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Role of Melatonin in Morpho-Physiological, Ionic and Biochemical Acclimation of Maize (*Zea mays* L.) Challenged with Salt Stress

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Abstract: Melatonin (N-acetyl-5methoxytryptamine) is a crucial biological hormone associated with many physiological and biochemical processes in plants and also enhanced resistance against various abiotic stresses. However the mechanism underlying the role of melatonin in mitigating salt stress in maize is still poorly understood. Present study was determined to understand the protective role of melatoninin relation to growth, physiological, ionic and antioxidant attributes in two maize hybrids under saline condition. The growth medium was salinized with three salinity levels (Control, 6dS m^{-1}NaCland 12 dS m^{-1}NaCl) and two levels of melatonin (0.5 μM L^{-1} and 1.0 μM L^{-1}) which was applied exogenously in combined and sole form.Salt stress significantly reduces plant dry matter accumulation, chlorophyll contents, RWC, MSI, K^+/Na^+ ratio and activities of antioxidant enzymes (POD, SOD, APX, CAT and GR) and these effects are more prominent at high salt level (120 dS cm^{-1}). Addition of melatonin alleviated salt toxicity is more conspicuous in maize hybrid 2225 as compared to 8711 as demonstrated by increasing RWC, MSI, K/Na ratio and activities of antioxidant enzymes. Our results suggested that melatonin induced salt tolerance in maize is due to enhancement of plant water relations, civilizing photosynthetic and anti-oxidative capacity along with ion homeostasis in maize. Moreover, there is still much that remains unknown and need to be elucidated in the future.

Key words: Melatonin・Maize・RWC・MSI・K/Na ratio・Antioxidant enzymes

INTRODUCTION

Soil salinity is one of the most threaten abiotic aspect to agricultural yield across the world, particular in arid to semi-arid regions [1]. Salinization is escalating rapidly and occupies 7% of world's land and will results in 50% loss of arable land up to 2050 [2]. High salt concentration in the growth medium imposes strong deleterious impacts on plant biomass [3], physiology [4] accumulation of mineral ions [5], destroy PSII reactions [6] and biochemical damage due to production of reactive oxygen species (ROS)which eventually leads to poor growth [7]. To improve salt tolerance, much efforts has been made to mitigate the hazardous effects of salt stress by various exogenous substances like salicylic acid [8], Polyamine [9], nitric oxide [10] and glycinebetaine [11].

Maize (*Zea mays* L.) is cultivated on more than 142 million ha providing raw material to industries, bio fuel and accounts for one third grain production globally [12] but normally submissive to salt stress. Due to its cross pollinated nature, maize is polymorphic crop in which salt resistance exists [13]. Increased salinized area with threaten climate change effects sustainable agriculture and arrest crop yield of a number of crops including maize [14]. Therefore studies on salt tolerance in maize aroused incisive interest.

Melatonin (N-acetyl-5methoxytryptamine) is an amphiphilic biological hormonefound throughout the plant kingdom. Melatoninplay a vital part in plant growth and development by regulating plant physiology and root regeneration [15], antioxidant activity [16], photosynthesis [17], senescence of leaves [18] and immunological enhancement [19]. Antioxidant capacity of melatonin fortify a variety of plantspecies from various abiotic stresses such as salinity [20], drought [21], chemical and disease resistance [22], low temperature [10] and heat stress [23] byaltering the expression of salt tolerance genes, up regulating antioxidant enzymes and

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direct scavenging of reactive oxygen species (ROS). Melatonin enhanced resistance against salt stress in various crops, including barley [24], rice [25], cucumber [26], apple [27] and soybean [28].

The present study was planned with the objective to explore the ameliorating role of melatonin on plant growth and physiological attributes, ions homeostatsis and activities of antioxidant enzymes in maize challenged with salt stress.We sought to clarify the involvement of exogenously applied melatonin in improving salt resistance and to use this information to get maximum production of maize from salt affected soils.

MATERIALS AND METHODS

Seed Material and Growth Conditions: A pot experiment was conducted in rain protected wire house of The Islamia University of Bahawalpur. Seeds of two maize hybrids 2225 identified as salt tolerant and 8711 as salt sensitive (from previous study) were sown in glazed earthen pots at the depth of 2 cm having well pulverized soil (12 kg/pot). Nitrogen, phosphorus and potassium were applied @ recommended dose of 150-100-50 kg ha^{-1} in the form of urea, DAP and SOP. Full dose of phosphorus and potassium with half dose of nitrogen was applied at the time of sowing while the remaining nitrogen was applied ten days after germination of seedlings. Before filling the pots, the required salt levels (6 dS m^{-1} and 12 dS m^{-1}) were developed by mixing calculated amount of NaCl. The experiment consists of the following treatments: control (T_1), 6 dS m^{-1}NaCl (T_2), 12 dS m^{-1}NaCl (T_3), 0.5 µM L^{-1} melatonin (T_4), 1.0 µM L^{-1} melatonin (T_5), 6 dS m^{-1}NaCl + 0.5 µM L^{-1}melatonin (T_6) and 12 dS m^{-1}NaCl + 1.0 µM L^{-1}melatonin (T_7). Melatonin was applied at seedling stage exogenously @ 0.5 and 1.0 µM L^{-1} in sole and combine form. Each treatment was replicated five times with complete randomized arrangement (CRD). Pots were irrigated with water having EC 0.68 dS m^{-1} when required.

Plant Harvesting and Analysis: Plants were harvested after 3 weeks of melatonin application. The roots and shoots were separated and growth attributes i.eroot and shoot length, fresh and dry weight with other physiological, photosynthetic, ionic and biochemical attributes by adopting standard protocols.

Physiological Attributes

Relative Water Contents: Fully expended youngest leaves of plant from each treatment were selected and relative water contents were measured by adopting the method of Lazcano-Ferrat and Lovatt [29] after taking fresh, dry and turgid weight.

RWC% = (Fresh weight- Dry weight) / (Turgid weight- dry weight) x 100

Membrane Stability Index: MSI was calculated by following the procedure demonstrated by Sairam and Saxena, [30]. Leaf disk (100 mg) was heated at 40°C in 10 ml water for thirty minutes in water bath and EC (C_1) was measured through EC meter. Then same sample was again heated at 100 °C for ten minutes and EC was observed as C_2. The value of MSI was measure by using the following formula:

$$MSI \% = [1-(C_1/ C_2)] \times 100$$

Chlorophyll Contents and Leaf Area: To determine the chlorophyll contents, leaf material (0.05 g) was placed in 10 ml dimethylsulfoxide (DMSO) and heated at 65 °C for four hours. The absorbance of extract were recorded at 660 nm and 665 nm for chlorophyll a and b [31] while carotenoid contents were estimated at 470 nm (Wellburn, 1994) by using spectrophotometer. Leaf area was calculated by using leaf area meter (Delta-MK-2).

Biochemical Attributes: Fresh leaf segments 0.5 gwas homogenized in 10 ml of an extracting buffer (50 mM phosphate, pH 7.8, 1 mM EDTA, 1 g polyvinylpyrrolidone (PVP) and 0.5 % Triton X-100). The prepared homogenate was centrifuge at 12, 000 rpm for 20 minutes and the supernatant used for the analysis of Superoxide dismutase (SOD), Peroxidase (POD), Ascorbate peroxidase (APX), Catalase (CAT) and Glutathione reductase (GR). All operations were carried out at 0-4°C [32].

Ionic Attributes: Sodium (Na^+) and potassium (K^+) concentration were determined by taking driedand ground shoot material (0.01 g). The samples were digested with hydrogen peroxide and sulphuric acid and heated at 350°C until fumes were produced and material was colorless according to the method explained by Wolf [33] by using Flame Photometer (Jenway- PFP-7- London, United Kingdom).

Statistical Analysis: The statistical analysis of data was carried out with statistical package Statistics 8.1 (Statistics. IL.USA).The bars in the graph show the mean values of 5 replicates and error bars are the standard deviations.

RESULTS

Plant Growth and Dry Biomass: The analyzed data under saline and non saline conditions regarding growth of maize hybrids (Figure 1) reveals that salt stress implemented strong negative impacts on root and shoot length, fresh and dry biomassof both maize hybrids used in the current project. However, reduction was maximum under high salt stress (12 dS m^{-1}) as compared to low salinity in the growth medium.Impact of salinization differ remarkably among both maize hybrids which were conclude with less reduction in growth of maize hybrid 2225 as compared to 8711. Exogenous application of melatonin marked change in growth pattern of both maize hybrids under salt stress and more mitigated role was observed under melatonin treatment @ 1.0 µM. Moreover, maize hybrid 2225 in term of growth attributes responses better to melatonin application as compared to 8711.

Physiological Attributes: Exalted salt applicationproved hazardous in terms of membrane stability index (MSI), relative water contents (RWC), leaf area and chlorophyll contents (Figure 2). Maximum values for MSI and RWC (88 and 95 %) were recorded under sole application of melatonin (1.0 µM). Melatonin feeding partially recovered membrane stability and plant water relations and this recovery was more prominent when high dose of melatonin was applied with elevated salt stress (12 dS m^{-1} + 1.0 µM melatonin).Both hybrids also exhibit significant variation in chlorophyll concentration and maximum reduction (50%) was noted under high salt stress. Maize hybrid 8711 present less MSI, RWC, leaf area and chlorophyll contents as compared to maize hybrid 2225 which reveals its more sensitivity towards NaCl stress treatment.

Ionic Attributes: Melatonin and salt stress application on ion homeostasis of two maize (*Zea maize* L.) hybrids are presented in Figure 3.Compared to control, increased salt concentration in the growth medium cause notable increase in Na^+ concentration while a pronounced decrease in K^+ level in shootsof examined maize hybrids. Highest Na^+ concentration was recorded in maize hybrid 8711 while least was marked in maize hybrid 2225. The tendency was opposite in case of K^+uptake by displaying least K^+ concentration at maximum salt level (12 dS m^{-1})

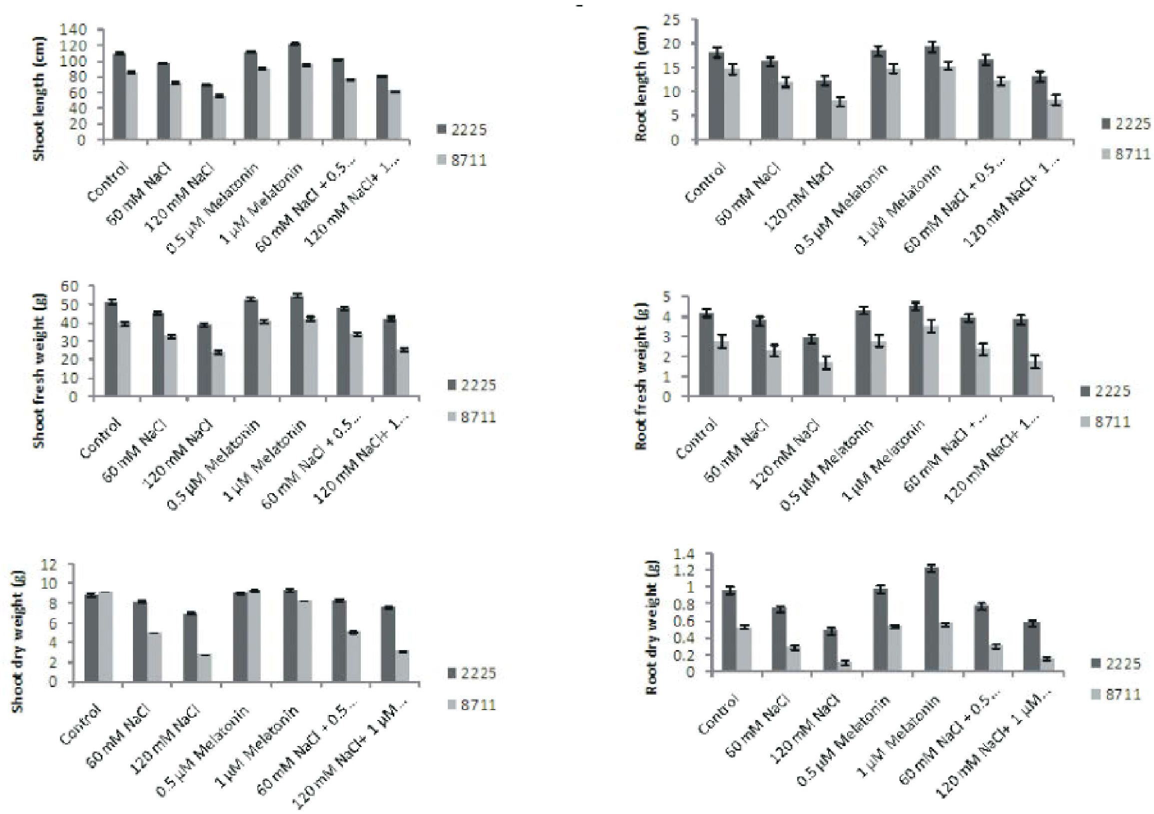

Fig. 1: Effect of melatonin on growth attributes of two maize hybrids under salt stress. Bars in the graph show average values of five replicates and the error bars are standard deviations

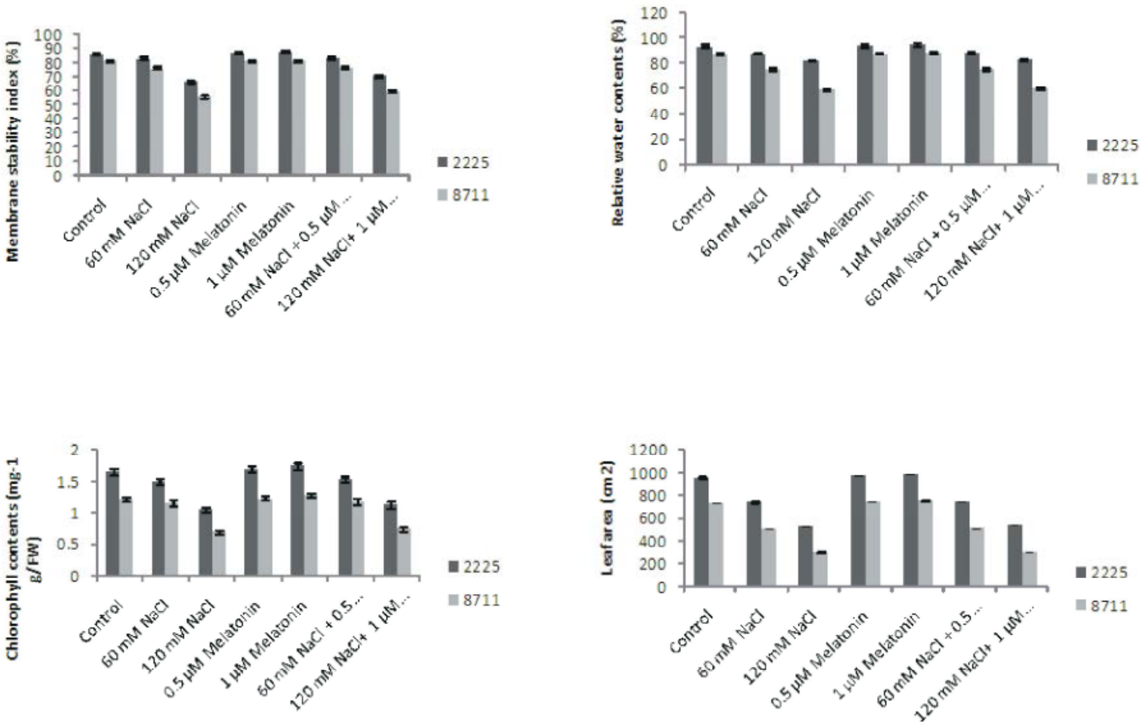

Fig. 2: Effect of melatonin on physiological attributes of two maize hybrids under salt stress. The bars in the graph show average values of five replicates and the error bars are the standard deviations

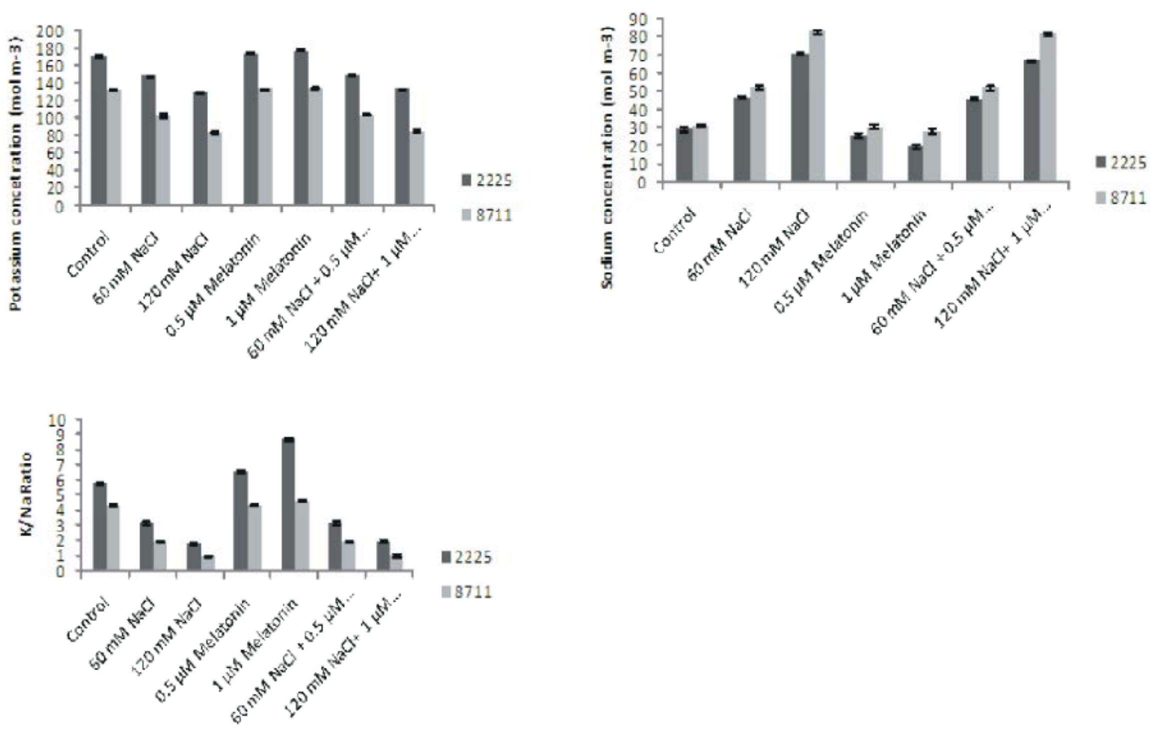

Fig. 3: Effect of melatonin on ionic attributes of two maize hybrids under salt stress. The bars in the graph show average values of five replicates and the error bars are the standard deviations

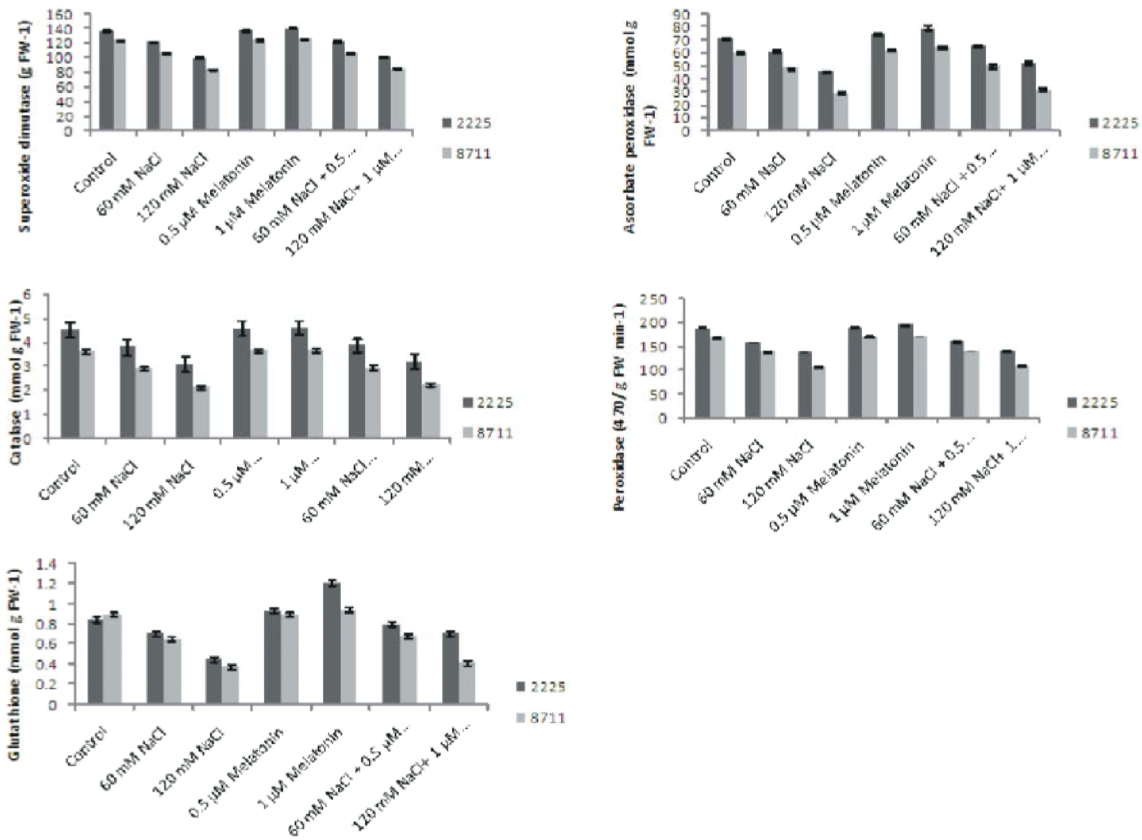

Fig. 4: Effect of melatonin on biochemical attributes of two maize hybrids under salt stress. The bars in the graph show the average values of five replicates and the error bars are the standard deviations

while maximum K^+ values was observed when melatonin @ 1.0 µM applied under non-stressed condition. Present study reveals that K^+contents were kept high in plants treated with melatonin under saline and non saline condition as compared to those without melatonin application. However, maize hybrid 2225 proved efficient in retaining maximum K^+ concentration under all levels of salt stress. The intensify uptake of Na^+ and decreasing K^+ concentration with increasing salinization concluded in inferior K^+/Na^+ratio. Melatonin exposure under low and high salt stress mitigated the adverse effects of salinity and maximize potassium uptake in both hybrids.

Antioxidant Enzymes Activity: Interactive effect of salinity and melatonin on biochemical enzymes activity in seedlings of both maize hybrids is illustrated in Figure 4. It is evident from the results that as compared to control, increased supply ofNaCl reduces the activities of APX, CAT, SOD, POD and GR. Maximum values were noted under sole application of melatonin (1.0 µM) while least activities of antioxidant enzymes were recorded under elevated salt stress (12 dS m^{-1}).However, melatonin treatment to salt affected plants leads to increase in activities of these enzymes. Furthermore, melatonin nutrition could not convey any eminent changes in peroxidase (POD) and catalase (CAT) activities under saline and non-saline environment. Maize hybrid 8711 showed minor response while maize hybrid 2225 displays superior response towards melatonin treatment under conducive and saline treatment which results in enhanced antioxidant enzymes activities.

DISCUSSION

Increased salinization in growth channel results in interruption of plant morphology, physiological characteristics, disturbs ions homeostatic and diminishes the activities of oxidative stress tolerant enzymes which ultimately disturbs the yield of agricultural crops while melatonin application significantly acclimate the deleterious effects of salinity. Decline in plant growth attributes is due to higher accumulation of Na^+ and Cl^-in the growth medium affecting permeability of membrane, disturbs photosynthetic activity, assembling of toxic ions at tissue level consequence in imbalance nutrients uptake, suspending the cell division and production of reactive oxygen species which remarkably reduces plant biomass [34, 35].

Application of exogenous melatonin play multifunctional role in improving plant physiology under stress and non-stressed environment. Reduced Na^+influx, acclimation of oxidative stress, up-regulation of antioxidant defense system and enhanced photosynthetic activity are important melatonin ramifications in improving salinity [36]. Moreover, melatonin contributes enlargement in growth traits of plants by normalizing the higher concentration of salts by altering efflux movement of Na^+ in roots of saltaffected plants [37].

Salinity disturbs plant water relations and deteriorates membrane stability as depicted in the current project and this reduction is more prominent in maize hybrid 8711 as compared to maize hybrid 2225. The increasing trend of NaCl in soil remarkably reduces the relative water contents (RWC) and membrane stability index (MSI) which results in reduced water uptake by plant, succulence of leaves, depreciate membrane reliability and cell membrane damage [38]. Melatonin nutrition markedly enhanced the plant water contents by improving the growth and ability of plant roots to absorb more water from growth medium, balanced osmotic adjustment, regulate transpiration rate with stomatal conductance and regulate plant water potential [24]. Melatonin enhanced antioxidant machinery by acting as scavenger against $H_2O_2$and O_2^-ensured ROS equilibrium at cellular level which promoted membrane stability and permeability [15].

Sodium (Na^+) aid in regulating plant metabolism but have drastic effects on plant morphology and physiologywhen exceeds from certain limit.Sodium leads to nutritional imbalance in maize due to its involvement in nutrients availability, competitive uptake and transport with in plant [39]. Presence of Na^+ transport channels in plasma membrane permits its entry into cell and serves as a key factor in sensitivity or tolerance of maize hybrid [40]. Potassium retaining capacity of plant cell is a major factor to check plant tolerance. Under saline environment, the major cause in reduced potassium uptake might be due to nutrient imbalance and competitive uptake of Na^+ and Cl^- with certain nutrients like K^+, Mn^{2+} and Ca^{2+} [41]. Higher K^+/Na^+ ratio reticence is a key factor that helps salt tolerant genotypes to perform well under saline environment and to preserve cellular metabolismby promoting protein synthesis, regulating enzymes activation, photosynthesis, osmoregulation and maintaining cell turgor [40]. In present study, melatonin application enables maize plant to maintain superior K^+ contents in shoot as compared to those without melatonin treatment which are parallel with the results of Jiang *et al*. [42]. Melatonin could restrict Na^+loading in roots and elevated retrieval of Na^+ from shoots contributed in more K^+ accumulation, osmotic adjustment and ion homeostasis and superior K^+/Na^+ratio under salt stress [20].

Induced salinity in the growth channel amends the activities and amount of antioxidant enzymes along with their ability to scavenge reactive oxygen species (ROS) results in cellular damage [15]. Melatonin has also been recognized to act as antioxidant and enhances the activities of SOD, APX and POD in bermudagrass leaves under salt stress [36]. Present study suggested that ameliorating activities of antioxidant enzymes influenced by addition of melatonin by elevating activity of antioxidant enzymes and direct free radical scavenging under salt stress [16].

CONCLUSION

Soil salinity has become aglobal agricultural constraint especially in arid to semi-arid regions which leads to desertification of large cultivated area and significant economic losses in maize and other crops. When exposed to salt stress, maize hybrids show disrupted growth, poor plant water relations and membrane de-stability, high cellular Na^+ contents and imbalance in mineral nutrition along with oxidative damage. Increase in plant biomass, improved water contents, higher K^+retention in leaves and activity of antioxidant enzymes revealed exogenous application of melatonin played multifunctional role in imparting salt tolerance in maize seedlings. Maize hybrid 2225 displays more K^+/Na^+ratio and high stress tolerant antioxidant enzymes activities along with improved morpho-physiological attributes under salt stress helps to declared it as salt tolerant as compared to maize hybrid 8711. To best of our knowledge, this is the first study on maize that will provide a novel approach to enhance salt tolerance of maize through application of melatonin and will help to get maximum production of maize on salt affected soil.

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