



Analysis of Water Stress in Different Varieties of Maize (*Zea mays* L.) at the Early Seedling Stage

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Authors' contributions

This work was carried out in collaboration among all authors. Authors KRP, PPS and SRS designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors SGW and KRP managed the analyses of the study. Authors SGR and SNH managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Maize (*Zea mays* L.) is a widely grown crop with a high rate of photosynthetic activity due to its C4 pathway leading to higher yields of grain and a potential for biomass. It is predominantly cross-pollinated crop, a feature that has contributed to its wide morphological variability and geographical adaptability. Abiotic stress such as drought stress negatively affects plant growth and development. The present study was designed to investigate the effects of drought stress on the morphological and biochemical content of seven different maize varieties. Here we have tested 7 different varieties of maize from the Marathwada region of Maharashtra, India. One week old plants have been affected by drought stress. After one week of drought stress, the plants were subjected to various morphometric and biochemical tests. The results showed that water stress treatment significantly affects root length, shoot length and fresh biomass of seven different varieties, *i.e.*

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TMMH 806, NMH 1008, DELTA 10V30, INDAM 1122, SRIKAR 3555, DKC 9141, SGA. Drought stress also had a negative impact on chlorophyll and proline content. Result further exhibited that based on drought tolerance index, variety SRIKAR 3555 could be ranked as drought tolerant and NMH 1008 as drought-sensitive, while remaining cultivator ranked as drought intermediates.

Keywords: Maize (*Zea mays L.*); drought tolerance; abiotic stresses.

1. INTRODUCTION

Crop production limitation and rapidly increasing human population, which is estimated to exceed nine billion people by 2050, is the biggest threat that we are facing now and we need to make necessary arrangements to feed this ever-increasing population [1]. Many efforts are already going on in the different corners of the world in plant research, but these are not only restricted in improving crop production in cereals (staple food) but also focused in doing basic plant science research with other experimental plants to develop methods inducing biotic and abiotic stress and molecular mechanism, further can then be applied in cereal crops with an ultimate objective of improving crop production [2-11]. Crop improvement efforts are ever-increasing toward feeding a life on earth.

Water is vitally needed for each organism in a specified quantity, and any deficiency in that particular quantity imposes stressful conditions. Water requirements vary across the tissues, and so far, there is no exception to the growth stages of the same crop species and maize crop. The assessment of optimum plant water requirements is a prerequisite for determining water deficiency in plants. Maize (*Zea mays L.*) belongs to the grass family (*Poaceae*). It is a tall annual plant with an extensive fibrous root system. In India, it is cultivated in most countries throughout all seasons and is the third-largest grain crop in India, after rice and wheat. Maize is an important multipurpose cereal crop used as food, feed, fuel and in the manufacture of industrial products [12-14]. Globally, it has grown in 184 Mha across 165 countries, with a total production of 1,016 MMT and average productivity of 5.52 t.ha⁻¹ [15]. Approximately 59% of total production is used as feed, while the remainder is used as industrial raw materials (17%), food (10%), exports (10%) and other uses (4%) [16]. The world is facing many of the most dangerous issues for crop production, including biotic stress with multiple pathogens and drought. Recently, the incidence of severe drought has increased in many countries. Drought places one of the most common and most significant constraints on

agricultural production, seriously affecting crop growth, gene expression, distribution, yield and quality [17]. Changes in rainfall patterns and rising temperatures are major causes of drought and have contributed to an appreciable decline in crop productivity [18-20]. As a result, significant agricultural losses have occurred due to the failure of drought-sensitive crops to grow under such conditions [21,22]. Increased population and changing climate conditions are more likely to increase water scarcity, which will further reduce crop productivity in the world. Current trends in climate change, for example, will increase water scarcity and reduce maize productivity by 15-30% [23]. The difference in plant responses to drought stress appears to be due to differences in morphological, anatomical and physiological characteristics. In response to drought stress, some plants may modify their leaf structure to reduce transpiration [24]. The main objective of the present study was, therefore, to evaluate the drought tolerance of maize during its early stages of growth and to select the maximum drought-tolerant cultivar among these cultivars.

2. MATERIALS AND METHODS

2.1 Plant Material

This study was performed at laboratory condition with seven-corn cultivar (TMMH 806, NMH 1008, DELTA 10V30, INDAM 1122, SRIKAR 3555, DKC 9141, SGA) with six replication. In this experiment, effect of drought induce by different concentration (0%, 5%, 10%, 15%, 20%, 25%) of polyethylene glycol 6000 (PEG 6000) treatments on Germination of corn were studied. In each level of stress, six seeds of each cultivar were selected and sterilized in mercuric chloride (HgCl₂) and then washed in distilled water for two times. The seeds of cultivars were germinated in Petri dishes on two layers of filter paper with an appropriate PEG treatment. The Petri dishes were kept in germinator for germination.

2.2 Germination

Percentage of germinated seeds was scored daily, based on emergence of radicles. After 10

days, the germination percentage was calculated by the formula given below ($Ge = n/N \times 100$), where, Ge = germination %, n= total no. of seeds germinated in each treatment, N= total number of seeds soaked in each treatment. The N= 120 for every variety and treatment, each set of an experiment repeated three times.

2.3 Crop Physiological Parameters

Root length and shoot length of each plant were measured by scale in cm. Roots and shoots of each plant were separated and were weighted in grams (g) by using the digital microbalance. Numbers of roots per plant were counted.

2.4 Chlorophyll Content

Chlorophyll content was measured by the method of Arnon et al. [25]. 0.1 g of fresh leaf was ground in 10 ml of 80% acetone. The extracts were filtered and absorbance was measured by spectrophotometer.

2.5 Proline Content

The proline was extracted from fresh leaves with 3% sulfosalicylic acid and estimation carried out using ninhydrin reagent according to the method of Bates and Waldren [26]. The layer was separated and red colour intensity was measured at 520 nm using a spectrophotometer. The concentration of proline was calculated using a calibration curve.

2.6 Statistical Analysis

The statistical analysis carried out using the one-way variance using the SPSS software to detect the difference between the different parameter of under stress condition. All measurement represents the means and standard error of six replicas.

3. RESULTS

Drought is one of the most damaging forms of abiotic stress in the world and significantly reduces the productivity of agricultural crops and Maize, a world's leading cereal crops, is vulnerable to drought stress. Productivity in Maize is affected by drought stress at different stages of growth in different regions. A decline in the rate of photosynthesis has been recorded in the literature due to the effect of water deficit. This could be due to a decrease in light interception as a result of decreased leaf spread or leaf senescence and due to a decrease in C-fixing per unit leaf area as stomata's will be

closed or it could be the result of photo-oxidative damages to the photosynthetic machinery. Here we have analyzed varieties performance of the combined analysis of different varieties in drought conditions. Proper seed germination depends on the availability of adequate moisture content for metabolic activation to break down dormancy or convert stored food to consumable form. As a first part of the study, we analyzed the germination percentage and all the maize varieties were germinated in between 5th and 7th day post sowing. The percentage germination of maize varieties on the 5th and 7th day of germination is mentioned below (Table 1). It suggests that the variety SRIKAR 3555 showing the best result of the germination percentage on the 5th and 7th day under the drought condition and variety NMH 1008 is susceptible to drought while the remaining five cultivars have been identified as intermediate drought (Table 1).

Root and shoot elongations are seedling growth parameters and are subject to drought stress reduction. At maize seedling stage, shoot elongation reduction is more than root elongation under drought stress. Reduction in the root length of all cultivators of maize was observed because of drought stress. Among all varieties M4 (INDAM 1122) and M5 (SRIKAR 3555) shows greater root length under drought condition and M2-NMH 1008 and M6-DKC 9141 shows shorter root length, others were intermediate (Fig. 1).

Due to the drought condition reduction in the shoot length among all varieties were observed. From the above table and graphical representation we conclude that variety M5 (SRIKAR 3555) showing the best result for shoot length. Analysis shows that, variety M2 (NMH 1008) is sensitive under drought condition for shoot length (Fig. 2).

Under drought condition, the fresh weight of root was decreased in all cultivators. Above graphical representation shows that M5 (SRIKAR 3555) showing the lesser reduction in fresh biomass of root, whereas, highly decrease in, M1 (TMMH 806) and M2 (NMH 1008), others were showing the moderate results (Fig. 3).

Above graphical representation shows that M4 (INDAM 1122) and M5 (SRIKAR 3555) showing the lesser reduction in fresh biomass of root, whereas, highly decrease in, M1 (TMMH 806) M2 (NMH 1008) and M3 (DELTA 10V30), others were showing the moderate results (Fig. 4).

Table 1. Germination percentage of maize varieties

Sr. no.	Varieties/ DAS	PEG concentration					
		0%	5%	10%	15%	20%	25%
1	TMMH 806						
	5 th day	80%	80%	80%	70%	50%	Nil
2	NMH 1008						
	7 th day	82%	82%	82%	70%	52%	16.66%
3	DELTA 10V30						
	5 th day	80%	71%	52%	40%	16.16%	Nil
4	INDAM 1122						
	7 th day	85%	73%	55%	41%	16.66%	Nil
5	SRIKAR 3555						
	5 th day	93%	93%	93%	93%	93%	740%
6	DKC 9141						
	7 th day	95%	95%	95%	95%	95%	78%
7	SGA						
	5 th day	90%	90%	90%	90%	89%	88%
	7 th day	95%	95%	95%	95%	93%	92%

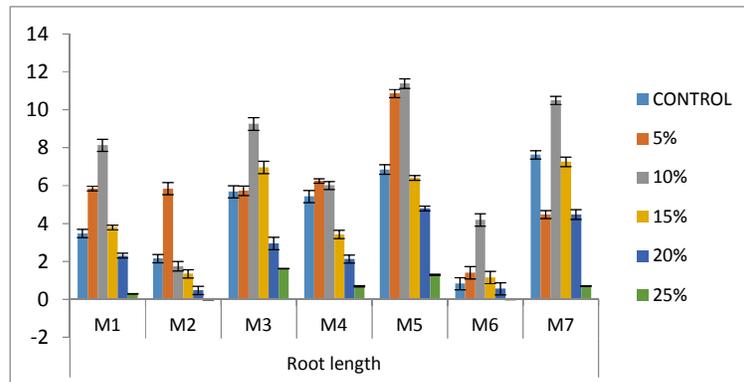


Fig. 1. Root length of maize varieties

Where, M1-TMMH806, M2-NMH1008, M3-DELTA10V30, M4-INDAM1122, M5-SRIKAR3555, M6-DKC9141 and M7-SGA

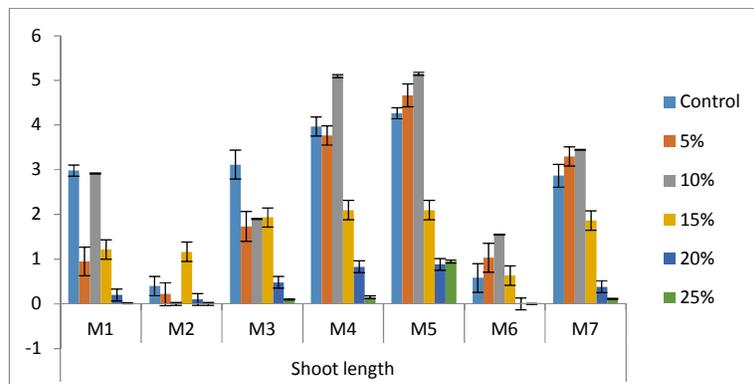


Fig. 2. Shoot length of maize varieties

Where, M1-TMMH806, M2-NMH1008, M3-DELTA10V30, M4-INDAM1122, M5-SRIKAR3555, M6-DKC9141, M7-SGA

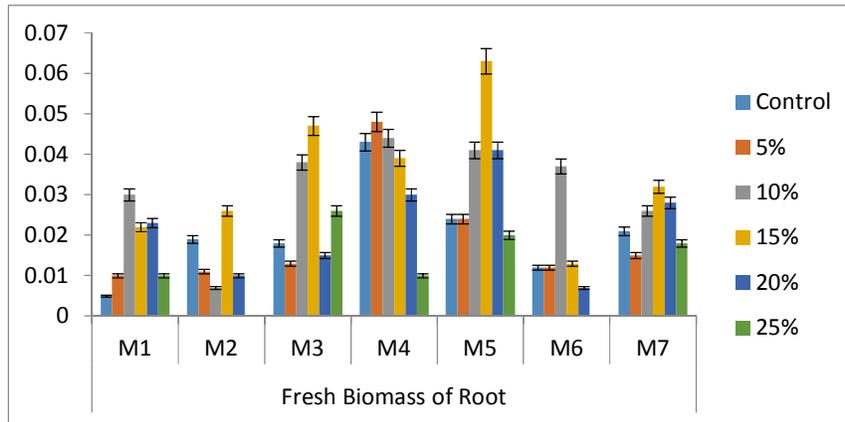


Fig. 3. Fresh biomass of root

Where, M1-TMMH806, M2-NMH1008, M3-DELTA10V30, M4-INDAM1122, M5-SRIKAR3555, M6-DKC9141 and M7-SGA

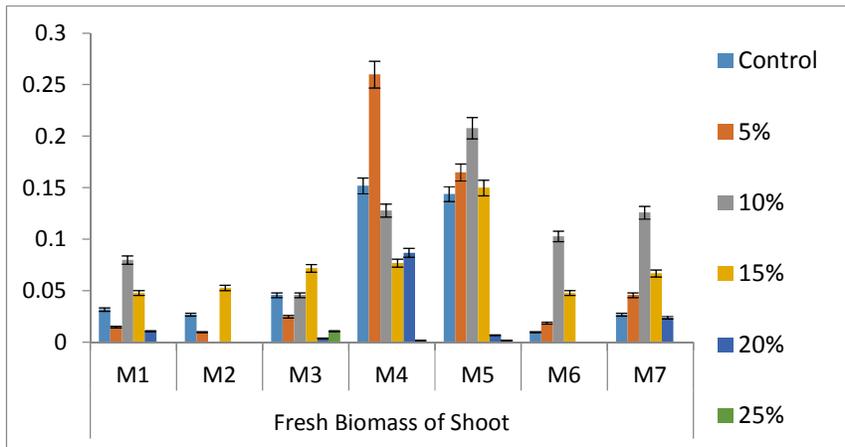


Fig. 4. Fresh biomass of shoot

Where, M1-TMMH806, M2-NMH1008, M3-DELTA10V30, M4-INDAM1122, M5-SRIKAR3555, M6-DKC9141 and M7-SGA

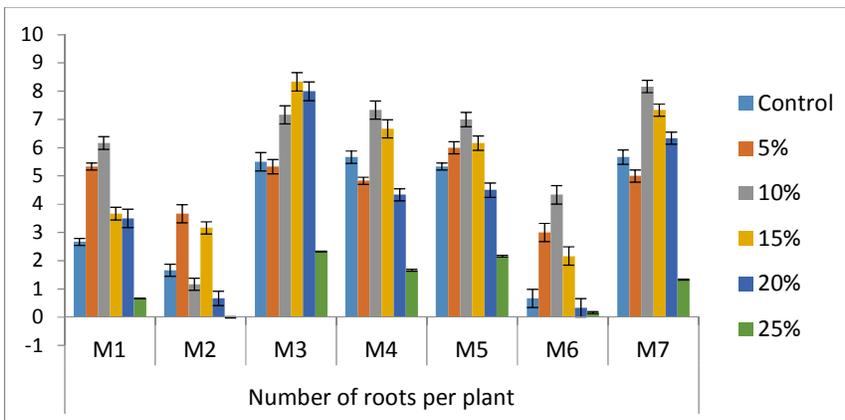


Fig. 5. Number of roots per plant

Where, M1-TMMH806, M2-NMH1008, M3-DELTA10V30, M4-INDAM1122, M5-SRIKAR3555, M6-DKC9141 and M7-SGA

In Fig. 6, shows that M6 (DKC 9141) showing the higher proline content whereas, reduced proline in M4 (INDAM 1122), were the M1 (TMMH 806) M2 (NMH 1008) and M3 (DELTA 10V30) showing the moderate results (Fig. 4).

The variety M 1 (TMMH) shows greater chlorophyll content than the other varieties M 2 NMH has lowest chlorophyll content (Fig. 7). As a result, it is evident from the above results that the potential for germination, root and shoot growth, and chlorophyll and proline content of maize are adversely affected by early-growing drought. Finally, the results also show a

decrease in the level of water stress, the percentage of germination, the root duration, shoot time, plant root number and fresh root and shoot biomass. In general, maximum germination decrease, root shooting length, fresh root weight and shooting were observed with the greatest water stress. The results showed that the SRIKAR 3555 is a drought-tolerant variety between all maize varieties based on morphological and biochemical characteristics. The NMH is also susceptible to drought. Drought-specific intermediate varieties are TMMH 806, DELTA 10V30, INDAM 1122, and SGA.

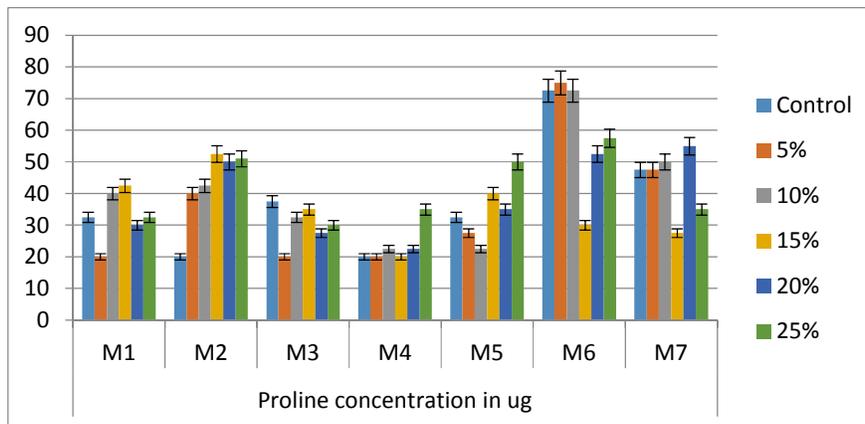


Fig. 6. Proline concentration

Where, M1-TMMH806, M2-NMH1008, M3-DELTA10V30, M4-INDAM1122, M5-SRIKAR3555, M6-DKC9141 and M7-SGA

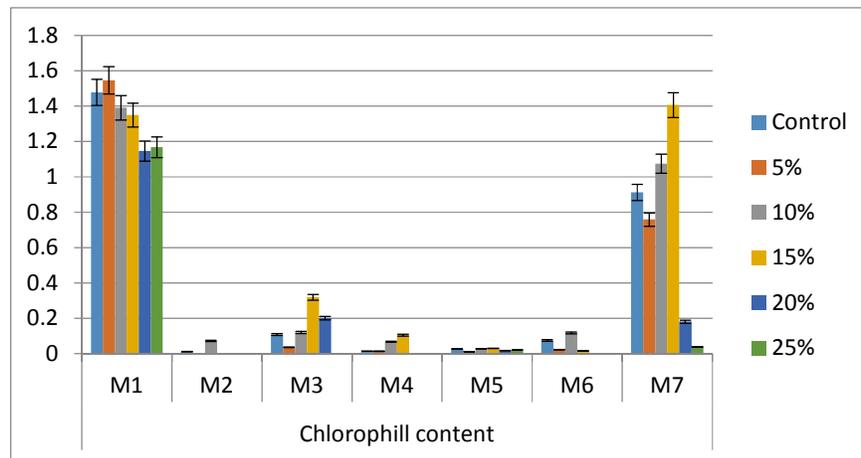


Fig. 7. Chlorophyll content

Where, M1-TMMH806, M2-NMH1008, M3-DELTA10V30, M4-INDAM1122, M5-SRIKAR3555, M6-DKC9141 and M7-SGA

4. DISCUSSION

Drought is one of the world's most detrimental abiotic stresses, seriously hampering agricultural crop productivity. Maize is one of the world's leading crops of cereals, but sensitive to drought. At various stages of growth in different regions, maize is affected by drought. Potential for germination, seedling growth, the establishment of seedlings, overall growth and development, pollen development, silk development, anthesis silking interval, pollination, embryo development, endosperm development, and kernel development are the events that are seriously hampered by drought stress in maize crop life [27]. Proper crop plant growth and development is important to establish a normal plant structure that conducts all physiological and metabolic processes and yields potential. Drought stress severely hampered maize growth and development. Growth and development included various parameters of components determined by different characteristics such as plant height, leaf area, root structural and functional characteristics, plant biomass, plant fresh weight, plant dry weight and stem diameter [28]. Height of the plant, diameter of the stem, the biomass of the plant and area of the leaf are reduced under the stress of drought.

In this study, higher percentages of germination 67% and 58% recorded by inbred lines SRIKAR 3555 and TMMH 806 respectively which indicate that these two genotypes were relatively able to maintain better plant water status within the water-stressed period during which measurement was taken. This shows that inbred lines SRIKAR 3555 and TMMH806 might not have only tolerated the drought but also might have avoided the drought as defined by Fisher and Sanchez [29] and also Otoole and Chang [30] that avoidance of drought is the ability of a plant to maintain relatively high water status despite the low moisture condition within the entire plant environment. According to González and González-Vilar [31], the subjective value accepted for LRWC is $\geq 80\%$. From the findings of González and González-Vilar [31], it can be deduced that all the other genotypes were apparently susceptible to drought when leaf relative water content was used as an indicator. Plant heights observed for the genotypes in the plant house were higher for the non-stress maize genotypes than the water stressed. The significant differences observed among the maize genotypes under the non-stressed condition as well as the stressed condition for the

other genotypes apart from inbred lines DELTA 10V30, INDAM 1122, DKC9 141, and variety SGA was by the findings of Olaoye [32], who observed that plant height of maize hybrid increased up to 45.38 cm at 100% field capacity 24 DAS (Days After Sowing), while it decreased up to 24.69 cm with decreasing field capacity. It was also reported by Abo-El-Kheir and Mekki [33], that the plant height of single cross maize hybrid was affected when deficit water was applied at different growth stages. The better performance of maize genotypes DELTA 10V30, INDAM 1122, DKC9 141, and variety SGA concerning root dry matter indicates their efficiency in resource acquisition particularly, water (Figs. 2, 3).

Maize genotypes SRIKAR 3555 and TMMH806 can be seen as having greater tendency to produce higher root dry matter under field conditions as concluded by Hurd [34] that measurement of roots in boxes of soil in the greenhouse gives a fair approximation of root growth in the field. Therefore, root growth at the seedling stage may, therefore, be useful in predicting root growth under drought stress at later growth stages. Camacho and Caraballo [35] also concluded that root dry mass was identified as the major criterion for selection of maize genotypes under drought conditions and this report again supports the higher drought tolerance level in inbred lines SRIKAR 3555 and TMMH 806. Significant lower dry matter yield was recorded by maize genotypes SRIKAR 3555 and TMMH 806. The significant lower dry matter yields recorded by these maize genotypes under water-stressed condition portends that the effect of the drought was severe to reduce leaf and stem growth as the crops intercepted less solar radiation (Figs. 3, 4). This observation agrees with the findings of Prabhu and Shivaji [36] who reported that the main effect of drought in the vegetative period is to reduce leaf and stem growth, so the crop intercepts less sunlight. In some cultivated cereals, the osmotic adjustment has been found to be one of the most effective physiological mechanisms underlying plant tolerance to water deficit [37]. Osmotic adjustment, as a process of active accumulation of compatible osmolytes in plant cells exposed to water deficit, may enable a continuation of leaf elongation, though at reduced rates. Osmoprotectants are classified into two main groups; the first group consists of nitrogen compounds such as proline, polyols, polyamines and glycinebetaine, while the second group consists of hydroxy compounds such as

polyhydric alcohols, saccharides and oligosaccharides [28]. The higher accumulation of proline content was observed in DKC 9141. Finally, the genotypes were ranked such that any genotype that had ≥ 3 out of the 4 indicators used was considered to be tolerant to drought (Figs. 4, 5, 6, 7). The following ranking was therefore obtained for the inbred lines and the varieties in decreasing order of drought tolerance; NMHSRIKAR 3555 > TMMH 806 > DELTA 10V30= INDAM 1122= DKC 9141 = SGA >>> NMH. These drought varieties can be modified by new technologies such as CRISPER-CAS9 and RNAi [11,39-41] to improve the development of improved abiotic and biotic stress-resistant crops.

5. CONCLUSION

From the above study, it is concluded that water stress of different level affects the growth of the maize. The result also shows that an increase in water stress level decreased the germination percentage, root length, shoot length, number of roots per plant and fresh biomass of root and shoot. Generally, maximum reduction in germination, root shoot length, fresh weight of root and shoot were observed in the highest water stress given. Result further exhibited on the basis of morphological and biochemical characteristics that the SRIKAR 3555 is drought tolerant variety among all varieties of maize. Moreover, the NMH is drought sensitive. While TMMH 806, DELTA 10V30, INDAM 1122, DKC 9141 and SGA is a drought intermediate varieties.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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