	1.43	3.02	0.68
NK6240	3.2 (27.8)	4.6 (-31.1)	4.1 (19.0)	3.7 (-198.4)	2.0 (-18.1)	0.92	2.46	0.69
900M Gold	3.4 (22.6)	3.8 (-8.00)	6.5 (-28)	3.3 (-170.7)	3.1 (-83.7)	2.23	3.77	0.67
PIO3396	4.4	3.5	5.05	1.2	1.7	3.09	2.87	0.59
SE(m)±	0.6	0.3	0.85	0.2	0.5	0.22	0.12	0.01
CD(0.05)	1.6	0.8	2.11	0.4	1.2	0.54	0.29	0.02
CV(%)	26.2	12.9	27.12	11.2	30.4	17.29	6.47	1.76

Figures in Parenthesis indicate % change over check

Table.6 Effect of water stress on cell membrane stability and chlorophyll stability index in different germplasm lines of maize

Treatment	Cell membrane stability(%)					Chlorophyll stability index (%)					Yield (t ha^{-1})
	45 DAS	5 DASi	15 DASi	75 DAS	90 DAS	45 DAS	5 DASi	15 DASi	75 DAS	90 DAS	
Z630-1	91.78 (0.25)	91.06 (0.68)	88.93 (-1.46)	89.06 (-3.10)	91.63 (-1.98)	72.91 (-4.59)	72.91 (-16.50)	68.16 (-8.20)	52.17 (-5.91)	52.78 (-29.68)	2.43 (3.65)
Z630-2	89.10 (-2.67)	83.85 (-7.29)	88.10 (-2.38)	88.25 (-3.98)	88.74 (-5.08)	71.72 (-6.15)	80.48 (-7.82)	81.13 (9.27)	51.98 (-6.25)	54.85 (-26.93)	2.07 (17.82)
Z630-3	86.62 (-5.38)	86.52 (-4.33)	86.03 (-4.68)	85.12 (-7.39)	85.74 (-8.28)	79.69 (4.28)	77.15 (-11.63)	73.65 (-0.81)	77.28 (39.38)	73.57 (-1.99)	1.88 (25.28)
Z630-4	89.14 (-2.63)	90.14 (-0.33)	84.87 (-5.96)	82.99 (-9.70)	75.87 (-18.84)	84.81 (10.99)	73.63 (-15.67)	75.59 (1.81)	70.53 (27.21)	70.32 (-6.32)	1.92 (23.77)
Z637-1	92.72 (1.29)	91.87 (1.58)	90.13 (-0.13)	89.13 (-3.02)	87.62 (-6.27)	73.80 (-3.42)	73.80 (-15.47)	65.71 (-11.51)	71.93 (29.73)	75.10 (0.05)	2.37 (5.90)
Z637-2	90.81 (-0.80)	90.46 (0.03)	90.70 (0.50)	90.04 (-2.04)	88.15 (-5.71)	81.99 (7.29)	75.78 (-13.21)	64.36 (-13.32)	71.31 (28.62)	92.35 (23.03)	2.44 (2.91)
Z695-1	86.78 (-5.20)	88.41 (-2.24)	84.03 (-6.89)	84.13 (-8.46)	81.97 (-12.32)	83.78 (9.63)	83.05 (-4.89)	72.31 (-2.61)	37.61 (-32.16)	64.57 (-13.98)	2.22 (11.83)
Z695-2	91.10 (-0.48)	85.55 (-5.40)	89.39 (-0.95)	87.75 (-4.53)	84.94 (-9.13)	78.51 (2.73)	78.22 (-12.82)	76.47 (2.98)	72.47 (30.71)	89.84 (19.69)	2.25 (10.63)
Z695-3	88.35 (-3.49)	86.85 (-3.97)	88.78 (-1.62)	88.12 (-4.12)	86.94 (-6.99)	86.21 (12.81)	70.73 (-18.99)	70.56 (-4.97)	77.37 (39.54)	79.35 (5.71)	2.65 (-5.21)
Z638-1	88.85 (-2.94)	89.09 (-1.49)	88.54 (-1.89)	87.98 (-4.27)	88.24 (-5.61)	54.51 (-28.67)	54.51 (-37.57)	77.23 (4.01)	48.59 (-12.36)	50.32 (-32.97)	2.35 (6.55)
Z638-2	87.37 (-4.56)	88.04 (-2.65)	86.02 (-4.68)	85.43 (-7.05)	83.87 (-10.28)	82.49 (7.95)	86.67 (-0.73)	84.93 (14.38)	87.63 (58.05)	86.08 (14.68)	2.61 (-3.61)
Z638-3	92.85 (1.43)	92.29 (2.05)	92.25 (2.22)	91.30 (-0.66)	91.77 (-1.83)	65.34 (-14.50)	65.34 (-25.17)	57.73 (-22.25)	65.31 (17.80)	69.05 (-8.01)	2.22 (11.84)
NK6240	88.27 (-3.58)	85.63 (-5.31)	86.50 (-4.15)	85.08 (-7.43)	84.94 (-9.14)	94.79 (24.03)	86.13 (-1.36)	57.96 (-21.94)	58.11 (4.80)	70.15 (-6.55)	2.21 (12.13)
900M Gold	90.46 (-1.18)	90.22 (-0.25)	88.36 (-2.09)	82.33 (-10.42)	67.67 (-27.61)	95.35 (24.77)	95.35 (9.21)	57.95 (-21.95)	72.33 (30.45)	70.26 (-6.40)	2.33 (7.44)
PIO3396	91.54	90.44	90.25	91.91	93.48	76.42	87.31	74.25	55.44	75.06	2.52
SE(m)±	0.16	0.80	0.77	1.06	0.69	3.91	5.44	4.56	7.37	14.00	0.06
CD(0.05)	0.40	1.99	1.91	2.62	1.70	9.70	13.49	11.30	18.26	34.70	0.14
CV(%)	0.31	1.56	1.51	2.10	1.39	8.60	11.81	11.20	19.74	33.88	4.37

Figures in Parenthesis indicate % change over check

Proline accumulation is considered as a sign of drought injury, the concentration of which builds up under stress due to hydrolysis of proteins or acceleration of its *de novo* synthesis (Levit, 1980). Data regarding proline content presented in Table 5 showed that water stress significantly affected proline accumulation. Increase in water stress was accompanied by increase in proline content. Maximum proline content (7.7 mg/g) accumulated 3.5 mg/g and 3.6 mg/g at 5 DASi and 15 DASi respectively. Proline accumulated in all the hybrid lines was significantly different. Proline accumulation induced by stress conditions is mediated by increase and reduced oxidation of amino acid. A sharp proline accumulation was observed, with a positive correlation between the drought stress imposed on the plants and the increments of proline (Icaballero *et al.,* 1998; Efeoğlu 2009; Sandra witt *et al.,* 2012).

The epi-cuticular wax content of the leaves was observed maximum at 15 DASi. The highest epi-cuticular wax content was recorded in the germplasm line Z695-2 (3.67mg/g). There was a significant increase in the epi-cuticular wax content with the increase in the water stress. The epi-cuticular wax showed close association with the yield and this was in the line with the work of Premachandra *et al.,* (1991).

The calcium content of the leaves after the attaining of physiological maturity was measured and was negatively associated with

the yield because the leaf calcium is translocated to the cob (Szczepaniak *et al.*, 2016). Cell membrane (CM) is central site for various cellular functions; especially those associated with membrane bound enzymes and transport of water and solutes. The CM function under desiccation to stress signal a hardening effect that is expressed in increased membrane stability under stress (Chohan *et al.*, 2012). CMS under stress has been shown to be positively correlated with yield under drought. The Cell membrane stability index was observed to be decreased at 15 DASi so there is a less decrease in the CMS value in tolerant germplasm lines which was supported by (Naveed *et al.*, 2016). Based on the yield studies the germplasm line Z695-3 (2.65t ha^{-1}) is considered to be most tolerant because of its yield capacity under water stress situation. That is followed by Z638-2 and Z637-2.

In the present study two germplasm lines namely Z695-3 and Z638-2 were selected as drough tolerant based on Physio-Biochemical parameters. RWC, Proline, LWP and CSI are the suitable traits which can be used for the drought screening in maize.

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