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Priming-mediated triggering of antioxidative response to induce drought tolerance in Maize (*Zea mays* L.)

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Abstract

Drought is a well-known issue in plants and it occurs when plants do not receive enough water to meet their requirements. Hence it alters the metabolic process of the plant, consequently reducing the yield. To overcome the loss of yield under prevailing situations, triggering of the antioxidative defense system is required which can mitigate the impact of drought on plants. The priming chemicals KNO_3, $Mg(NO_3)_2$ and GA_3 were evaluated with hydro-priming to know the mitigative response of priming against drought-induced stress in Maize plants. The morpho-physiological and biochemical parameters were used to evaluate the impact of priming-mediated triggers on the antioxidative response. The results of this work indicate that leaf area index (LAI), crop growth rate (CGR, mg g^{-1} day^{-1}), total chlorophyll, and chl'a' (mg g^{-1}) were recorded maximum in T_5 ($Mg(NO_3)_2$, 10 mM) while chl 'b' in T_4 (Mg $(NO_3)_2$, 7 mM). The maximum Membrane Stability Index (MSI %) and Membrane Injury Index (MII%) were recorded in T_5 and T_0 (Control). The osmoregulatory compound proline content (µg g^{-1}) and antioxidative enzyme catalase (nm H_2O_2 mg^{-1} min^{-1}) were detected in significantly highest quantity in T_3 (KNO_3, 15 mM) while the least amount of malondialdehyde (MDA nm g^{-1}) was found under the same treatment. The correlation studies amongst all the parameters reflected that MII % and MDA content (MDA nm g^{-1}) negatively correlated with the remaining parameters studied. This study has reflected that out of all the sources of priming treatments, KNO_3 in 15 mM and $Mg(NO_3)_2$ in 10 mM has the potential to trigger the antioxidative defense mechanism to mitigate the response of drought in Maize.

Keywords

Catalase activity, Drought, Leaf Area Index, Malondialdehyde, Proline content, Membrane Stability Index

Introduction

Maize (*Zea mays* L.) is recognized as the queen among the cereal crops because of its high-yielding potential (1). United States of America is the most significant contributor of maize (35%) worldwide and followed by Brazil, China, and Mexico (2). Drought is one of the critical constraints that alters the entire sequence of biochemical reactions and antioxidative defense mechanisms, consequently limiting crop growth, development, and yield as per the severity and intensity of drought (3, 4).

Scarcity of water during plants' growth period may cause ROS burst (O^{-2}, H_2O_2, and OH); consequently, ROS-mediated damage occurs at the

molecular level in the cell in which membrane, protein, and nucleic acid are few of them. However the trigger of certain osmolytes that is low in molecular weight and high in water-solubility such as trehalose, proline, glycine-betaine and antioxidative enzymes i.e. catalase (CAT), superoxide dismutase (SOD), ascorbate peroxidase (APX) has shown their potential to protect the plant from the damage that occurs due to drought (5-8).

Priming techniques are an effective strategy that opens a new window to enhance the yield of a crop by accelerating the entire growth phase even under stressful conditions (9). Moreover, it is cost-effective, environment-friendly and easy to apply. Large groups of priming agents are known that perform effectively under a wide range of climatic conditions in favor of morphological, physiological, biochemical, and molecular alteration in various crops *i.e.,* hydropriming, $Ca(NO_3)$, $NH_4(SO_4)_2$, NaCl, KCl, KH_2PO_4, $CaCl_2$, $ZnSO_4$: Salicylic acid, $MgSO_4$, GA_3, KNO_3 Mg $(NO_3)_2$(10-12). Optimizing the priming chemicals concentration in relation to knowing the sequential event of the metabolic process of maize plant at the molecular level under drought conditions is most needed.

Materials and Methods

Treatment details

An experiment was planned and executed over the Research Farm of Lovely Professional University in 2019-20 in the summer season. The seeds of maize variety (AHC-1212) were received from the certified shop of Jalandhar, Punjab. This experiment was laid out in Randomized Block Design (RBD) with a total of eight different combinations of seed priming treatment (T_0= Control, T_1= Priming with Distilled water, T_2= KNO_3, 10mM, T_3= KNO_3, 15mM, T_4 = Mg $(NO_3)_2$, 7 mM, T_5= Mg $(NO_3)_2$, 10 mM, T_6= GA_3, 3 mM and T_7= GA_3, 4 mM) in three replications. Surface sterilized seeds of maize were used to prime with respective priming agents like hydropriming, KNO_3, $Mg(NO_3)_2$, and GA_3, in which each priming chemical was used in two different concentrations. Artificial drought is a process in which water supply to a particular area is intentionally reduced or stopped to study the effects of drought on plants (13). However, in the present piece of work, it was created before the flowering while standard agronomic and cultural practices were adopted during the cultivation of crops.

Crop allometry

The observations of leaf area index (LAI) and crop growth rate (CGR) were recorded at two intervals, i.e., 60 and 90 DAS for LAI while 30-60 and 60-90 DAS for CGR by adopting the standard procedure given by Watson DJ. (14).

LAI = Total Leaf Area (cm^2) / Total Ground Area (cm^2)

CGR = $(W_2\text{-}W_1 / T_2\text{-}T_1)$ X (1/A) g cm^2 day^{-1}

Estimation of MSI and MII %

The estimation of the Membrane Stability Index (MSI %) was carried out according to Sairam RK *et al.,* (15), while the Membrane Injury Index (MII%) was calculated by subtracting the value of MSI %.

Membrane stability index (MSI)% = 100 $[1- C_1 / C_2]$

Estimation of Total chlorophyll, chla and chlb

The total chlorophyll content was analyzed according to the method of Arnon DI (16). However, the calculation of total chlorophyll, chl 'a' and chl'b' was carried out as per the formula below.

$$\text{Total chlorophyll} = (20.2\,(D\,645) + 8.02\,(D\,663)) \times \frac{V}{1000 \times W}\ \text{mg g}^{-1}$$

$$\text{Chl 'a'} = (12.7 \times A663) - (2.69 \times A645) \times \frac{V}{1000 \times W}\ \text{mg g}^{-1}$$

$$\text{Chl 'b'} = (22.9 \times A645) - (4.68 \times A663) \times \frac{V}{1000 \times W}\ \text{mg g}^{-1}$$

Estimation of Proline

The determination of proline content was carried out according to Bates LS (17), 0.5 g leaf sample was pulverized in 5 ml of 3 % Sulphosalicylic Acid using a mortar and pestle and homogenate was centrifuged at 3000 rpm for 10 minutes. A 2 ml of extracted sample, 2 ml of glacial acetic acid and 2 ml of ninhydrin reagent were pipette out in a respected test tube and placed in a water bath at 100 ^{0}C for 30 minutes. Thereafter, the proline was separated with help of 6 ml toluenein a separating funnel. The absorbance of chromophore-containing color was measured at 520 nm while the standard of proline was used to calculate the presence of proline in the samples.

Estimation of Malondialdehyde (MDA)

Malondialdehyde was determined by measuring the thiobarbituric acid (TBARS content) as per the protocol (18). Leaf samples 0.5 g were homogenized in 10 ml of 0.1% TCA. The homogenized samples of maize were centrifuged at 10000 rpm for 15 minutes. Thereafter, 1 ml of the extracted sample and 4 ml of 0.5 % TBA were added to each test tube. The mixture of the samples was heated in an electric oven for half an hour followed by cooling at room temperature. The absorbance of the sample was recorded at 532 and 600 nm while the content of MDA was calculated by using the extinction coefficient (155 mM^{-1} cm^{-1}).

Catalase activity

Catalase activity was assayed according to (19), in which one unit of CAT activity was defined as nmol H_2O_2 min^{-1} (extinction coefficient 36 mM cm^{-1}). 0.1 g of maize leaf sample were taken and homogenized in 5 ml of phosphate buffer (0.1M) and the extract was centrifuged at 10000 rpm for 20 minutes. The activity of the enzyme was assayed by taking 2.6 ml, of phosphate buffer (0.1M), 0.1 ml of enzyme extract and 0.1 ml H_2O_2 (1%) while a blank sample was

prepared by taking phosphate buffer (0.1 M) into reaction mixture instead of enzyme extract. Absorbance was recorded at 240 nm at intervals of seconds up to minutes.

Statistical analysis

All the data were subjected to statistical analysis by adopting a Randomized Block Design (RBD). The least significant differences (LSD) were calculated to compare the significance among the treatments at probability ($p \leq 0.05$) of significance by SPSS software 23rd version by Duncan's multiple range test (DMRT).

Results and Discussion

In the present work, we attempted to screen out highly beneficial priming-mediated triggering of biochemical and antioxidative responses in maize under drought conditions. As a result, it was depicted in (Table1) that the T_5(Mg $(NO_3)_2$, 10 mM) had the significantly highest growth in terms of leaf area index (LAI) 6.23, 5.40 and crop growth rate (CGR) 8.09, 9.54 mg g^{-1} day^{-1} at both the intervals (60, 90 and 30-60, 60-90 DAS) as compared to rest of the treatment which was followed by T_3> T_4> T_2> T_6>T_1> T_7>T_0. The significantly highest amount of total chlorophyll content and chl 'a' were detected in T_5at all observations 1.36, 2.83, 1.28, and 1.02, 2.19, 1.15 mg g^{-1}(Fig. 1 and 2). However, the amount of chl 'b' was significantly highest in T_5 at 30 DAS, and T_4 was noticed at maximum at 60 and 90 DAS (Fig. 3). The performance of the rest treatments for the total chlorophyll and chl 'a' were observed as T_3> T_4> T_2> T_6> T_1> T_7>T_0,but the performance of the treatments for the chl 'b' was recorded as significantly different as compared to total chlorophyll and chl a. It was evident that the drought-induced membrane injury index of leaf cells (Fig. 4), while the priming treatments, tried to recover the rate of membrane injury index as compared to the control one. Out of all the priming treatments, T_5 was noticed to have the least amount of MII %, 25.67 and 31.52 % significantly. It effectively ameliorated the impact of drought, which was also reflected in MSI % 74.33 and 68.48 % Fig. 4. The second most effective treatment was recorded in T_4, followed by T_3 in terms of both MSI% and MII%.A remarkable increase in proline content was detected in the entire set of treatments due to the influence of drought conditions at all the DAS(Fig.5). In contrast, a significantly sharp increase of proline content was recorded in T_3 72.26 and 92.30 µg g^{-1}at 60 and 90 DAS, followed by T_5> T_2> T_4> T_6> T_1> T_7 compared with T_0 (Non primed seeds).

The production of malondialdehyde (MDA) is an indication of rapid lipid peroxidation and cell damage. It was evident that drought accelerates the production of MDA in leaf cells. It was depicted in Fig. 6, the significantly least amount of MDA content was recorded in T_3 21.48 and 47.81 nm g^{-1} at both the intervals 30 and 60 DAS, which was followed by T_5> T_2> T_4> T_6> T_1> T_7> T_0. The activity of CAT was measured at two regular intervals *i.e.,* 30 and 60 DAS after inducing drought during the crop growth period. The data depicted in Fig. 6 shows that out of all the priming treatments the significantly highest activity of CAT was measured in T_3 290.54 and 424.75 n mole H_2O_2 mg^{-1} min^{-1}at both the intervals, which were followed by T_5> T_2> T_4> T_6> T_1> T_7 as compared to T_0(control). The activity of CAT increased sharply at 60 DAS as compared to 30 DAS in all sets of priming treatments while the least difference in CAT activity was recorded in the control (T_0).

Data depicted in (Table 2) reveals the study of the correlation coefficient among the studied parameters representing the effect of priming treatments under the influence of drought in maize. It was evident that LAI, CGR, total chlorophyll, chl a, chl b, and MSI had a negative correlation with MII and MDA, while the MII with proline content and catalase, Proline with MDA, and MDA with catalase also had a negative correlation among them, however, rest of the parameter combinations were positively correlated with each other.

Drought is one of the leading causes behind the yield reduction in most crops in the dry land area where the rainfall occurs below the threshold level compared to the crop demand (20). Drought is a kind of stress influencing the entire growth phase, from seed germination to crop harvesting. Morphologically it delayed the rupturing of the seed coat and the emergence of healthy seedlings by reducing the rate of cell division and multiplication (21), reducing plant height, leaf area, LAI, and CGR (22) while biochemically altering the metabolic process during seed germination due to the scarcity of water, reduces the content of chlorophyll hence, rate of photosynthesis reduced subsequently and limited the production of photosynthetic assimilates. Moreover, a drastic reduction in antioxidative enzymes and the accumulation of osmolytes under severe drought (23). Therefore, in the prevailing conditions, hydropriming, nitrate salts of Mg, K and growth hormone GA_3were used to decipher the significance of priming treatments concerned with drought. Several studies have been done in the past with

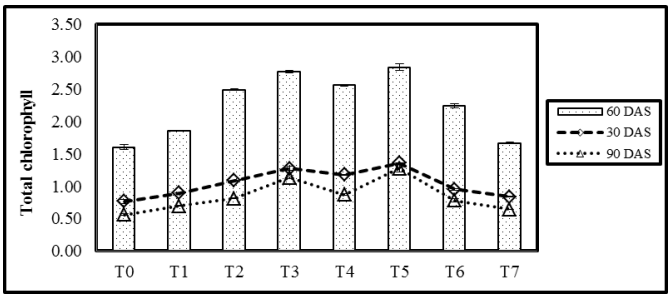

Fig. 1. Effect of priming treatments on total chlorophyll (mg g^{-1}) in maize.

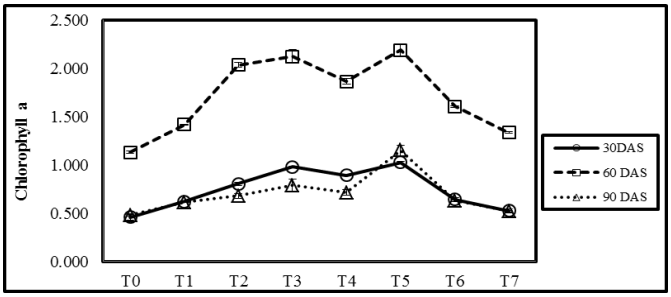

Fig. 2. Effect of priming treatments on chlorophyll a (mg g^{-1}) in maize.

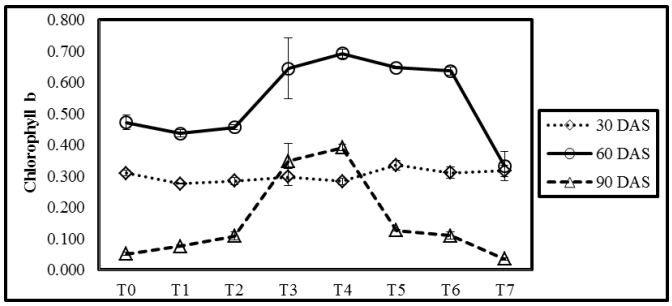

Fig. 3. Effect of priming treatments on chlorophyll b (mg g^{-1}) in maize plant

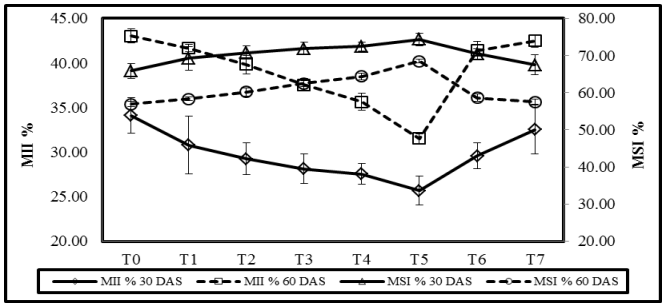

Fig. 4. Effect of priming treatments on MII and MSI % in maize

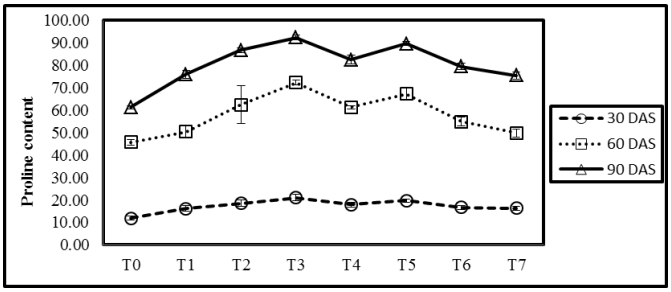

Fig. 5. Effect of priming treatments on proline content (µg g^{-1}) in maize.

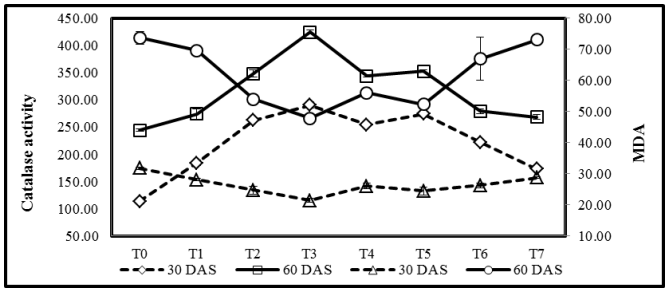

Fig. 6. Effect of priming treatments on catalase activity (nmole H_2O_2 mg^{-1} min^{-1}) and MDA (nm g^{-1}) in maize.

priming agents to overcome the effect of drought who reported that priming with Mg $(NO_3)_2$, KNO_3 and GA_3 is not only effective upto seed germination, seedling growth, LAI and CGR (23, 24) but it is also participating in the synthesis of chlorophyll (25). Similar results were obtained from the current study indicating that priming treatments specially KNO_3 followed Mg $(NO_3)_2$ positively influenced and enhanced the LAI and CGR (Table 1), total chlorophyll content, and chl a and chl b Fig. 1 to 3. Mg and K both are critical elements in the plant that helps in upregulate many metabolic pathways under drought conditions in which Mg is a central atom of chlorophyll hence it is one of the key elements for the synthesis of chlorophyll (26) while K enhances a plant's tolerance to drought by improving water uptake and regulation within the plant, reducing water loss through transpiration, and strengthening the plant's stem and stomata. Potassium also helps in maintaining proper turgor pressure in plant cells, which is necessary for maintaining the structure and function of the plant during drought. Additionally, potassium activates various stress-responsive genes, which help the plant cope with adverse conditions such as drought. Therefore, adequate levels of potassium in the soil can help plants overcome drought stress and improve their survival and productivity(27, 28).Recent findings on the impact of priming on aquaporins are recognized as mediators for improving water status in drought conditions (29). A positive relationship among the LAI, CGR, and chlorophyll content has already been established in favor of plant growth because of the collective efforts of these parameters to reform the status

Table 1. Effect of priming treatments on LAI and CGR (mg cm^2 day^{-1}) in maize

Treatments detail	LAI		CGR (mg cm^2 day^{-1})	
	60DAS	90 DAS	30-60DAS	60-90DAS
T_0	3.79^{a}	3.36^{a}	5.80^{a}	6.88^{ab}
T_1	4.63^{bc}	3.65^{b}	6.43^{abc}	8.11^{abc}
T_2	4.94^{cd}	4.26^{de}	7.41^{bcde}	8.45^{bc}
T_3	5.50^{e}	4.63^{f}	7.84^{de}	9.10^{c}
T_4	5.12^{d}	4.33^{e}	7.58^{cde}	8.44^{bc}
T_5	6.23^{f}	5.40^{g}	8.09^{e}	9.54^{c}
T_6	4.84^{cd}	4.22^{d}	6.89^{abcd}	8.02^{abc}
T_7	4.40^{b}	3.82^{c}	6.38^{ab}	6.57^{a}
SE(m)±	0.115	0.032	0.360	0.545
CD (*p<0.05*)	0.346	0.096	1.090	1.649

Table 2. Correlation studies among the parameters studied in maize

	LAI	CGR	Total chl.	Chl. a	Chl. b	MSI %	MII %	Proline	MDA	Catalase
LAI	1									
CGR	0.91	1								
Total chlorophyll	0.91	0.93	1							
Chlorophyll a	0.91	0.91	0.98	1						
Chlorophyll b	0.65	0.73	0.78	0.63	1					
MSI%	0.92	0.84	0.86	0.82	0.70	1				
MII %	**-0.92**	**-0.84**	**-0.86**	**-0.82**	**-0.70**	**-1.00**	1			
Proline	0.88	0.88	0.96	0.97	0.66	0.78	**-0.78**	1		
MDA	**-0.84**	**-0.89**	**-0.96**	**-0.97**	**-0.66**	**-0.79**	0.79	**-0.98**	1	
Catalase	0.77	0.81	0.90	0.91	0.59	0.69	**-0.69**	0.97	**-0.97**	1

of plant growth in prevailing conditions(30). Results depicted from (Fig. 4 and 5) showed an impact of priming treatments where the minimum injury and maximum stability index were recorded compared to control hence, the accumulation of proline in most of the priming treated sets were recorded. The priming with nitrate salts efficiently reduces membrane injury and increases the proline content in drought and salinity stress as reported by (31, 32). Moreover, the synthesis of the least quantity of MDA and high catalase activity in priming treatments reflect an additional benefit in favor of drought tolerance in maize plants (Fig. 6). The above results are as per the findings of (33), who reported that the maximum stability in the membrane and the synthesis of the least amount of MDA along with the accumulation of osmolytes (proline content) and catalase activity in priming treatments reform the damage of morphological as well as biochemical to overcome from drought conditions (34, 35). Furthermore, analysis of the correlation coefficient of studied parameters also provides a glimpse of the positive and negative relationships with priming treatments for drought tolerance (Table 2).

Conclusion

This study was centralized to screen out a potential source of priming treatments with optimum concentrations to protect the maize plant from drought conditions by triggering the antioxidative defense mechanism. Out of all the sources of priming agent, Mg $(NO_3)_2$ with 10 mM concentration was found better in favor of phenological parameters, *i.e.,* LAI and CGR. However, $KNO_3$with 15 mM concentration was superior to triggering the antioxidative response by accelerating the synthesis of proline and catalase activity and at the same reducing lipid peroxidation in terms of MDA. Thereby the significance of priming treatments as a potential source to overcome the drought was also reflected during the correlation studies.

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Authors contributions

This research work, manuscript drafting, statistical analysis and their proof readings are collective efforts and contributions of all the authors.

Compliance with ethical standards

Conflict of interest: The authors do not have any conflict of interest.

Ethical issues: None.

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