Mitigation of Drought Stress in Maize by Natural and Synthetic Growth Promoters

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ABSTRACT

Drought is an important factor-limiting yield in maize. Cytokinins are well reported for reducing the perilous effects of drought stress in maize (Zea mays L.). A net house study was planned to compare the efficacy of synthetic cytokinins, benzyl amino purine (BAP) with a natural source of cytokinins i.e., leaf extract of Moringa olifera as foliar application on maize seedlings subjected to different levels of drought (75% & 50% of field capacity). On weekly basis, the plants were sprayed with BAP (50 mg L⁻¹) and moringa leaf extract (MLE) (30 times diluted with water) @ 25 mL/plant. The Plants applied with normal irrigation (100% field capacity) and water spray were taken as control. The crop was analyzed for seedling vigor, growth, water relations, physiological attributes and enzymatic antioxidants. The results showed that drought stress significantly affected the leaf area, plant height; rooting density, root fresh and dry weights, shoot fresh and dry weights, cell membrane thermostability (CMT), leaf temperature, osmotic and turgor potentials, peroxidase (POD) activity and chlorophyll a contents. Foliar applications significantly improved the leaf area, plant height, root fresh and dry weights, CMT and chlorophyll a, b contents. BAP alleviated the drought stress better than other treatments as it increased the root fresh and dry weights, CMT, chlorophyll a and b contents significantly. MLE increased leaf area, plant height, chlorophyll a and b contents under severe drought stress (i.e., 50% field capacity) and root fresh and dry weights under mild stress. Under normal and mild drought stress levels, MLE found as a best stimulus for plant growth that triggered the physiological and biochemical attributes, while under severe drought stress BAP (50 mg L⁻¹) gave the best results for mitigation of drought effects in maize.
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Key Words: Drought stress; Cytokinins; Chlorophylls; Cell membrane stability; Maize

INTRODUCTION

Maize is the third most important cereal after wheat and rice all over the world serving as staple food for many countries (Frova et al., 1999). In Pakistan, maize is grown on an area of 950,000 ha with the production of 3,487,000 tones and average grain yield of 3671 kg ha⁻¹ (Government of Pakistan, 2010).

Maize is cultivated in both spring and autumn seasons and it is best suited in existing cropping scheme. However, yield potential of maize is highly prone a biotic stresses (Drought, salinity, extreme temperatures, flooding, pollutants & poor or excessive irrigation) which are important factors towards limiting the crop productivity (Misovic, 1985; Lawlor, 2002). Among the abiotic stresses, drought is the most severe limitation to maize production (Sallah et al., 2002). To a careful estimate, only drought reasons for 50% or more reduction in average yields worldwide (Wang et al., 2003).

Water stress reduces crop yield regardless of the growth stage at which it occurs (Jensen & Mogensen, 1984). Drought causes numerous physiological and biochemical changes in plants like reduced leaf size, stem extension, root proliferation, reduced water use efficiency (Farooq et al., 2009), alteration in metabolic activities (Lawlor & Cornic, 2002), inhibition of enzymatic activities (Ashraf et al., 1995), ionic imbalance and disturbances in solute accumulation (Khan et al., 1999) or a combination of all these factors.

In maize, drought reduces leaf area, leaf chlorophyll contents, photosynthesis and ultimately lowers the grain yield (Athar & Ashraf, 2005). At flowering, drought widens the anthesis silking interval (ASI) in maize, which severely reduces the kernel set (Emeadeas et al., 2000). Under drought leaf senescence is also accelerated to decrease the canopy size (Mooney & Duplesis, 1970) severely affecting the crop yield. However delayed leaf senescence affects positively for reducing the harmful effects of drought on crop yield (Rivero et al., 2007).
There are different approaches to mitigate the drought hazards, which include the development of stress tolerant plants by selection of stress resistant varieties (Ahlloowalia et al., 2004), in vitro selection, use of plant growth hormones (ABA, GA, cytokinin, SA), antioxidants (ascorbic acid, H₂O₂) and osmoprotectants as foliar application and seed treatment (Senaratna et al., 2000; Farooq et al., 2009). Among growth regulators, Cytokinins have critical role for the promotion of cell division, chlorophyll biosynthesis and modification in apical dominance in plants (Taiz & Zeiger, 2006). Cytokinin application under abiotic stressful conditions can delay the leaf senescence directly by scavenging free radicals (Miller, 1992; Grossman & Leshem, 2006). When maize plants genetically modified with IPT gene subjected to drought stress, then IPT gene was over expressed and induced the endogenous cytokinin production, which resulted in stay green characteristic and ameliorated the adverse effects of drought (Robson et al., 2004).

Cytokinins are commercially available in the form of benzyl amino purine (BAP) or Trans-zeatin, but their use is very much costly. Moringa (Moringa oleifera L.) is a tree commonly known as “the miracle tree”. It is well known for its nutritional and medicinal properties. Leaves of moringa are rich in vitamins (A, B, C), essential minerals (K, Ca, Fe), antioxidants (Ascorbate, Phenolics), proteins and zeatin (Foidle et al., 2001). Hence, its leaf extract either prepared in 80% ethanol or in water contains growth-enhancing substances and can be used as natural source of growth promoter (Fuglie, 2000), which may reduce the adverse effects of drought stress on maize by delaying the leaf senescence and scavenging the reactive oxygen species. Plants treated with this spray exhibited more pest and disease resistance, vigorous life-span, heavier roots, stems and leaves, bigger fruits, higher sugar levels and an overall 20-35% increase in yield (Makkar & Becker, 1996).

Few reportings are available on the use of alternate naturally occurring leaf extracts for the mitigation of stressful conditions especially drought. Use of moringa as a growth enhancer was found potential source to mitigate the deleterious effects of drought. Therefore, the present study was planned to evaluate the potential of moringa leaf extract as growth enhancer and delaying the leaf senescence of maize under drought stress conditions, while exploring improved drought tolerance on physiological and biochemical basis.

**MATERIALS AND METHODS**

Experiment regarding was conducted under net house conditions in the department of Crop Physiology, University of Agriculture, Faisalabad, Pakistan during 2009-2010. Hybrid maize seed of line R-3333, obtained from Rafhan Maize Products, Faisalabad, was used as planting material. The experiment was laid out in Completely Randomized Design (CRD) with factorial arrangements using three replications. Maize seedlings were grown in the clay pots containing soil and compost (1:1). Four seeds were sown in one pot. At four leaf stage thinning was done and one plant per pot was maintained till physiological maturity. Drought stress was imposed after four weeks of sowing when seedlings attained the four leaf stage. Three drought levels i.e., 100% field capacity (FC), 75% FC and 50% FC were maintained on gravimetric basis (Nachabe, 1998).

Three foliar treatments viz Water spray (Control), MLE (1:30) Optimized dilution level by Iftikhar (2009) and Benzyl amino purine (BAP) @ 50 mg L⁻¹; application was started one week after imposition of drought stress, onward it was applied on weekly basis. For each spray 25 mL BAP or MLE was applied per plant. Moringa leaf extract was prepared by grinding the young frozen leaves of moringa. The extract obtained was diluted 30 times by adding the distilled water. For the preparation of BAP solution, BAP was first dissolved in ethanol and then distilled water was added to get the 50 mg L⁻¹ concentration.

**Cell membrane thermostability (CMT):** For determination of CMT fourth leaf from top of each plant was used an CMT was measured by the method proposed by Rehman et al. (2004).

**Plant water relations:** A fully expanded 3rd leaf of each plant from each treatment was used to determine the leaf water potential (Ψw) and osmotic potential (Ψs). Leaf water potential was measured early in the morning (6 a.m. to 6.30 a.m.) with a Scholander type pressure chamber. The remaining leaf was frozen in a freezer at -20°C for seven days and then the leaf material was thawed with the help of a disposable syringe. The sap so extracted was used to determine the osmotic potential by using an Osmometer (VAPRO-5520, Wescor). Leaf turgor pressure was calculated as the difference between osmotic potential (Ψw) and water potential (Ψs) values. For relative water contents (RWC) 0.5 g of leaf (FW) was taken and soaked in distilled water overnight to get them saturated. Then the leaf samples were weighed again to get the turgid weight (TW). The samples were then dried in an oven at 80°C for 24 h and weighed (DW). The relative water contents were measured as following:

\[
RWC = \frac{(FW-DW)}{(TW-DW)} \times 100
\]

**Biochemical analysis:** The chlorophyll a and b content (mg g⁻¹ F. wt.) were determined with the method as described by Amon (1949) by using the following formulae.

Chlorophyll a (mg/100 mL) = 0.999 A663 - 0.0989 A645

Chlorophyll b (mg/100 mL) = 0.328 A663 + 1.77 A645

The carotenoid contents of maize plants were determined with the method described by Nagata and Yamashita (1992) by using the formula:

\[
\beta-\text{Carotene (mg g}^{-1} \text{ F wt)} = 0.216 A_{436} - 1.22 A_{455} - 0.304 A_{469} + 0.452 A_{453}
\]

However, total phenolic contents were measured by using the protocol of Waterhouse (2001).
Statistical analysis: The data was analyzed statistically using Fisher’s analysis of variance technique (Steel et al., 1997) and treatment means showing F-values significant were compared using LSD value at 5% probability level using Statistix software version 9.0.

RESULTS AND DISCUSSION

Drought stress significantly affected the leaf area, plant height; rooting density, root fresh and dry weights, shoot fresh and dry weights, cell membrane thermostability (CMT), leaf temperature, osmotic and turgor potentials, peroxidase (POD) activity and chlorophyll a content at 1% level of probability (Table I). However, effect of drought foliar application and their interaction was found nonsignificant on number of leaves, stem diameter, water potential, relative water content, total soluble proteins and carotenoid content.

Leaf area: Comparison of means indicate that mild drought stress triggered the leaf area and produced maximum leaf area (Table I). Severe drought stress highly reduced the leaf area, which is due to accelerated leaf senescence caused by drought stress. As earlier reported that drought stress reduced the leaf area, radiation use efficiency and harvest index of maize significantly by degenerating the membrane structures and overproduction of ROS and accelerated leaf senescence (Nogues & Baker, 2000; Earl & Davis, 2003). MLE sprayed plants showed the maximum leaf area followed by BAP treated plants, whereas control plants produced the minimum leaf area (Table I). Cytokinins have the strongest effect in retardation of the leaf senescence either applied exogenously or produced endogenously; delay the leaf senescence by scavenging the free radicals involved in the process of senescence and increase the photosynthetically active leaf area (Miller, 1992; Galuszka et al., 2001). Since MLE is rich in zeatin, ascorbates, carotenoids, phenols, potassium and calcium (Foidle et al., 2001), it might have plant growth promoting capabilities, which delayed the leaf area by decreasing the chlorophyll degeneration. For interactive effect of drought and foliar applications maximum, the plants sprayed with MLE under normal watering produced leaf area followed by plants applied with MLE at 75% FC. The minimum leaf area was measured in plants sprayed with BAP at 50% FC. This might be due to the presence of cytokinins in the MLE, which improved the leaf area under normal and mild stress conditions. Foidle et al. (2001) reported the role of MLE as growth promoter under normal conditions; however, it might be possible that there is less amount of cytokinins in MLE, the reason the plants could not performed well under severe drought.

Plant height: Increase in drought intensity resulted in reduced plant height (Table I). This is due to the adaptation of the maize plants to cope with drought stress. With the initial effects of drought stress, the maize plants started to divert the assimilates from stem and utilized them for increased root growth in order to increase the water absorption. Hence, the plant height was affected significantly, which is in accordance with the earlier findings (Itoh & Kumara, 1986; Hamada, 2001; Liu et al., 2004). Plants treated with MLE spray exhibited maximum plant height, while BAP applied plants responded with minimum plant height. These results support our hypothesis that MLE contains growth hormones and cytokinins, which increased the plant growth and height. As reported earlier that spraying the leaves of crop plants with MLE prepared in 80% ethanol and then diluted with water produced heavier and longer stems and leaves, bigger fruits and higher sugar levels in maize, sorghum, onions, bell pepper, soya, coffee, tea, chili, melon etc. (Foidle et al., 2001). However, interactive effect of drought levels and foliar applications remained non-significant (Table I).

Root density: Drought levels significantly reduced the root density of maize plants. The plants grown at 75% field capacity show maximum root density which is statistically at par with root density of normal irrigated plants (Table I). These results show that mild drought stress triggered the plants to increase their root length, root thickness and increased number of lateral roots, which is considered as most important phenomenon for adaptation to drought conditions. While under severe drought stress the root growth was severely reduced due to reduced root penetration into dry soil and physical damage to young root tips a potential source of cytokinins. The results match with Nejad et al. (2010) and Matsuura et al. (1996). Effect of foliar application and foliar x drought interaction was found non-significant (Table I).

Root fresh and dry weight: Drought stress significantly reduced the root fresh and dry weight. Current study exhibits that highest root fresh and dry weights were observed in maize plant exposed to mild drought stress whereas, severe drought stress markedly reduced root fresh and dry weights (Table I). This increase in root fresh and dry weight of maize plants under the conditions of mild water stress is an important adaptive feature due to assimilation of photosynthates used in the stem for increasing the root growth and increase in root respiration efficiency (Fitter & Hay, 2002; Hamayun et al., 2010). However, severe drought significantly reduced the root growth by resisting the root penetration into the dry soil and reducing the root respiratory efficiency (Borrell & Hammer, 2000; Thomas & Howarth, 2000). BAP applied plants accumulated maximum root fresh and dry weight followed by MLE treated plants (Table I). This is due to the reason that young root tips are the potential site for cytokinin biosynthesis, since cytokinin is reported to be involved in the cell division, cell enlargement and elongation; hence it improved the root growth (Pospisilova et al., 2000).

Interactive effect of drought and foliar applications showed that BAP application under severe drought (50% FC) accumulated maximum root fresh and dry weight (10.71 g) followed by MLE spray (9.59 g) under mild stress.
level (75% FC), Though, least root fresh and dry weight (4.25 g) were recorded in severely stressed plants, in reducing the adverse effect of drought on the root growth by increasing the root respiration efficiency, root elongation and cell division. The results are in contrast with the results of Yousef (1998) who observed that application of seaweed extract (rich in cytokinins) to maize seedlings under 50% field capacity reduced the root growth, however under 75% of field capacity seaweed extract treatment resulted in increased root and shoot fresh or dry weights (Bilgin et al., 2008). This shows that BAP was more effective under severe drought stress conditions.

**Shoot fresh and dry weight:** Drought stress reduced the shoot fresh and dry weight significantly. Where maximum shoot fresh weight was observed at 75% FC and dry weight at 100% FC however, severe drought stress produced the minimum shoot fresh and dry weights (Table I). This reduction in the shoot fresh and dry weight with the increase in drought stress is due to the adaptation of maize plants to drought stress. As drought comes plants tend to reduce the shoot growth by reducing the leaf area, no of leaves and plant height and increase root growth (Schuppler et al., 1998). Shoot fresh and dry weights in maize and soybean plants also significantly reduced when exposed to drought

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**Table I: No of leaves (NL) leaf area (LA) stem diameter (SD) plant height (PH) root dry weight (RDW) shoot fresh weight (SFW) shoot dry weight (SDW) cell membrane thermostability (CMT)**

<table>
<thead>
<tr>
<th>Drought</th>
<th>NL</th>
<th>LA</th>
<th>SD</th>
<th>PH</th>
<th>RD</th>
<th>RFW</th>
<th>RDW</th>
<th>SFW</th>
<th>SDW</th>
<th>CMT</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% FC</td>
<td>15.22</td>
<td>491.13b</td>
<td>1.16</td>
<td>138.39a</td>
<td>232.11ab</td>
<td>51.53b</td>
<td>7.3644b</td>
<td>185.41a</td>
<td>49.87a</td>
<td>13.32a</td>
</tr>
<tr>
<td>75% FC</td>
<td>14.78</td>
<td>524.90a</td>
<td>1.18</td>
<td>115.44b</td>
<td>244.78a</td>
<td>63.20a</td>
<td>9.1744a</td>
<td>187.28a</td>
<td>47.14a</td>
<td>11.25ab</td>
</tr>
<tr>
<td>50% FC</td>
<td>14.56</td>
<td>584.84c</td>
<td>1.13</td>
<td>97.44c</td>
<td>2004.67b</td>
<td>48.78b</td>
<td>7.0166b</td>
<td>131.27b</td>
<td>30.01b</td>
<td>9.46b</td>
</tr>
</tbody>
</table>

| LSD (D) | 19.37 | 7.49 | 345.52 | 5.84 | 1.3067 | 32.73 | 6.41 | 2.763 |

**Treatments**

| Control | 14.11 | 458.34c | 1.15 | 119.44a | 2082.22 | 48.75b | 6.7644b | 164.62 | 44.12 | 13.88a |
| MLE     | 15.22 | 526.94a | 1.18 | 122.28a | 2230.56 | 52.94b | 9.1744a | 187.28a | 43.8  | 8.15b  |
| BAP     | 15.22 | 489.59b | 1.13 | 109.56b | 2373.78 | 61.82a | 9.28a  | 159.48 | 39.1  | 12.00a |

| LSD (T) | 19.37 | 7.49 | 345.52 | 5.84 | 1.3067 | 32.73 | 6.41 | 2.763 |

**Interaction**

| D1xT1  | 14  | 435.69fg | 1.179 | 142.67 | 2274.67 | 54.88bc | 7.38bcd | 194.266ab | 54.98a | 17.48a |
| D1xT2  | 15.33 | 558.95a | 1.192 | 150.83 | 2367.33 | 61.16ab | 9.1744a | 179.82abc | 51.02abc | 17.92a |
| D1xT3  | 16.33 | 478.74de | 1.161 | 2338.33 | 55.76bc | 7.8666c | 19.25ab | 46.86bc | 9.31bc |

| LSD (DxT) | 0.746 | Ns  | 1.51  | Ns   | Ns   | Ns   | 0.00255 | Ns  | 0.221 | Ns  |

**Table II: Leaf temperature (LT) water potential (WP) osmotic potential (OP) turgor potential (TP) relative water contents (RWC) total soluble proteins (TSP) total phenolic contents (TPC) carotenoids (CAR)**

<table>
<thead>
<tr>
<th>Drought</th>
<th>LT</th>
<th>WP</th>
<th>OP</th>
<th>TP</th>
<th>RWC</th>
<th>TSP</th>
<th>TPC</th>
<th>CAR</th>
<th>Chl a</th>
<th>Chl b</th>
</tr>
</thead>
<tbody>
<tr>
<td>100% FC</td>
<td>37.05</td>
<td>-0.52</td>
<td>-3.91a</td>
<td>3.38b</td>
<td>77.95</td>
<td>0.396</td>
<td>1.7991a</td>
<td>0.4056</td>
<td>2.15b</td>
<td>0.9611</td>
</tr>
<tr>
<td>75% FC</td>
<td>37.65</td>
<td>-0.55</td>
<td>-6.62b</td>
<td>6.07a</td>
<td>77.69</td>
<td>0.184</td>
<td>1.7972a</td>
<td>0.11</td>
<td>2.04b</td>
<td>1.0766</td>
</tr>
<tr>
<td>50% FC</td>
<td>38.83</td>
<td>-0.38</td>
<td>-4.49a</td>
<td>3.99b</td>
<td>81.46</td>
<td>0.186</td>
<td>1.7931b</td>
<td>-0.0044</td>
<td>2.77a</td>
<td>1.59</td>
</tr>
</tbody>
</table>

| LSD (D) | 0.746 | Ns  | 1.51  | Ns   | Ns   | Ns   | 0.00255 | Ns  | 0.221 | Ns  |

**Treatments**

| Control | 38.02 | -0.38 | -4.42 | 3.91 | 78.57 | 0.174 | 1.7968 | 0.2867 | 2.44a | 1.0766 |
| MLE     | 37.85 | -0.54 | -4.71 | 4.16 | 78.79 | 0.187 | 1.7972 | 0.223  | 1.87b | 1.0566 |
| BAP     | 37.66 | -0.52 | -5.89 | 5.37 | 79.74 | 0.357 | 1.7954 | 0.0011 | 2.65a | 1.6622 |

| LSD (T) | 0.746 | Ns  | Ns   | Ns   | Ns   | Ns   | 0.00255 | Ns  | 0.221 | Ns  |

**Interaction**

| D1xT1  | 37.73 | -0.49 | -3.82 | 3.33 | 78.85 | 0.161 | 1.798 | 0.3166 | 2.33bc | 0.9467bc |
| D1xT2  | 36.9 | -0.54 | -3.73 | 3.18 | 78.70 | 0.181 | 1.7933 | 0.4533 | 1.82d | 0.9433bc |
| D1xT3  | 36.53 | -0.54 | -4.17 | 3.63 | 76.29 | 0.706 | 1.798 | 0.4466 | 2.32bc | 0.9933bc |
| D2xT1  | 37.4 | -0.51 | -4.66 | 4.14 | 74.28 | 0.169 | 1.7976 | 0.4646 | 2.18cd | 0.8233bc |
| D2xT2  | 37.43 | -0.57 | -5.96 | 5.38 | 77.57 | 0.90 | 1.798 | 0.3833 | 1.13e | 0.4733c |
| D2xT3  | 38.13 | -0.56 | -9.25 | 8.69 | 81.25 | 0.191 | 1.796 | -0.5 | 2.81a | 2.42a |
| D3xT1  | 38.93 | -0.52 | -4.78 | 4.26 | 82.59 | 0.192 | 1.7986 | 0.0966 | 2.81a | 1.46b  |
| D3xT2  | 39.23 | -0.51 | -4.44 | 3.93 | 80.10 | 0.191 | 1.7983 | -0.166 | 2.66ab | 1.75ab |
| D3xT3  | 38.33 | -0.46 | -4.25 | 3.79 | 81.69 | 0.175 | 1.7983 | 0.0566 | 2.84a | 1.57ab |

| LSD (DxT) | 0.746 | Ns  | Ns   | Ns   | Ns   | Ns   | 0.00255 | Ns  | 0.221 | Ns  |

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Chlorophyll a (Chl a) chlorophyll b (Chl b)
due to reduced shoot growth, increased senescence and switching over of the plant growth from shoot growth towards root growth (Sharp et al., 1988; Hamayun et al., 2010). However, interaction of foliar application and drought non-significantly affected the shoot fresh and dry weights of maize plants.

**Cell membrane thermostability:** A decreasing trend in CMT was observed in maize plants in response to increasing drought levels (Table I). This is due to damage to membrane structure caused by drought stress, which resulted in solutes leakage from cell hence CMT was reduced. *Brassica napus* L. exposed to drought stress resulted in 21% reduction in CMT at flowering stage under stress as compared to 83% under well watered conditions due to rise in leaf temperature from 1 to 2°C and damage to the cell membrane structure by the drought stress (Hashem et al., 2008). BAP application and water spray maintained the higher percentage of CMT under our experimental conditions (Table I). The water spray might had reduced the leaf temperature and hence reduced the coupled effect of drought stress and high temperature on cellular membranes and hence maintained higher CMT percentage. Cytokinins (BAP) are well recognized for enhancing the antioxidant activities, which scavenge the ROS production and ultimately reduce the membrane damage and improved the CMT (Sayed et al., 2010). For interactive means, Control at 75% FC resulted in maximum CMT value, which is statistically at par with BAP and control treatments at 100% FC. Least membrane thermostability was recorded in plants applied water at 50% FC (Table I). Application of fortified seaweed extract, a rich source of natural cytokinins on perennial ryegrass (*Lolium perenne* L.) enhanced the CMT by changing the cell membrane composition both under drought and normal conditions, which could be assumed from the higher leaf water potential in plants applied with seaweed extract (Yan et al., 1997).

**Leaf temperature:** Extreme drought resulted in highest recording of leaf temperature, whereas control and mild drought conditions were statistically at par (Table II). Under water limited conditions this increase in leaf temperature might be due to the reduced transpiration and lesser cooling effect due to non availability of water. Maize and other plants subjected to severe drought stress gave the highest leaf and canopy temperatures due to inhibited transpiration and increase in the boundary layer resistance to transpiration (Siddique et al., 2000; Hirayama et al., 2006). However, foliar applications and interaction of foliar applied cytokinins and drought levels expressed non-significant effect on leaf temperature of maize crop (Table II).

**Osmotic and turgor potential:** The plants at mild drought stress level maintained least osmotic potential and highest turgor potential. Under severe drought stress less negative osmotic potential than mild stressed plants was found, whereas lesser turgor potential than mild stressed plant leaves (Table II). These results indicate that the maize plants have done osmotic adjustment and maintained a higher turgor potential in response to the mild drought stress. Osmotic adjustment is an adaptive feature of plants facing the abiotic stresses, which helps the plants to maintain their turgor potential in case of water shortage. However, under severe drought stress plants failed to maintain the turgor (Table II), this might be due to the excessive water loss through transpiration required to reduce the leaf temperature. Similar results were found when triticale varieties were exposed to drought stress plants exhibited the more negative osmotic potential and maintained the turgor potential, indicating better osmotic adjustment (Hura et al., 2007). In another study, leaf relative water content (RWC), water potential (Ψw), osmotic potential (Ψp), turgor potential (P) and osmotic adjustment (OA) were significantly decreased under severe drought stress due to the excessive water loss (Machado & Paulsen, 2001).

**Total phenolic contents:** Total phenolic contents of maize plants significantly decreased with the increase in drought levels. Whereas, foliar spray and drought x foliar application had no significant effect on the total phenolic contents of maize plants (Table II). Maximum phenolic contents (1.799) were explored in plants applied with normal irrigation (100% FC) with statistically at par observation in plants (1.799) applied with 75% FC, while least phenolic contents (1.79311) were estimated in plants exposed to the severe drought stress i.e., 50% FC (Table II). Total phenolic contents were decreased with the increasing drought stress (Table II). This decrease in phenolic contents is due to the degradation of photosynthetic pigments under drought stress. Results are supported by a study in which when maize plants were exposed to drought stress, the level of phenolic compound i.e., ferulic acid was decreased significantly due to the break down of the chlorophylls and phenolic pigments with the increase in drought intensity (Hura et al., 2008). Drought conditions reduced phenolic contents in cotton leaves at boll formation due to increased leaf senescence and degeneration of photosynthetic pigments (Ahmed et al., 2008). Foliar application had no significant impact on total phenolic contents.

**Chlorophyll a:** Maximum chlorophyll *a* (2.77 mg g⁻¹ F. wt) content was found in plants exposed to 50% FC followed by plants applied with 100% FC (2.15 mg g⁻¹ F. wt), which is at par with plants with mild stress of 75% FC (2.04 mg g⁻¹ F. wt). Drought causes the chlorophyll breakdown and accelerates the leaf senescence. Studies show that when maize plants were subjected to PEG induced drought stress their chlorophyll *a, b* and total chlorophyll contents were significantly decreased due to leaf senescence acceleration (Mohammadkhan & Heidari, 2007; Efeoglu et al., 2009). However, according to our results maximum chlorophyll *a* contents were measured in the plants given the 50% FC, this increase may be due to the effect of the foliar applications. Among foliar applications, BAP application produced the maximum chlorophyll contents (Table II). The role of BAP in reducing the chlorophyll breakdown, increasing the cell division, cell elongation, increasing the chlorophyll
biosynthesis and delaying the leaf senescence is also reported (Synkova et al., 1997).

Maximum chlorophyll a contents were observed in plants applied with BAP at 50% FC. Similarly, increased chlorophyll contents were observed when plants were applied with BAP in 5 μM concentration under drought stress due to increased cell division, cell elongations and increased stay green foliage (Pandey et al., 2003). Application of benzyl adenine (BA), a cytokinin at the concentration of 5 mg L⁻¹ to ungrazed tallgrass prairie increased the higher mean chlorophyll contents (Towne & Owensby, 1983). Cytokinin applied creeping bentgrass showed the increased leaf chlorophyll content under heat stress because of reduced degeneration of chlorophyll and production of new cells (Liu & Huang, 2002). Least chlorophyll a content was recorded in plants applied with MLE at mild stress of 75% FC (Table II).

Chlorophyll b: For chlorophyll b content, non-significant effect of drought and foliar application was explored while; their interactive effect was found significant (Table II). For interactive effects of drought and foliar applications, BAP at 75% FC (2.42 mg g⁻¹ F. wt) induced highest chlorophyll b content followed by MLE treatment at severe drought of 50% FC (1.753 mg g⁻¹ F. wt), which is statistically at par with BAP at same drought level (1.57 mg g⁻¹ F. wt). MLE at 75% FC i.e., mild stress is the combination resulting in minimum chlorophyll b content (0.47 mg g⁻¹ F. wt) as shown in the Table II.

CONCLUSION

Moringa leaf extract (30 times water diluted) proved the best stimulus for plant growth and triggered physiological and biochemical attributes under normal and mild stressful conditions, while under severe drought stress BAP (50 mg L⁻¹) gives the best results for mitigation of drought effects in maize. Findings of this study are however, in need of reconfirmation in order to identify dose and critical stage of maize crop for commercial use.

REFERENCES


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