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Responses of *Zea mays* L. Cultivars to PEG induced Drought Stress

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Water deficit stress is one major environmental constraint having a devastating impact on crop productivity. Improving plant tolerance to drought is imperative to ensure food security. Drought stress during maize seedling establishment stage lowers the seedling survival rate and increases post pollination embryo abortion rate. Thus, an experiment was carried out as factorial in randomized complete design with three replicates to evaluate the tolerance mechanism of five local maize cultivars at Department of Life Sciences (Botany), Manipur University, Manipur during February to April, 2022. The maize seeds were allowed to germinate under control environment conditions: 14h light/10h dark, 25±2°C temperature and 60% relative humidity using a growth chamber (Tanco PLT-149 Plant Growth Chamber). At seven days after emergence, different concentrations of PEG-6000 were used to impose water deficit stress to maize seedlings. It was observed that drought stress substantially inhibited growth and development across all cultivars. Relative water content (RWC) as well as chlorophyll pigment concentration showed gradual decline under different drought stress levels with cultivar M002 being the least affected. Drought stress also triggered significant augmentation of osmolytes and antioxidant activity with maximum accumulation in cultivar M002. Overall findings from this study indicate that cultivar M002 possess promising drought tolerance characteristics and can perform successfully in water scarce regions.

Key words: Maize, PEG-6000, Drought, Osmolyte, Antioxidant, DPPH

With the changing environmental condition, cultivated crop plants often encounter different abiotic stress during their growth and development. Drought stress is responsible for reduction of nearly 50% of world agronomic production (Lamaoui *et al.,* 2018). As per FAO estimation, water scarcity accounts for a loss of USD 29 billion to agriculture in developing countries from 2005-2015 (FAO, 2021). Hence, concrete efforts towards development of high yielding, abiotic stress tolerant crop variety is critical to ensure food security. To achieve this, understanding crop tolerance and response to drought stress is imperative.

Severe drought during seedling stage cause visible leaf curling and stunted growth in Maize plants (Effendi *et al.,* 2019). Despite seedling establishment stage being one of the most vulnerable growth stages, previous studies have proven that drought stress during this initial growth phase of plant life cycle can substantially improve tolerance to water scarcity in later growth stages (Auler *et al.,* 2021). Several germination and seedling growth indices are therefore used frequently as basis for screening tolerant crop varieties (Queiroz *et al.,* 2019). Hence, identifying and selecting drought tolerant cultivar during seedling establishment stage can help improve crop yield (Ru *et al.,* 2022).

PEG-6000, a non-ionic water soluble polymer extensively used to stimulate drought stress in plant was utilised to induce varying degree of drought to the germinating seedlings. This study explores the relative significance of osmolyte accumulation and antioxidant activity in maize seedling subjected to PEG induced drought stress. It aims to provide a better understanding and highlight the impact of drought stress on maize seedling establishment, recognising that the seedling stage in maize is particularly sensitive to drought stress and improving the mechanism of seedling stage drought tolerance for better crop establishment is the main target for maize drought resistant breeders.

MATERIALS AND METHODS

The present study was carried out at Department of Life Sciences (Botany), Manipur University during February – April, 2022. Five maize cultivars with different genetic backgrounds were selected to analyse the impact of drought on early seedling stage (Table 1). Viable seeds were initially surface sterilised using 1% (v/v) sodium hypochlorite and rinsed twice in double distilled water. The seeds were allowed to germinate in a growth chamber (Tanco PLT-149 Plant Growth Chamber) under control environment conditions: 14h light/10h dark, 25±2°C temperature and 60% relative humidity. At seven day after emergence (DAE), the seeds were treated with different concentrations (0, 10%, 20% & 30%) of PEG-6000 which corresponds to 0, -0.15, -0.49 and -1.03 MPa osmotic potential respectively. The osmotic potential of PEG 6000 was determined using the equation of Michel and Kaufmann (1973). The PEG solution was replaced every two days and treatment was continued for 7 days. Fourteen day old seedlings were used to carry out different biochemical analysis. All experiments were laid out in Randomised Complete Design with three replicates. Root and shoot lengths were measured in centimetre using a graduated scale.

Relative Water Content

Relative water content (RWC) was determined following the method of Khaleghi et al. (2019). RWC% was calculated according to Turner (1981) using the formula:

$$RWC\% = \frac{Fresh\,weight - Dry\,weight}{Turgid\,weight - Dry\,weight} \times 100$$

Chlorophyll Content

Chlorophyll content of fresh maize leaves was determined according to the method by Lichtenthaler and Wellburn (1983). Chlorophyll a, chlorophyll b and total chlorophyll content were calculated as described by Arnon (1949).

Proline determination

0.2g fresh leaf sample was used to estimate proline content using ninhydrin reagent according to the method of Bates et al. (1973).

Total Phenolics

Khanam et al. (2012) method for phenol estimation using folin-ciocalteau reagent was used to quantify total phenol content in fresh maize leaves.

Total Soluble Sugar

Anthrone method was used to estimate the total soluble sugar content in leaves of maize seedling exposed to drought stress. Total sugar content was estimated from glucose standard curve.

Soluble Protein and Amino Acid

Soluble protein content in leaves for each treatment was estimated according to method by Lowry et al. (1951) and amino acid content was assessed in fresh leaf sample using the method by Yemm and Cocking (1955).

DPPH assay

The DPPH radicle scavenging activity was evaluated using the method described by Hatano et al. (1988). Final capacity to scavenge DPPH radicle was calculated using the formula by Son and Lewis (2002).

$$\text{Radicle Scavenging Assay (RSA) \%} = \frac{Ao - A1}{Ao} \text{ X } 100$$

Where A0 is absorbance of the control reaction and A1 is absorbance of the sample at 517nm.

Determination of antioxidant activity

To determine the antioxidant enzyme activities (SOD, POD and CAT) in maize leaf, enzymatic extract was prepared using 0.5 g of fresh leaf sample in 5 ml of 50 mM potassium phosphate buffer (pH 7.8) under ice cold condition. The homogenate was centrifuged at 10000 x g for 30 min at 4°C. The resulting supernatant was collected and used as crude extract.

SOD activity was assayed according to Tang et al. (2010) by measuring the enzyme ability to inhibit photochemical reduction of nitro blue tetrazolium (NBT).

POD activity was determined according to Upadhyaya et al. (1985). One POD unit (U) was defined as the change of 0.01 OD per min at 37°C under the assay condition.

For CAT activity, 0.2ml enzyme extract was added to an assaying mixture containing 2ml of 50mM phosphate buffer (pH 7.5) and 1ml of hydrogen peroxide. Change in absorbance at 240 nm per min was recorded.

All data collected were statistically analysed using SPSS 20.0. The mean value of three replicates and standard error were determined using LSD-Duncan test at $p \leq 0.05$.

RESULTS

In the present study, five maize cultivars with diverse genetic backgrounds were evaluated for their morphological, physiological and biochemical response to osmotic stress induced using different concentrations of PEG 6000.

Impact of drought on plant morphological characteristics

Drought stress substantially inhibited growth and development in all cultivars. Compared to control (0MPa), 30% PEG (-1.03MPa) treatment resulted in significant reduction of plumule length and marked reduction in seedling length across all the five cultivars (Table 2). While all the morphological parameters exhibited a gradual decline with increase in severity of drought stress, radicle length of most cultivars tend to increase under drought stress. Radicle length of cultivars M001, M002 and M003 increases along with increase in drought stress while cultivar M004 and M005 depicted marked decline in radicle length (Table 2). Photographic depiction of changes in morphological patterns under progressive drought stress in all cultivars at 7 days after treatment (DAT) is shown in Fig 4, 5, 6 & 7.

Impact of drought on Relative Water Content (RWC)

Reduction in RWC of plant leaves is a primary physiological response to drought stress. Table 2 data shows the effect of drought stress on RWC for all five maize cultivars. When compared to control, drought induction caused significant reduction in RWC and lowest RWC % was recorded under the 30% PEG treatment. Among the cultivars, most significant decline in RWC was observed in cultivar M003 followed by M004.

Effect of drought on photosynthetic pigment content

Chlorophyll concentration is reduced when plants are exposed to abiotic stress as a result of increased oxidative stress and photo oxidation of chlorophyll pigments (Allakhverdiev 2020). To understand the extent of drought impact on photosynthetic attributes, chlorophyll a, b and total chlorophyll content were measured for all the tested cultivars. It was observed

that PEG induced drought stress resulted in gradual decline of chlorophyll content in maize cultivars although no significant difference (p≤0.05) was observed across all treatment levels. Among the cultivars, it was observed that M002 showed the least reduction in chlorophyll content (Table 2).

Osmolyte Accumulation under drought stress

Drought stress triggered a rapid production and build-up of different osmolytes across all maize cultivars. The concentration of proline, phenol, soluble sugar, protein and free amino acid were considerably increased with increase in drought severity (-1.03MPa> -0.49MPa>-0.15MPa> 0MPa). The accumulation of osmolytes varied among the cultivars and maximum accumulation was recorded in cultivar M002 (Fig 1A, B, C, D & E) indicating that M002 might perform well under drought condition.

DPPH radicle scavenging assay

The DPPH radicle scavenging assay was evaluated in order to estimate the antioxidant activity of maize leaf extract under induced drought stress. Under control (0MPa) and -0.15MPa osmotic potential, rise in DPPH scavenging activity of the cultivars showed no significant difference (p≤ 0.05) (Fig 2). However, a sudden spike in antioxidant activity in all the cultivars was observed under -0.49 MPa and -1.03 MPa osmotic potential, the highest scavenging activity was recorded in M002 and M005.

Table 1. List of local maize cultivars used in the study

Cultivars	Local Name
M001(W)	*Chujak Angouba*
M002(V)	*Chujak Arangba*
M003(R)	*Chujak Angangba*
M004(B)	*Chujak Amuba*
M005(N)	*Nepali Chujak*

Table 2. Data represents mean of three replicates ± SE. Values followed by similar letters within a column are not significantly different at p≤0.05.

Maize Cultivar	PEG Treatment	Radicle Length (cm)	Plumule Length (cm)	Seedling Length (cm)	RWC (%)	Chl A (mg/gFW)	Chl B (mg/gFW)	Total Chl (mg/gFW)
M001 (W)	0MPa	6.5±1.1^{de}	14.67±0.89^{b}	22.67±1.13^{cd}	83.19±0.09^{b}	0.51±0.03^{abc}	0.59±0.05^{a}	1.06±0.08^{ab}
	-0.15MPa	6.7±2.6^{de}	12.16±0.23^{c}	19.27±0.68^{de}	74.52±0.28^{e}	0.45±0.03^{bcde}	0.31±0.05^{bcd}	0.77±0.08^{cdef}
	-0.49MPa	7.1±0.9^{cd}	7.5±1.15^{ef}	14.2±2.13^{ghi}	47.17±0.13^{l}	0.33±0.03^{ghij}	0.28±0.02^{bcd}	0.64±0.12^{efgh}
	-1.03MPa	8±2.2^{bc}	2.26±1.03^{i}	8.73±0.3^{k}	46.39±0.79^{l}	0.26±0.03^{jk}	0.25±0.004^{bc}	0.5±0.01^{hi}
	Mean	7.08	9.15	16.22	62.82	0.39	0.36	0.74
M002 (V)	0MPa	8.4±2.1^{bcd}	17.96±0.57^{a}	29.07±0.68^{a}	79.13±0.46^{d}	0.59±0.02^{a}	0.64±0.02^{a}	1.23±0.01^{a}
	-0.15MPa	9.4±0.8^{abc}	16.13±0.45^{ab}	26.5±1.81^{ab}	70.6±0.25^{f}	0.43±0.04^{cdef}	0.53±0.04^{a}	0.81±0.07^{cde}
	-0.49MPa	10.4±2.8^{ab}	15.76±2.4^{b}	25.17±1.79^{bc}	65.33±0.35^{h}	0.36±0.01^{efgh}	0.33±0.07^{bc}	0.72±014^{defg}
	-1.03MPa	11.1±1.2^{a}	9.36±3.3^{de}	17.67±2.97^{efg}	62.41±0.38^{i}	0.36±0.05^{efgh}	0.29±0.04^{bcd}	0.58±0.06^{efgh}
	Mean	9.83	14.8	24.6	69.36	0.44	0.45	0.84
M003 (R)	0MPa	5.8±0.49^{e}	15.2±0.1^{b}	24.73±0.73^{bc}	88.55±0.35^{a}	0.4±0.05^{defg}	0.51±0.02^{a}	0.97±0.07^{bc}
	-0.15MPa	6±0.7^{de}	10.73±0.8^{cd}	18.3±0.7^{ef}	55.03±0.71^{g}	0.37±0.04^{efgh}	0.36±0.05^{b}	0.61±0.004^{efg}
	-0.49MPa	8.6±0.7^{cde}	7.86±1.45^{ef}	13.87±0.97^{hij}	67.64±0.54^{j}	0.27±0.01^{ijk}	0.29±0.02^{bcd}	0.63±0.03^{efgh}
	-1.03MPa	9.3±1.2^{abc}	2.5±0.62^{i}	7.77±0.29^{k}	36.08±0.34^{n}	0.25±0.02^{k}	0.18±0.01^{d}	0.53±0.05^{ghi}
	Mean	7.4	9.07	16.17	61.83	0.32	0.34	0.69
M004 (B)	0MPa	6±1.5^{de}	10.06±1.17^{cd}	16.43±1.46^{efgh}	83.3±0.61^{b}	0.48±0.02^{bcd}	0.57±0.03^{a}	1.05±0.04^{ab}
	-0.15MPa	5.8±0.6^{de}	7.96±1.33^{ef}	13.8±0.53^{hij}	70.9±0.12^{f}	0.35±0.01^{efgh}	0.33±0.04^{bc}	0.76±0.12^{cdef}
	-0.49MPa	5.4±1.2^{e}	5.43±0.35^{gh}	10.2±0.4^{jk}	47.84±0.22^{l}	0.31±0.05^{hij}	0.23±0.02^{bcd}	0.63±0.04^{efgh}
	-1.03MPa	4.8±0.45^{f}	2.76±0.45^{i}	8.17±0.9^{k}	40.08±0.13^{m}	0.27±0.02^{ijk}	0.19±0.01^{cd}	0.44±0.03^{i}
	Mean	5.5	6.55	12.15	60.53	0.35	0.33	0.72
M005 (N)	0MPa	8.1±0.36^{bcd}	10.43±0.46^{cd}	18.53±0.3^{ef}	80.68±0.47^{c}	0.54±0.03^{ab}	0.52±0.05^{a}	1.1±0.02^{ab}
	-0.15MPa	8.3±1.2^{bcd}	7.03±0.32^{fg}	15.37±0.5^{fgh}	66.85±0.3^{g}	0.45±0.03^{bcde}	0.36±0.09^{b}	0.88±0.03^{bcd}
	-0.49MPa	5.4±1.1^{ef}	5.13±0.57^{gh}	10.5±0.76^{ijk}	63.97±1.4^{h}	0.36±0.03^{efgh}	0.36±0.07^{b}	0.68±0.09^{defg}
	-1.03MPa	4.3±0.4^{f}	3.63±0.23^{hi}	7.97±0.12^{k}	52.07±0.32^{k}	0.34±0.02^{fghi}	0.3±0.03^{bcd}	0.55±0.07^{fgh}
	Mean	6.53	6.56	13.09	65.89	0.42	0.39	0.8

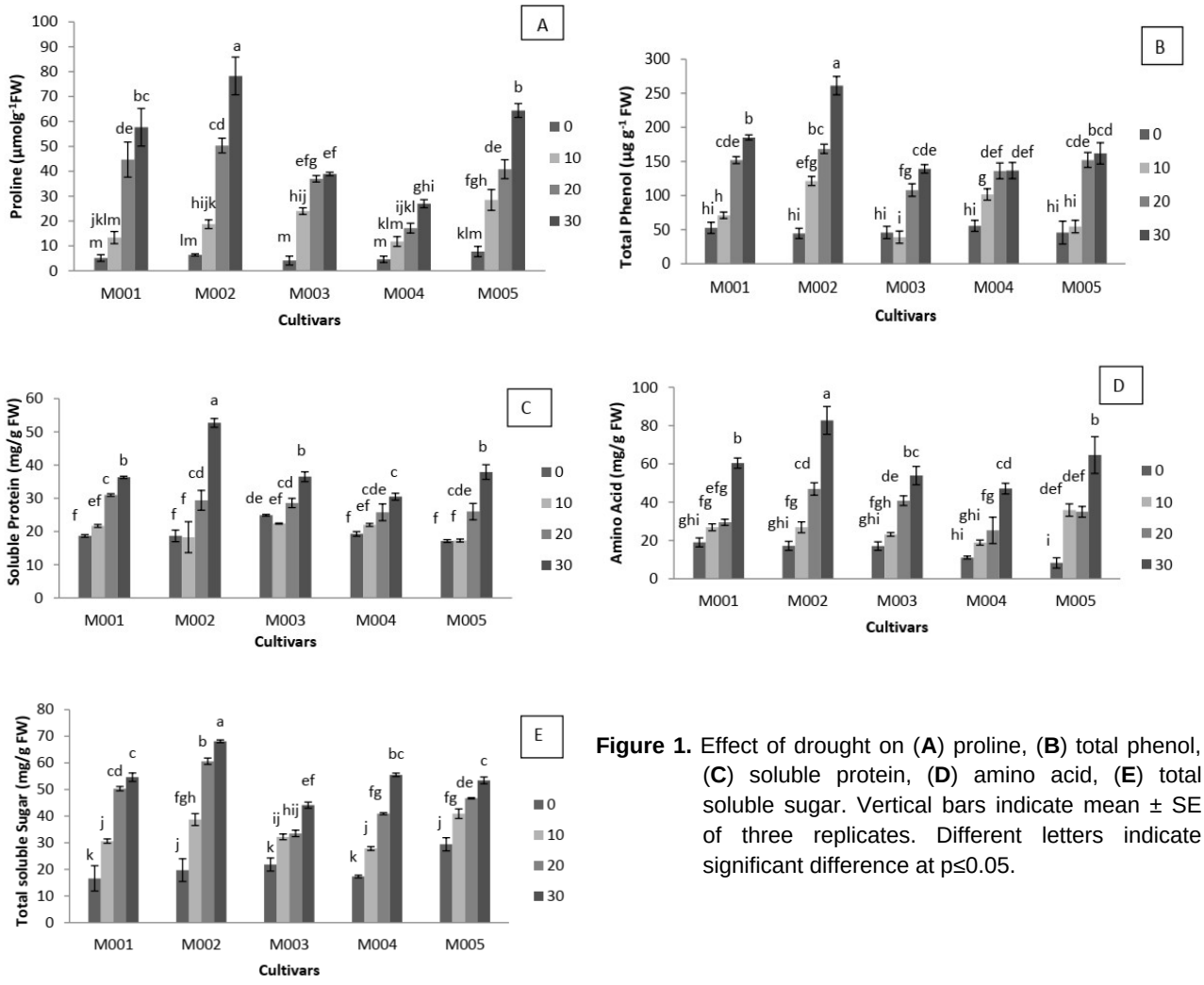

Figure 1. Effect of drought on (**A**) proline, (**B**) total phenol, (**C**) soluble protein, (**D**) amino acid, (**E**) total soluble sugar. Vertical bars indicate mean ± SE of three replicates. Different letters indicate significant difference at p≤0.05.

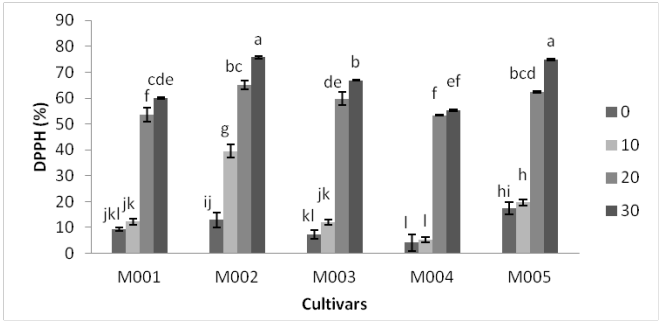

Figure 2. Effect of drought on DPPH radicle scavenging activity of five maize cultivars. Vertical bars indicate mean ± SE of three replicates. Different letters indicate significant difference at p≤0.05.

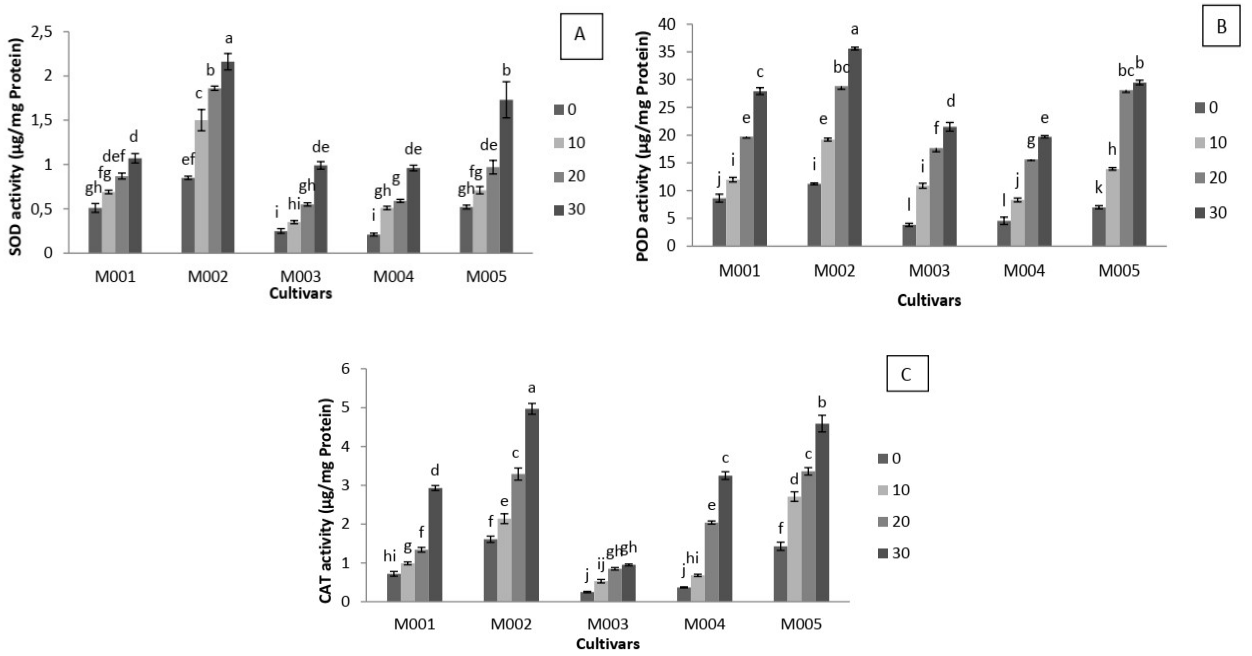

Figure 3. Effect of drought on (A) SOD, (B) POD, (C) CAT activities of five maize cultivars. Vertical bars indicate mean ± SE of three replicates. Different letters indicate significant difference at p≤0.05.

Figure 4. Photographs comparing the performance of different maize cultivars at 7 DAT under control (0MPa) condition. W=M001, V=M002, R=M003, B=M004 and N=M005.

Figure 5. Photographs comparing the performance of different maize cultivars at 7 DAT under 10% PEG treatment (-0.15MPa). W=M001, V=M002, R=M003, B=M004 and N=M005.

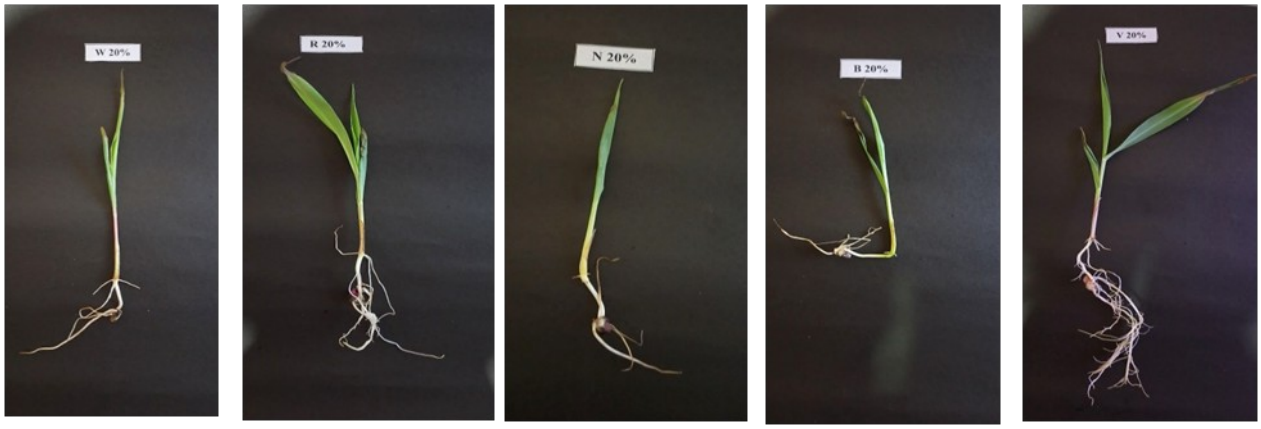

Figure 6. Photographs comparing the performance of different maize cultivars at 7 DAT under 20% PEG treatment (-0.49MPa). W=M001, V=M002, R=M003, B=M004 and N=M005.

Figure 7. Photographs comparing the performance of different maize cultivars at 7 DAT under 30% PEG treatment (-1.03MPa). W=M001, V=M002, R=M003, B=M004 and N=M005.

Impact of drought stress on antioxidant activity

Drought stress induces significant ($p \leq 0.05$) variation in antioxidant activity among the five maize cultivars. A gradual rise in SOD activity was observed in all cultivars with increase in severity of drought and maximum value was recorded in cultivar M002 (Fig 3A). Similarly, a significant rise in POD and CAT activities was also evident across all the cultivars (Fig 3B, 3C). Maximum activities of POD and CAT were recorded in M002 and M005 under -1.03MPa treatment.

DISCUSSION

Prevalence of drought and its detrimental impacts on plants necessitates studies on exploring plant drought tolerance mechanisms to mitigate significant yield losses under water stress (Singh et al. 2014). In this study, five maize cultivars with diverse genetic backgrounds were evaluated for their morphological, physiological and biochemical response to osmotic stress induced using different concentrations of PEG 6000. Although drought significantly reduced shoot growth in all cultivars, radicle length of most cultivar increases under drought except for cultivar M004 and M005. As mentioned by Leishman and Westoby, 1994, ability to extend radicle length is an important trait for selection of drought resistant cultivars. In this study, M002 cultivar with highest root extension can be considered as a tolerant cultivar.

Conspicuous decline in leaf water content was observed across all cultivars under drought stress. However, the negative impact of drought was more pronounced in sensitive cultivars compared to tolerant cultivars. The observed reduction in RWC of maize seedling agrees with previous findings reported by Nayyar and Gupta (2006).

Drought stress displayed differential influence on chlorophyll content in leaves of all five maize cultivars. Notably, different cultivars exhibited distinct sensitivity to PEG induced drought stress. Decline in chlorophyll level under drought stress have been reported in other species such as wheat and rice. According to Herbinger et al. (2002), the observed degradation of light absorbing pigments under drought stress might contribute towards effective avoidance of ROS production in plants.

Plants generally accumulate osmolytes such as proline, soluble sugar, protein, amino acid and phenol chiefly in cytoplasm in response to drought stress. This enable plants to effectively scavenge ROS, maintain optimum water potential and protect cellular components from lipid peroxidation (Raza et al. 2019).

Proline accumulation in plants has been extensively studied and reported to be an adaptive response to osmotic stress (Hare et al. 2002). A significant ($p<0.05$) rise in proline concentration was observed in all the maize cultivars with increase in drought level. This observation is in accordance with previous reports in other plants such as *Arabidopsis thaliana* (Sperdouli and Moustakas 2012), *Capsicum annuum* (Anjum et al. 2012) and *Oryza sativa* (Dien et al. 2019). Proline not only regulates osmotic potential under stress condition but also functions as scavenger for free radicles and protects plant leaves against lipid peroxidation (Gill and Tuteja 2010). Highest spike in proline accumulation was recorded under -1.03MPa osmotic potential this might have helped in effective recovery of plants after stress.

Analysis of phenol content in this study showed a significant increase in total phenol content across all cultivars which is in alignment with reports made earlier in this context. Saad Allah et al. (2022) and Sarker and Oba (2018) reported a similar progressive increase in total phenol content in plants under drought stress.

It was observed that the level of soluble sugar, protein and amino acid in all maize cultivars rise gradually with increase in drought level. Compatible solutes such as soluble sugar, protein and amino acid accumulate in plant tissue chiefly in cytoplasm in response to limited water availability signifying their role in improving osmotic adjustment, ROS detoxification and cell membrane protection in plants under osmotic stress (Raza et al. 2019; Reddy et al. 2004). Soluble sugar has been widely acknowledged as the main osmolyte contributing directly in mitigating oxidative damage in plants species (Dubey and Singh 1999). In the present study maize cultivars respond to drought stress with increased accumulation of soluble sugar which might have improved the ROS scavenging potential in maize cultivars. Hence, it can be concluded that drought sensitivity of cultivars might be associated with lower level of soluble sugar accumulation since sugar starved plants have been reported to produce more ROS (Couee 2006).

DPPH assay was carried out to evaluate the free radicle scavenging potential of the cultivars. Plants with higher radicle scavenging potential tend to perform better under unfavourable climatic conditions. In this study, a gradual rise in RSA% was observed with increase in drought severity. The result indicates that there is a significant decrease in DPPH radicle due to rise in scavenging activity of antioxidants present in maize cultivars under drought stress.

Similarly, an enhanced accumulation of enzymatic antioxidants such as SOD, POD and CAT in plants under oxidative stress act as powerful defence shield against oxidative damage. High antioxidant content in plant leaves confer significant drought tolerance (Aslam et al. 2015). In the present study, drought treatment significantly increased antioxidant activity in all maize cultivars. It is evident that the cultivars showed varied response to induced drought stress with cultivar M002 showing the highest antioxidant activities while cultivar M003 showed the least antioxidant activity.

CONCLUSION

Drought significantly impacted on all measured parameters across all osmotic stress levels. In the

present experiment, cultivar M002 performed well under drought stress in terms of growth, development and biochemical parameters. Compared to rest of the cultivars, M002 accumulated abundant osmolytes along with enhanced antioxidant activities even under severe drought stress (-1.03MPa) condition. Therefore, it can be concluded that cultivar M002 is superior in terms of drought tolerance and after further field level performance analysis, this cultivar could be advised for large scale cultivation in drought prone areas.

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CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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