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ROLE OF FOLIAR APPLICATION OF SALICYLIC ACID AND L-TRYPTOPHAN IN DROUGHT TOLERANCE OF MAIZE

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ABSTRACT

Yield of maize is adversely affected by drought. Exogenous application of different chemicals may reduce stress induced inhibition of plant growth. A pot experiment was conducted to determine drought mitigating effect of Salicylic acid and L-Tryptophan. Maize seeds were sown in pots filled with soil. Salicylic acid and L-Tryptophan were sprayed at 3-4 leaves stage @ 100, 150, 200 ppm and 5, 10, 15 ppm, respectively. Drought stress was induced by with holding water after five days of Salicylic acid and L-Tryptophan application. Significantly higher relative water content, leaf membrane stability index, chlorophyll and potassium content were found in plants treated with 100 ppm Salicylic acid and 15 ppm L-Tryptophan compared with other treatments and control plants. Results suggest that foliar application of Salicylic acid and L-Tryptophan can play a role to reduce the effect of drought in maize.

Keywords: Drought, Salicylic Acid, L-Tryptophan, Foliar Application, Maize.

INTRODUCTION

Maize (*Zea mays* L.), the third most important cereal crop after wheat and rice, is the major kharif (summer) crop of rain-fed areas in Pakistan. More than 35% of the crop is planted under rainfed conditions with high probability of drought during its growth period. Yield of maize crop is sensitive to abiotic stresses and may cause more than 50% yield reduction (Cakmak, 2005). Drought, the major abiotic stress, affects every aspect of plant growth and is mainly responsible for limiting crop production (Golbashy *et al*., 2010).

Plants tend to adapt to drought by accumulation of cyto-compatible organic osmolytes (Rhodes and Hanson, 1993) such as polyols, proline and betaines. Seed treatment or foliar application of chemicals like glycinebetaine, kinetin, salicylic acid (Gunes *et al*., 2007; Karlidag *et al*., 2009) may increase yield of different crops due to reduction in stress induced inhibition of plant growth (Elwana and El-Hamahmyb, 2009), enhanced photosynthetic rates, leaf area and plant dry matter production (Khan *et al*., 2003).

Salicylic acid, a naturally occurring plant hormone acting as an important signaling molecule adds to tolerance against abiotic stresses. It plays a vital role in plant growth, ion uptake and transport. Salicylic acid is also involved in endogenous signaling to trigger plant defense against pathogens (Khan *et al*., 2003). Salicylic acid can also play a significant role in plant water relations (Barkosky and Einhelling, 1993), photosynthesis, growth and stomatal regulation (Khan *et al*., 2003; Arfan *et al*., 2007) under abiotic stress conditions. L-Tryptophan is known to be a physiological precursor of auxins in higher plants. It is investigated that L-Tryptophan has more positive effect on plant growth and yield as compared to pure auxins (Zahir *et al*., 1999). L-Tryptophan is an amazing amino acid. It may act as an osmolyte, ion transport regulator, modulates stomatal opening and detoxify harmful effects of heavy metals (Rai, 2002). Several studies have been conducted to evaluate the influence of exogenous application of salicylic acid and L-Tryptophan on plant growth, development and stress tolerance. Nonetheless, less attention has been paid to determine the role of L-Tryptophan and salicylic acid in drought tolerance. Application of salicylic acid and L-Tryptophan may increase stress tolerance of plants by positively altering physiological phenomena in plants. Therefore, the present study was undertaken to investigate the protective role of salicylic acid and L-Tryptophan against drought stress in maize.

Abbreviations: SA (salicylic acid), TRP (Tryptophan), MSI (membrane stability index), RWC (relative water content).

MATERIALS AND METHODS

The study was carried out in the Crop Physiology Laboratory, Department of Agronomy, Pir Mehr Ali Shah Arid Agriculture University Rawalpindi, Pakistan during the year 2010. Seeds of maize cultivar “Islamabad Gold” were sterilized with hydrogen peroxide (H_2O_2) and sown in pots containing well mixed soil. Germinated plantlets were irrigated regularly till seedling establishment. Treatments consisted of different levels of

Salicylic acid (SA) @ 100, 150, 200 ppm and L-Tryptophan (L-TRP) @ 5, 10 and 15 ppm, sprayed at 3-4 leaves stage along with one check i.e. without foliar application of SA and L-TRP. There are four pots per replication and five plants per pot. The experiment was conducted using completely randomized design (CRD) with three repeats. Drought stress was induced by withholding irrigation after 5 days of chemical application. After five days data for following parameters were recorded of stress period and analyzed statistically.

Relative Water Content (%): Relative water content of leaf was determined by method developed by Barrs and Weatherley (1962). Second leaf of randomly selected four plants was used for determining relative water content. Fresh weight (FW) immediately recorded, and then leaves were soaked for 4 hours in distilled water at room temperature under a constant light and saturated humidity. Turgid weight (TW) was recorded followed by drying for 24 hours at 80 ºC for dry weights (DW). Relative water content (RWC) was calculated according to the following formula:

$$RWC = [(FW - DW) / (TW - DW)] \times 100$$

Leaf Membrane Stability Index: The leaf strips (0.2 g) of uniform size were taken in two sets of test tubes containing 10 ml of distilled water. Test tubes in one set were kept at 40 ºC in a water bath for 30 min and electrical conductivity of the water containing the samples were measured (C1). Test tubes in the second set were incubated at 100 ºC in the boiling water bath for 15 min and electrical conductivity (C2) was measured. MSI was calculated by following formula:

$$MSI = [1-(C1/C2)] \times 100$$

C1 = Electrical conductivity of water containing the sample in test tube of set 1.

C2 = Electrical conductivity of water containing the sample in test tube of set 2.

Chlorophyll Content (µM): Five leaves from each experimental unit were selected randomly and detached from the plant and frozen. Two gram frozen leaves from each sample were put into a mortar and finely ground with pestle in 80 % acetone and kept in dark for few hours to allow the leaf tissues to be thoroughly homogenized. Samples were then centrifuged for 10 min @ 6000 rpm. Absorbance of supernatant was determined at 647 nm for chlorophyll 'a' and at 664 nm for chlorophyll 'b' by UV spectrophotometer. Absorbance values were used in following expression to quantify chlorophyll as reported by Coombs *et al*., (1987).

Chlorophyll "a"(µM) = 13.19 A664 - 2.57 A647

Chlorophyll "b"(µM) = 22.10 A647 - 5.26 A664

Total Chlorophyll Content (µM) = 7.93 A664 + 19.53 A647

Potassium Contents (ppm): Potassium was determined following the procedure described by Allen *et al*., (1986). Dried leaf sample (0.1 g) was taken and digested in 2 ml of sulphuric acid digestion mixture until a clear and colorless solution was obtained. Volume of sample was made 25 ml with distilled water. Potassium was determined with flame photometer. Potassium standards i.e. 5, 10, 15, 20 and 25 ppm were made from KCl. Then a potassium standard curve was plotted from standard solutions and potassium contents of leaf samples were standardized with the help of standard curve.

Statistical Analysis: Analysis of variance was performed and means were compared by employing Duncan Multiple Range Test (DMRT) at 5 % level of probability. The statistical work was done using the computer based statistical package MSTATC following Steel *et al*., (1997).

RESULTS AND DISCUSSION

Relative Water Content: Data pertaining to relative water content (RWC) illustrate that all the treatments differ significantly from each other at 5 % probability level (Table 1). Application of 100 ppm salicylic acid (SA) and 15 ppm L-Tryptophan (L-TRP) maintained highest RWC i.e. 79.37 % and 70.11 %, respectively followed by 150 ppm SA and 10 ppm L-TRP. RWC dropped to 52.27 % in check plants. It becomes evident that foliar treatment of organic chemicals had significant effect on RWC under osmotic stress conditions. Plant and cell water balance is determined by the difference of water absorbed from the soil and transpirational water loss to the atmosphere. RWC tend to decline when transpiration exceeds water absorption under drought condition (Tas and Tas, 2007) leading to decrease in cell turgor. Maintenance of high RWC under drought due to relatively more growth of the roots than shoots and/or abscisic acid induced reduction in stomatal opening (Makoto *et al*., 1990) tends to maintain cell turgidity, chlorophyll content (Keyvan, 2010) and photosynthesis. Foliar application of SA (100 ppm) and L-TRP (15 ppm) may regulate stomatal openings and reduce transpirational water loss under drought conditions enabling the plants to maintain turgor, carry on photosynthesis and be productive under water deficit conditions. He *et al*., (2005) and Sakhabutdinova *et al*., (2003) postulated that salicylic acid increased the production of photosynthetic apparatus that produced more photosynthates. This enhanced photosynthetic activity increased sap production in the leaf lamella which resulted in maintenance of relative water content in leaf and better growth. L-TRP it is an endogenous hormone which stimulates plant growth and is associated with increased amount of water content in the cell (Hala, 2005). Similar findings have also been reported by other investigators (Yildirim *et al*., 2008; Frankenberger *et al*., 1990).

Leaf Membrane Stability Index: Significant differences were found among various levels of SA and L-TRP for their effect on leaf membrane stability index (MSI) of maize (Table 1). Significantly higher MSI (87.06) was observed with foliar application SA-100ppm followed by L-TRP-15ppm (52.55) than other treatments which yielded MSI lower than those of control plants. Drought significantly affects integrity of cell membranes manifested by lower values of MSI (Tas and Tas, 2007) and is correlated with growth responses of wheat seedlings (Bajji *et al*., 2001) and water use efficiency (Franca *et al*., 2000). Membrane stability, a major component of drought tolerance (Sairam *et al*., 2001), is a reliable trait to evaluate drought tolerance of plants under osmotic stress. Lower amount of ion leakage (high MSI) indicates the stability of leaf membrane (Jaleel *et al*., 2007). Application of SA increases the accumulation of Ca^{+2} which can maintain membrane integrity (Khan *et al*., 2010). SA treatment ameliorates the impact of abiotic stress through improving antioxidant system necessary to reduce oxidative damage and ion leakage from membranes (Yusuf *et al*., 2008). Our findings suggest that application of SA and L-TRP may have favorable effect on growth and development of crop under stress conditions.

Chlorophyll Content: Different concentration of SA and L-TRP differed significantly for their effect on chlorophyll content of maize seedlings (Table 1). Foliar application 100 ppm of SA followed by 15 ppm L-TRP significantly increased chlorophyll contents (63.62 µM and 51.25 µM, respectively) over control (26.98 µM) under stress conditions. Similar results were reported by Arfan *et al*., (2007) and Yildirim *et al*., (2008). Limited water supply usually causes a reduction in chlorophyll content (Paknejad *et al*., 2007). Being positively correlated with yield **(**Zaharieva *et al*., 2001) relatively high chlorophyll content may contribute to the plant productivity under stress conditions. Therefore, foliar application of SA (100 ppm) may contribute to drought tolerance and sustain productivity. Foliar application of SA is also involved in stomatal regulation thereby controlling photosynthetic rate (Khan *et al*., 2003). However, the beneficial effect of SA application depends on genotype (Bezrukova *et al*., 2004). Exogenous application of L-TRP at higher concentration was found effective for abiotic stress tolerance by increasing photosynthetic pigments in *Catharanthus roseus* (Talat *et al*., 2005). Our findings are also supported by work of El-Bassiouny (2005).

Potassium Content of Leaf: Statistical analysis of data for potassium content revealed that all the treatments differ significantly from each other at 5 % level of probability (Table 1). Lowest level of leaf K (9.47 ppm) was observed in control plants when subjected to water stress. However the plants treated with different levels of SA and L-TRP accumulated significantly higher K compared with that of control plants. Highest potassium contents (19.70 ppm) observed in leaves of the plants treated with SA 100 ppm followed by SA 150 ppm. Potassium is key osmoregulator which plays a major role in maintaining the cellular water balance under osmotic stress conditions and reduced supply of potassium causes a decrease in leaf water potential. It is the primary element in osmotic adjustment. Accumulation of ABA associated with decreased potassium up take of plants (Szepesi *et al*., 2008) may reduce stomatal conductance and activity of ribulose bisphosphate carboxylase (Zhao *et al*., 2001) leading to decrease in photosynthesis of K-deficient plants under drought. Application of 100 ppm SA to the plants subjected to drought may help maintain higher leaf K and avoiding drought induced damage to photosynthesis. These results are in line with many other researchers who reported an increased amount of almost all elements in roots and shoot with application of SA and L-TRP (Karlidag *et al*., 2009; Zahir *et al*., 2000).

Table 1: Effect of foliar application of Salicylic Acid and L-Tryptophan on relative water content, membrane stability index, chlorophyll content and potassium content of Maize crop.

Treatments	Relative Water Content (%)	Membrane Stability Index (%)	Chlorophyll Content (µM)	Potassium Content (%)
Control	52.27 c	41.75 c	26.98 c	9.47 g
Salicylic Acid 100 ppm	79.37 a	87.06 a	63.62 a	19.70 a
Salicylic Acid 150 ppm	64.07 b	33.75 d	20.39 d	16.53 b
Salicylic Acid 200 ppm	32.13 e	4.26 g	21.23 d	13.60 d
L-Tryptophan 5 ppm	41.59 d	12.90 f	20.39 d	10.73 f
L-Tryptophan 10 ppm	51.87 c	21.06 e	29.68 c	12.53 e
L-Tryptophan 15 ppm	70.11 b	52.55 b	51.25 b	14.83 c

Means sharing a letter in a column did not differ significantly $P < 0.05$

Conclusion: SA and L-TRP seem important protectants against drought stress in maize. Foliar application of these chemicals is an effective way to augment adverse effects of drought in plants. However, further experimentation is required for comprehensive conclusion for practical purpose.

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