Screening of Some Selected Indian Maize Cultivars to Simulated Drought Condition

Iwuala Emmanuel¹, ², Odjegba Victor³, Umebese Caroline⁴, Aqeel Hasan Rizvi⁵, Tapan Kumar⁶, Afroz Alam⁷

ABSTRACT
Drought is a major constraint to get an optimum yield of any crop under changing environment. A total of 12 Indian accessions of maize seedlings was screened initially by performing relative water content (RWC) under water deficit condition. Three (RJ-2020, BPCH-6 and EC-3161) landraces that showed a higher RWC than DTSYN11 were finally selected for further studies. Four landraces maintained RWC above 80% under 4th and 8th day stress periods. Higher contents of chlorophyll were recorded significantly in EC-3161 and in RJ-2020 as compared to DTSYN11 under the 8th day of stress. RWC showed a decline in BPCH-6 (72.54%) and in RJ-2020 (72.65%) and proline was significantly increased up to 10 and 13-fold, respectively. Therefore, this research has identified three unfamiliar maize landraces that possess the capacity to be drought tolerance when under water deficit and watering condition. In addition to their useful applications in breeding program, they are valuable resources to elucidate genetic profiling that enhance the capacity to exhibit drought tolerance in Indian maize cultivars.

Key words: Chlorophylls, Drought, Genotypes, Proline, Zea mays.

INTRODUCTION
Drought is known to cause the utmost negative effect on agricultural practices globally (Vinocur and Altman, 2005) affecting about one-fourth of Mediterranean areas meant for maize cultivation (Rabaut and Ragot, 2007). About 70% maize cultivars are grown in conditions of rain-fed, with possibilities that alleviate limited water deficit conditions (Rabaut and Ragot, 2007). Thereby, a need exists to develop varieties tolerant to drought either through genetic engineering to meet the demands of maize grain production to feed both animals and humans (Parry et al., 2005). Daryanto et al. (2016) estimated that most often at the developmental and vegetative phases of maize production, maize yields were reduced by 39.3% when exposed to mid-season droughts. It is observed that the alarming increase in temperatures and climate change have a huge implication in maize production, thereby reducing food supply and create poor livelihoods for smallholder farmers (lobell et al., 2011). The adaptation to recent climatic changes is essential for adequate supply of food and security. Currently, one of the recent adaptation strategies is the production of maize drought-tolerant (DT) varieties. During field and controlled experiment, the use of DT maize varieties has been confirmed not only to possess drought tolerance capacity, but likewise produce high yield more than other commercial hybrids (CIMMYT, 2013). Plants adequately acquired certain myriad of metabolic or developmental signals to enhance uptake of water and effectively balance the utilization of water during their vegetative and reproductive stages causing tolerance to drought. Plants are known to exhibit several biochemical and physiological responses to alleviate drought effects (Tuinstra and Pereira, 2003; Bray et al., 2000).

The response of plants under moisture deficit condition could be seen in a reduced relative content of water. Relative content of water is the status of the amount of water in plant under stress and how interruptions occur, causing injury leading to death (McKersie and Leshem, 1994). Several mechanisms could activates under environmental stresses in plants which include increased amounts of photosynthetic pigments and osmolytes (Britton, 1995; Malkin and Niyogi, 2000). The objective of the present study is to screen and identify Indian maize landraces and to determine their physiological parameters when under water deficit and mild re-watering condition.

MATERIALS AND METHODS
A total of (12) selected maize (Zea mays L.) seeds were collected from ICAR-IARI (Indian Council of Agricultural Research-Indian Agricultural Research Institute), New Delhi,
Screening of Some Selected Indian Maize Cultivars to Simulated Drought Condition

India and (1) drought tolerant line from International Institute of Tropical Agriculture, Nigeria (Table 1 list of Genotypes). Four seeds of each genotype namely DTSYN11 (Drought Tolerant), 12 LRNC126 (Drought Sensitive) landraces, were sown in pots of 12 cm in diameter. The pots were kept in the growth chamber facility in Banasthali Vidyapith, Rajasthan. The facility had a photoperiod of 16 h duration of light and 8 h dark. The temperature was kept at a range of 26–29° C. Three replicates were used with three different stress periods.

Drought stress at vegetative stage

Seeds were sown in petri dishes, 4 kg of soil mixed with 500 ml of water was kept in a tray of 30×20 cm diameter. After growth, seedlings of 15 cm that are one-week old was now transferred into 60 earthen pots. Prior to planting, the earthen pots were totally filled with 1 kg autoclaved soil and moistened with 250 ml of water. Till the 8th weeks (60 days), seedlings were irrigated daily using 100 ml until the start of imposed drought regimes. The control pots were regularly irrigated with tap water. During the investigation, three-day stress period was evaluated: 1st and 4th day water stress condition, 2nd and 8th day water stress condition while at 3rd day, 120 ml mild re-watering was added at about 6 hours before harvest of seedlings on the 9th day of treatment.

Relative water content (RWC)

Leaf relative water content (RWC %) was measured according to (Turner, 1981) on 13 maize lines. Soil moisture content (SMC) was determined daily by the use of a soil moisture probe device 65 mm depth (DFM Software Solutions CC, RSA), all measurements of triplicates was taken from each single earthen pot. Each fourth leaf from midrib were collected and weighed adequately to find out its initial fresh weight followed by immersing the excised leaves alone in autoclaved H2O in the absence of light. It was taken from the water after 24h, dried with a sterilized blotting paper followed by weighing to find out their weight at turgid stage. Subsequently the leaves were then placed in labelled bags and left at 65°C in an automated oven for 72h, subsequently, their whole dry weight (DW) was taken.

RWC was determined using the formula by Turner (1981)

\[
\text{RWC} = \left( \frac{\text{Fresh Weight} - \text{Dry Weight}}{\text{Turgid Weight} - \text{Dry Weight}} \right) \times 100
\]

Quantification of total chlorophyll, carotenoid and proline

Total chlorophyll was extracted in 5ml of ice-cold 80% acetone by crushing drought stressed leaves. The homogeneous mixture was then put in a centrifugation machine for 3000 rpm at 4°C for 10 min. The optimum absorbance of the supernatant was taken at the wavelength of 480, 645 and 663 nm. The estimated chlorophyll and carotenoid (ca) pigments were reported, according to the procedure of (Arnon, 1949; Lichtentaler and Buschman, 2001) respectively.

For calibration curve, stock solution of pure proline (0.1mg/1ml) was prepared and its serial dilutions were used. The content was expressed as mg Proline/g fresh wt. and calculated according to the formula by Bates et al. (1973).

\[
\text{Proline (mg.g-1 FW)} = \left( \frac{\text{[μg proline/ml} \times \text{4 ml toluene}]}{0.5 \text{ g sample/2.5}} \right) / 1000.
\]

Statistical analysis

All obtained data were evaluated by SPSS 16.0 program (SPSS Inc., Chicago, USA). Interactions in three ways were executed within the selected genotypes. For each and every output variable multiple-comparison Turkey's p≤0.05 post test was performed to compare data from identical varieties. Sigma plot (8.0) was used to draw graphs. The data represent means ± standard error.

RESULTS AND DISCUSSION

Drought stress condition at early seedling period

A total of 12 maize lines were screened at seedling stage for drought tolerance using percentage RWC in comparison
Screening of Some Selected Indian Maize Cultivars to Simulated Drought Condition

Maize genotypes

Fig 1: Changes in relative water content (RWC%) of maize genotypes compared with a drought resistant line DTSYN11 during seedling stage at 2, 4 and 6 day drought stress. Data ± SE (n=3) Genotypes names (DTSYN11, GK3150, RJ-2020, FSCH18, PRO-385, PH-1974, MCH46, EC-3161, BPCH-6, MAIZE-310, NMH-731, NMH-1242, FH 3554).

Fig 2: Changes in plant height and relative water content (RWC%) of maize genotypes (DTSYN11, BPCH-6,RJ-2020 and EC-3161) during vegetative stage at 4, 8 day of drought stress and a mild. Data ± SE (n=3) 4 day C-control 4 day T-treated; 8 day C-control; 8 day T-treated; RW-C-control; RW- T-treated.

to a drought tolerant line DTSYN11 (Fig 1). During 6th day of drought stress, RJ-2020, BPCH-6 and EC-3161 recorded a high RWC as compared to DTSYN11 (Fig 1.7.2, 10.3, 12.7% and 9.8%). RJ-2020, BPCH-6 and EC-3161 were only the landraces that exhibited superior drought tolerance performance in comparison to DTSYN11 under drought condition. Essentially, it is indispensable that seedlings tolerant to drought stress could impact stability in most annual crop undergoing growth (McKersie and Leshem, 1994).

Responses of maize during drought stress condition at vegetative stage

A significant difference exists as SMC range between 37 and 45% at 4th day, 22-27% during 8th day, while 53-70% during mild re-water treatments. In each maize landrace, SMC of control pots showed similarity statistically (Table 2). SMC in DTSYN11, BPCH-6 and EC-3161 control condition range between 61 and 72%, 77-84% and 62-75%, respectively. RJ-2020 and BPCH-6, the SMC of control condition was measured for 84-88% and 78%, respectively. The record of SMC among treatment and control pots showed a significant difference, thereby indicating that a measure of limited water was available to drought treated plants. The shoot height of all maize landraces at the time of maturity shows significant difference that exists in the height of the plant between maize landraces. The data revealed that RJ-2020 was phenotypically more significant taller as compared to DTSYN11, BPCH-6 and EC-3161 (Fig 2).

Measurement of relative content of water (RWC %) at vegetative stage

During harvest time, RWC recorded significant differences between maize landraces (Table 2) which range about 76-81% at 4th day, 71-77% at 8th day, while 74-87% at mild re-watering treatments. During mild re-watering, a significant difference was observed between RJ-2020 and BPCH-6 which is less statistically as compared to other landraces (Fig 2). While in control, a low data of RWC was observed for RJ-2020 and EC-3161 (7.4% and 8.2%) respectively. Therefore, RWC for all four maize landraces were above the value of 76% across all treatments therefore this process enables the water potential of the soil to be greater than root tissues undergoing process of water potential. Furthermore, process of re-hydration consequently affects damage of leakage ions due to reversible changes within the cell membranes due to consequence from drought condition (Ristic, 1996; Triparthy et al., 2000). Similarly, Iwuala et al. (2019) reported that certain amount of water in plant tissues ranges between 72% to 88%.

Responses of drought to proline, carotenoid and chlorophyll

Proline content revealed a significant difference between maize landraces in control and stressed plants (Table 3). Proline content measured on the 4th day was between 2.23 (mg.g⁻¹ FW) (BPCH-6) and 1.7 (mg.g⁻¹ FW) (DTSYN11) in
drought treated plants and 1.6 (mg.g\(^{-1}\) FW) (BPCH-6) and also 1.3 (mg.g\(^{-1}\) FW) (EC-3161) in controlled plants. On the 8\(^{th}\) day, proline content measured in treated plant range within 2.5 (mg.g\(^{-1}\) FW) (BPCH-6) and 3.5 (mg.g\(^{-1}\) FW) (EC-3161) in contrast to control plants which recorded value of 1.17 (mg.g\(^{-1}\) FW) (BPCH-6) and 0.8 (mg.g\(^{-1}\) FW) (DTSYN11). Proline content under mild re-watering were between 1.52 (mg.g\(^{-1}\) FW) (BPCH-6) and 3.94 (mg.g\(^{-1}\) FW) (EC-3161), while in control plants it range between 1.21 (mg.g\(^{-1}\) FW) (RJ-2020) and 0.56 (mg.g\(^{-1}\) FW) (DTSYN11). There was a remarkable increase in proline as indicated during mild re-watering for RJ-2020 and BPCH-6. The genotype RJ-2020 recorded a significant high content of proline for about (11.14 mg.g\(^{-1}\) FW) making an increase of 14-fold in comparison to its value of control which is 1.21 (mg.g\(^{-1}\) FW). While BPCH-6 evidently showed a high content of proline of 10.2 (mg.g\(^{-1}\) FW) under drought also an increase 11-fold was observed as compared to the control (1.52mg.g\(^{-1}\) FW) in contrast to the controlled plant of EC-3161 (Table 3).

Significant differences were recorded in content of total chlorophyll (Tchl) among maize landraces for both control and treated plants. Tchl between maize landraces was similar statistically at 4\(^{th}\) day ranging from 1.4 (mg.g\(^{-1}\) FW) (BPCH-6) and 2.02 (mg.g\(^{-1}\) FW) (EC-3161) in drought treated plants while 2.9 (mg.g\(^{-1}\) FW) (BPCH-6) and 3.13 (mg.g\(^{-1}\) FW) (EC-3161) was recorded in the plants under control. At 8\(^{th}\) day, a significant low contents of Tchl were recorded in plant under drought treatment for DTSYN11 (0.90 mg.g\(^{-1}\) FW) and (BPCH-6) (2.1 mg.g\(^{-1}\) FW) when compared to RJ-2020 (1.7 mg.g\(^{-1}\) FW) and EC-3161 (2.02 mg.g\(^{-1}\) FW). The Tchl was between 1.7 (mg.g\(^{-1}\) FW) (BPCH-6) and 3.1 (mg.g-1 FW) (RJ-2020) recorded in plant under drought condition at 8\(^{th}\) day. The measured values of Tchl for mild-re watering treatment, showed a similarly which is 1.01 (mg.g\(^{-1}\) FW) (DTSYN11) and 2.29 (mg.g\(^{-1}\) FW) (RJ-2020) in plants treated with

| Plant     | Day | Genotype   | Soil moisture content | Total chlorophyll (mg g\(^{-1}\) FW) | Carotenoid (mg g\(^{-1}\) FW) | Proline (mg g\(^{-1}\) FW) |
|-----------|-----|------------|-----------------------|-------------------------------------|-----------------------------|--------------------------|
| Zea mays  |     |            | Control               | Treated                             |                             |                           |
|           | 4   | DTSYN11    | 51.22\(^{+v}\)        | 31.42\(^{+v}\)                      |                             |                           |
|           |     | BPCH-6     | 70.26\(^{+v}\)        | 54.17\(^{+v}\)                      |                             |                           |
|           |     | RJ-2020    | 72.45\(^{+v}\)        | 52.33\(^{+v}\)                      |                             |                           |
|           | 8   | EC-3161    | 61.28\(^{+v}\)        | 43.70\(^{+v}\)                      |                             |                           |
|           |     | DTSYN11    | 63.54\(^{+v}\)        | 21.45\(^{+v}\)                      |                             |                           |
|           |     | BPCH-6     | 77.42\(^{+v}\)        | 41.26\(^{+v}\)                      |                             |                           |
|           |     | RJ-2020    | 79.55\(^{+v}\)        | 44.65\(^{+v}\)                      |                             |                           |
|           |     | EC-3161    | 68.34\(^{+v}\)        | 37.86\(^{+v}\)                      |                             |                           |
| Re-watering|     | DTSYN11    | 77.47\(^{+v}\)        | 41.43\(^{+v}\)                      |                             |                           |
|           |     | BPCH-6     | 85.71\(^{+v}\)        | 64.16\(^{+v}\)                      |                             |                           |
|           |     | RJ-2020    | 91.59\(^{+v}\)        | 73.54\(^{+v}\)                      |                             |                           |
|           |     | EC-3161    | 85.34\(^{+v}\)        | 58.44\(^{+v}\)                      |                             |                           |

Mean = 3 ± SE.; Alphabets denote a significant value following test by Tukey post hoc: non-similar alphabet denote significant value at (P < 0.05). Bold text highlights statistically significant values.

Table 3: Changes in chlorophyll, Carotenoid and Proline content of maize genotypes at 4, 8 day of drought stress and mild re-watering.

Table 2: Changes in soil moisture content (SMC) of maize genotypes at 4, 8 day of drought stress and mild re-watering.
drought as compared to 3.46 (mg.g⁻¹ FW) (DTSYN11) and 3.66 (mg.g⁻¹ FW) (EC-3161) in the control plants (Table 3). Previously Xu et al. (2010) reported that in cereal crops, a reduction of 21% in total chlorophyll content in drought-resistant genotype was recorded between drought stress and in control plants. Therefore, all landraces sustained significant contents of chlorophyll as compared to their controls at all days of treatments. At 4th day, carotenoid was similar statistically between maize landraces for all control and treated plants which range from 3.53 (mg.g⁻¹ FW) (BPCH-6) to 3.66 (mg.g⁻¹ FW) (RJ-2020). At 8th day, there was a low value for carotenoid (ca) in DTSYN11 as compared to their control. This is similar to the report by Iwuala et al. (2019) on the effects of drought on carotenoid content in pearl millet seedlings. Carotenoid records the highest value at 8th day in treated plant which was observed in EC-3161 and RJ-2020 respectively for 2.53 and 3.11 mg.g⁻¹ FW. The landraces EC-3161, RJ-2020 and BPCH-6 when under mild re-watering, showed a similar statistical value of (3.2, 4.73 and 3.73 mg.g⁻¹ FW) than in DTSYN11 (3.1 mg.g⁻¹ FW) at all stress days (Table 3). Also Blum, (2011) reported that plant height is a dominant character affecting transpiration of plants, when sown in pot that has equal soil volume, thereby causing a large plant to require frequent application of water than in a small plant. Overall, under consistent water deficit conditions, the four maize landraces investigated showed that phenotypically, plant height were influenced by water use efficiency demand and the duration of the period of water stress observed. Also, tall crops selectively grown by farmers in Africa after could be utilized in fuel production (Maiti et al., 2012). The content of carotenoid maintained a significant difference between water stress and control plants in all the treatments, excluding DTSYN11 at 8th day, which showed a significant reduction of 32.4% during water stress condition. Carotenoids play a major role in photosynthesis, which involves the ability to signal oxygen damaging radicals known to be produced readily by complexes during the light splitting process of photosynthesis (McKersie and Leshem 1994; Malkin and Niyogi 2000; Santabarbara et al., 2013). Similarly, Takele (2010) investigated the reduction of both carotenoid and chlorophyll in drought-resistant maize at pre-flowering growth stage under dehydration. However, levels of chlorophyll and carotenoid were maintained under water deficit and mild re-watering condition which indicates that no damage occurred in the apparatus of photosynthesis process in all the maize landraces during the water stress condition. Previously, Hare et al. (1998) reported that plants accumulate so much proline under multiple stress conditions which is due to induced synthesis and reduced degradation. Accumulation of proline under drought stress is a change capable of alleviating the effects of damage to membrane (Iwuala et al., 2019 Bray et al., 2000; Coruzzi and Last, 2000). However, accumulation content of solute proline under drought stress present in both RJ-2020 and in EC-3161 underscores that these maize landraces have some measure of antioxidant activity which inhibits death of plant.

**CONCLUSION**

Screening of three selected lesser known Indian maize landraces at seedling stage has revealed that they have significant tolerance against drought stress. It has been proved they have remarkable potential to water use managed which efficiently maintain the high RWC compared to previously known tolerant drought line, DTSYN11. Therefore, the studied landraces can be further used in various breeding programmes that are specially working on the development of drought tolerant cultivars. All parameters investigated in the present study indicate that the selected landraces could be used as a reservoir of a better custom-made genome for generating tolerant breeding lines to grow in drought hit regions worldwide.

**Conflict of Interest:** The authors declare no potential conflict of interest.

**Contributions:** IE conceived the idea, designed, wrote the draft; AA, OV, UC designed, analyzed, interpreted the data and revised similarly; AHR and TK compiled the article.

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Screening of Some Selected Indian Maize Cultivars to Simulated Drought Condition

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