

Alleviation of salt stress-induced adverse effects on maize plants by exogenous application of indoleacetic acid (IAA) and inorganic nutrients – A field trial

Cengiz Kaya^{1*}, Muhammad Ashraf², Murat Dikilitas³ and Atilla L. Tuna⁴

¹Harran University, Agriculture Faculty, Soil Science and Plant Nutrition Department, Şanlıurfa – TURKEY

²Department of Botany, University of Agriculture, Faisalabad, Pakistan

³Harran University, Agriculture Faculty, Plant Protection Department, Şanlıurfa – TURKEY

⁴Muğla University, Biology Department, Muğla – TURKEY

*Corresponding author: c_kaya70@yahoo.com, ckaya@harran.edu.tr

Abstract

The effects of indoleacetic acid (IAA) and inorganic nutrients (K and P) on some physiological parameters and kernel yield of maize (*Zea mays* L.) cultivar DK 647 F1 were investigated in two parallel experiments conducted in the same growth season in a saline field. Sodium chloride equivalent to 100 mM was added to the irrigation water and saline water applied to the field using a drip irrigation system. Indoleacetic acid was applied as foliar spray. Potassium and P were applied to the soil at the sowing time as mono-potassium phosphate at 200 kg ha⁻¹. Salinity significantly reduced shoot dry mass, cob yield, total kernel yield, weight of 1000 kernels, chlorophylls “a” and “b” and relative water content in the maize plants, but increased proline accumulation, activities of the key antioxidant enzymes superoxide dismutase (SOD; EC 1.15.1.1), peroxidase (POD; EC. 1.11.1.7), catalase (CAT; EC. 1.11.1.6) and polyphenol oxidase (PPO; 1.10.3.1), and electrolyte leakage. However, application of K and P or foliar spray of IAA mitigated the adverse effects of salinity on maize plants. The most promising effect of IAA or K and P on alleviation of salt stress on maize was found when they were applied in combination. Leaf sodium (Na⁺) concentration increased substantially, but leaf K⁺, Ca²⁺ and P concentrations decreased markedly in the salt stressed maize plants. However, exogenous application of nutrients, IAA, or their combination considerably reduced Na⁺ concentration and significantly improved K⁺, Ca²⁺, and P levels in the salt stressed maize plants. The exogenously applied inorganic nutrient- or auxin-induced growth promotion in maize plants was found to be associated with increased photosynthetic pigment concentration and leaf Na⁺/K⁺ ratio, reduced membrane permeability, and altered activities of some key antioxidant enzymes such as SOD and CAT under saline conditions.

Keywords: oxidative stress; potassium; phosphorus; *Zea mays*; indoleacetic acid.

Abbreviations: CAT- catalase; SOD- superoxide dismutase; POD-peroxidase; PPO- polyphenol oxidase; IAA- indoleacetic acid.

Introduction

Soil or water salinity is known to cause considerable yield losses in most crops, thereby leading to reduced crop productivity (Ashraf, 2009; Cha-um et al., 2011). The salinity-induced crop yield reduction takes place due to a number of physiological and biochemical dysfunctions in plants grown under salinity stress which have been listed in a number of comprehensive reviews on salinity effects and tolerance in plants (Ashraf et al., 2008; Munns and Tester, 2008; Jamil et al., 2011; Krasensky and Jonak, 2012). Scientists have been vying for the last many decades to overcome the problem of salinity by employing a variety of strategies. Of the various strategies currently under exploitation, improvement in salinity tolerance of crops through exogenous application of different types of chemicals including plant growth regulators, osmoprotectants and inorganic nutrients seems to be an efficient, economical and shot-gun approach (Ashraf et al., 2008). The use of such substances has resulted in a substantial increase in both growth and yield of many crops grown under saline conditions (Ashraf et al., 2008; Kaya et al., 2010).

Of the various plant growth regulators which regulate growth under normal or stress conditions, indoleacetic acid (IAA) plays a vital role in maintaining plant growth under stress conditions including salt stress (Gulnaz et al., 1999; Iqbal and

Ashraf, 2007). Recently, while examining the ameliorative effect of IAA on salt stressed plants of blackgram (*Phaseolus mungo* L.), Guru Devi et al. (2012) found that foliar-applied IAA (15 mg l⁻¹) considerably ameliorated the adverse effects of salt on these plants. Similarly, a few years earlier Egamberdieva (2009) examined the effect of IAA producing bacteria on salt stressed wheat plants and reported considerable alleviation of salt-induced adverse effects on these plants. Exogenous application of inorganic essential nutrients as foliar spray or through the root growing medium has also been reported to be an economical and efficient means of mitigating the adverse effects of salt stress on different crops (Ashraf et al., 2008; Akram et al., 2009). Of different major essential nutrients, potassium (K) and phosphorus (P) play vital roles in plant growth and regulate various metabolic reactions (Taiz and Zeiger, 2010). In view of the literature, the effects of IAA and inorganic nutrients such as K and P on different crops exposed to saline stress have been studied separately. There is little information available in the literature on the effects of both chemicals applied in combination. Thus, the premier objective of the present investigation was to examine the effects of K + P and IAA individually as well as in combination on maize plants grown in NaCl induced saline field.

Results

Plant dry weight and yield

Salinity significantly reduced shoot dry weight, total kernel yield, cob yield, weight of 1000 kernels and relative water content in the maize plants (Table 1). However, application of potassium (K) and phosphorus (P) and foliar spray of IAA mitigated partially the adverse effects of salinity on maize plants. The most promising effect of IAA and K and P on alleviation of salt stress on maize was found when they were applied in combination.

Chlorophyll contents

Plant pigments such as chlorophylls a and b decreased markedly in maize plants due to salinity stress (Table 2). However, exogenous application of IAA and/or K and P significantly alleviated the inhibitory effects of salt stress on both pigments. Again the combination of both nutrients and IAA was found to be very effective in improving pigment contents in the maize plants subjected to saline medium.

Electrolyte leakage and free proline

Electrolyte leakage from the cellular membranes of maize plants increased considerably under saline regime. However, exogenous application of K and P and/or IAA checked the electrolyte leakage significantly in the maize plants exposed to saline stress (Table 2). The combined application of K, P and IAA proved to be highly effective in counteracting the adverse effect of saline stress on membrane permeability. Salt stress caused a marked increase in the levels of free proline in maize plants, but the exogenous application of either of the two nutrients, IAA, or their combination significantly reduced the levels of free proline under saline conditions with reference to the proline levels found in the salt stressed maize plants receiving no treatment of nutrients or IAA.

Ion contents

Leaf sodium (Na^+) concentration increased substantially in maize plants exposed to saline stress, but exogenous application of nutrients, IAA or their combination considerably reduced Na^+ concentration in the leaves of maize plants grown under saline stress (Table 3). Leaf K^+ , Ca^{2+} and P concentrations decreased markedly in the salt stressed maize plants, but application of both nutrients or IAA significantly improved K^+ , Ca^{2+} , and P levels. However, the combined application of the nutrients and IAA proved to be very effective to further improving K^+ , Ca^{2+} , and P levels in the salt stressed maize plants. Leaf Na:K ratio increased markedly in the leaves of salt stressed maize plants, but exogenous application of the two inorganic nutrients or IAA significantly decreased leaf Na:K ratio, and the more promising effect being due to the combined application of nutrients and IAA.

Enzyme activities

The activities of all four antioxidant enzymes, superoxide dismutase (SOD), catalase (CAT), peroxidase (POX), and polyphenol oxidase (PPO) appraised in the present investigation increased markedly in the leaves of salt stressed maize plants (Table 4). However, there had been differential effects of all exogenous chemical treatments on the activities of the four enzymes. For example, exogenous application of

K and P caused a marked decrease in SOD activity, whereas that of IAA resulted in a phenomenal increase in the enzyme activity. The combined application of inorganic nutrients and IAA resulted in lowering the SOD activity compared with that in the salt regime receiving no any exogenous treatment. The CAT activity remained unaffected in the salt stressed maize plants due to exogenously applied IAA, but in contrast, addition of K and P to the root growing medium caused a significant decrease in CAT activity in the salt treated maize plants. In case of POX activity, all exogenous treatments proved to be either non-effective or they caused a decline in the enzyme activity in the leaves of salt stressed maize plants. Although salt stress caused a marked increase in PPO activity, all exogenous chemical treatments brought the PPO activity to the level recorded in the non-stressed maize plants.

Discussion

A variety of strategies for counteracting the adverse effects of salt stress on plants are currently in practice. Of these, exogenous application of different types of plant growth regulators, osmoprotectants, and inorganic salts is contemplated to be an economical and shot-gun approach to alleviate the harmful effects of salinity on plant growth (Ashraf et al., 2008). Of various plant growth regulators, indoleacetic acid, one of the key auxins occurring naturally in plants, is known for its beneficial effects on growth of plants subjected to stress conditions including salt stress (Iqbal and Ashraf, 2007; Guru Devi et al., 2012). Similarly, of different essential inorganic nutrients, K and P have been reported to play a vital role in mitigating the harmful effects of salinity on most plants (Kaya et al., 2001; Naheed et al., 2007). In the present study, K and P as well as IAA were found to be effective in improving growth of maize plants exposed to saline medium. However, the most promising effect of the two nutrients and IAA in promoting growth of maize plants under saline regime was recorded when the two types of chemicals were applied in combination. This shows that IAA and inorganic nutrients (K and P) had synergistic effect in terms of improving growth and yield of maize plants stressed with salinity (Tables 1 and 2). Such type of synergistic effect has already been observed particularly that of IAA and K in maize plants (Cocucci and Rosa, 1980). The synergistic effect of IAA and K and P could be explained in view of the growth promoting role of both types of chemicals not only in plants grown under normal non-saline conditions but also in those under saline conditions. Auxin is believed to stimulate the uptake of sugar (Baker and Ray 1965) and inorganic nutrients (Haschke and Lutttge 1973) in plants suggesting that the phenomenon of osmoregulation is under hormonal control. Thus, it is highly likely that both IAA and K and P when applied in combination might have enhanced osmoregulation thereby promoting growth of salt stressed maize plants. Long ago, Munns (1993) proposed a viable two-phase model of responses of plants to salt stress. In view of this model, in the first phase, salt stress imposes osmotic effect on plants, whereas in the second phase salt stress causes ion toxicity in plants. However, the second phase has been contemplated to be more injurious to plant growth than the first phase. Although this two-phase model was originally proposed for wheat, it has been reported to be partially valid for maize as well (Fortmeier and Schubert, 1995). In maize particularly, Fortmeier and Schubert (1995) have shown that Na^+ toxicity is the predominant process during the second phase of salt stress. Furthermore, Schubert et al. (2009) reported that grain yield formation of maize exposed to saline conditions could suffer due to osmotic stress and Na^+ toxicity

Table 1. Shoot dry weight, kernel yield and some yield components, and leaf relative water content (RWC) in maize plants grown in saline soil with or without applied potassium and phosphorus, and sprayed with IAA.

Treatments	Kernel yield (kg ha ⁻¹)	Cob Yield (g cob ⁻¹)	Weight of 1000 kernels (g)	Shoot DW (g plant ⁻¹)	RWC (%)
Control (C)	9570a	141a	362a	412 a	85.4 a
Salt (S)	7200e	121c	331c	160 e	72.6 d
S + KP	7580d	125 c	340b	301 b	75.6 c
S + IAA	7840c	122c	336b	283 c	77.2 b
S + IAA+KP	8640b	132 b	343b	315 b	78.9 b

Mean values followed by different letters within each column differ significantly at $P \leq 0.01$. Control (non-saline); S: 100 mM NaCl; IAA: 2 mM indoleacetic acid applied as foliar spray; KP : 200 kg mono-potassium phosphate ha⁻¹ applied to soil.

Table 2. Chlorophyll a and b content (mg kg⁻¹ FW), electrolyte leakage (%) and free proline (μmol g⁻¹ FW) content in maize plants grown in saline soil with or without applied potassium and phosphorus, and sprayed with IAA.

Treatments	Chl. a	Chl. b	Electrolyte leakage	Free proline
Control (C)	1145 a	972 a	14.42 d	2.18 d
Salt (S)	920 e	745 d	28.56 a	4.06 a
S + KP	1020 c	838 c	23.64 bc	3.44 b
S + IAA	1054 b	856 bc	23.15 bc	3.06 c
S + IAA+KP	1070 b	876 b	21.45 c	3.14 c

Mean values followed by different letters within each column differ significantly at $P \leq 0.01$.

C: Control (non-saline); S: 100 mM NaCl; IAA: 2 mM indoleacetic acid; KP : 200 kg mono-potassium phosphate ha⁻¹ applied to soil.

in the first and second phase, respectively. In our study, we subjected the maize plants to saline stress on a long-term basis, i.e. plants were grown in saline stress up to maturity, so it is expected that the maize plants experienced both phases of salt stress and thus may have experienced both osmotic stress and ion toxicity. From the results of leaf Na⁺ content presented here, it is obvious that the maize plants accumulated very high amount of Na⁺ in their leaves, so the ion (Na⁺) toxicity effect of salt stress on maize plants cannot be ruled out. Furthermore, exogenous application of both IAA and inorganic nutrients (K and P) promoted growth of salt stressed maize plants as well as reduced the levels of Na⁺ in their leaves. The IAA- or inorganic nutrient-induced reduction in leaf Na⁺ could have been partly due to the dilution effect as salt stressed maize plants treated with IAA or nutrients had significantly higher relative content compared with the salt stressed maize plants not treated with IAA or nutrients (Table 1). Such a salt-induced dilution effect has earlier been observed in different crops, e.g. *Lycopersicon esculentum* (Al-Karaki et al., 2001), *Spinacia oleracea* (Kaya et al., 2001a), *Physalis peruviana* (Miranda et al., 2010), as well as in *Zea mays* (Collado et al., 2010). Membrane permeability (MP) usually appraised as electrolyte leakage is a key indicator of membrane integrity in plants subjected to stress conditions (Farkhondeh et al., 2012; Mansour, 2012). In the present study though salt stress caused a phenomenal increase in electrolyte leakage in maize plants, the exogenous application of the two inorganic nutrients or IAA considerably reduced electrolyte leakage, but the most significant effect of both types of chemicals was observed when applied in combination, again showing the synergistic effect of the nutrients and IAA on maintenance of membrane integrity in maize plants exposed to saline regime. From the data for electrolyte leakage and chlorophyll contents, it is evident that a strong relationship exists in these two physiological attributes. Such a sound relationship has already been drawn in different studies (Chen et al., 1991; Kaya et al., 2001b) showing that maintenance of membrane integrity partly depends on chlorophyll content, because stress-induced reduction in chlorophyll content leads to leaf senescence thereby causing membrane to be permeable (Niu et al., 1995; De Araújo et al., 2006). As in most mesophytes, salt stress caused a substantial increase in leaf Na⁺ content,

but a decrease in leaf K⁺, Ca²⁺ and P contents in maize plants. However, exogenously applied inorganic nutrients or IAA proved to very effective in altering the concentrations of all these nutrients in salt stressed maize plants; they caused considerable reduction in leaf Na⁺ and increase in leaf K⁺, Ca²⁺, and P compared with the concentrations of these nutrients in salt stressed plants receiving no any exogenous treatment. Ion homeostasis is one of the key mechanisms involved in plant salt tolerance (Ashraf, 2004; Singh et al., 2011). Enhanced uptake of K⁺ and/or Ca²⁺ at the cost of reduced uptake Na⁺ in the cells of salt stressed plants is considered vital for maintaining high cellular K⁺/Na⁺ ratio (Ashraf, 2004; Kavitha et al., 2012). Exogenous application of inorganic nutrients (K and P) or IAA showed ameliorating effects in terms of lowering the levels of Na⁺ and elevating those of K⁺, Ca²⁺ and P thereby maintaining higher K⁺/Na⁺ ratio as compared to that in salt stressed plants receiving no any external treatment; the most distinctive effect on ion homeostasis could be observed when the two inorganic nutrients and IAA were applied in combination. This again shows the synergistic effect of K + P and IAA on ion homeostasis in maize plants under saline medium. Salt stress elevated the activities of all four antioxidant enzymes SOD, CAT, POX, and PPO in maize plants exposed to saline stress suggesting that oxidative defense system in salt stressed maize plants had been very operative to counteract various reactive oxygen species (ROS) produced due to salt stress (Ashraf, 2009; Kaye et al., 2011). Exogenous application of IAA further increased the activities of SOD and CAT in the salt stressed maize plants, whereas such effect was not observed on POX or PPO. The application of K and P and combined treatment of these nutrients with IAA were found to be ineffective in enhancing the activities of all four enzymes in salt stressed maize plants. These results show that only exogenously applied IAA had a promising effect in enhancing the activities of some key enzymes of the antioxidative defense system. This suggests that plant growth regulators including IAA can regulate the activity/synthesis of key antioxidant enzymes, and some of their isoforms are also involved in the metabolism of plant growth regulators (Synkova et al. 2004; Tognetti et al., 2012). Reactive oxygen species and auxins (IAA) are known to play a vital role in the regulatory networks of the mechanism of salt tolerance,

because both are significantly influenced by stress conditions. But on the other hand, the underlying mechanisms of the crosstalk between ROS and auxins are not well elucidated yet (Tognetti et al., 2012). Thus, it is not yet fully understood how far IAA can crosstalk with ROS or antioxidant defense system including antioxidant enzymes. This issue needs to be fully researched.

Material and methods

Plant material and experimental details

A field experiment was conducted at the Agricultural Research Station of the University of Harran, Şanlıurfa on a clay loam soil using a maize cultivar DK 647 F1: DEKALP. Various physico-chemical properties of the soil used were: water content at field capacity 30.28%, water content at the permanent wilting point 24.4%, dry bulk density 1.38 g cm^{-3} , pH 7.1, organic matter content 1.2%, and electrical conductivity (EC) of the top 0.3 m of soil 1.10 dS m^{-1} . The quality of the irrigation water (EC = 0.52 dS m^{-1} and pH 7.2) used for the experiment was reasonably good. Soil EC content was monitored weekly. The maize kernels were sown in rows in plots, each 6.0 m X 2.8 m. Ninety-six plants were sown in each plot representing a replicate. The plant to plant distance within each row was 0.25 m and between rows 0.70 m. All treatment units were arranged in a randomized split-block design with three replicates. The main plants represented the salinity treatment and the subplots IAA or K + P. All plots were watered through a drip irrigation system at 10:00 to 17:00 at a rate of $4 \text{ L h}^{-1} \text{ m}^{-1}$ for two weeks to allow establishment of roots before the application of salinity treatment. A single-drip irrigation tube with 4.0 L h^{-1} and 0.5 m emitter spacing was laid on the soil surface on each row. The operating pressure of the drip irrigation system was maintained at 100 kPa throughout the experiment. Tensiometers were installed at 30 and 45 cm soil depths in between two plants in each row so as to appraise the irrigation schedule. The tensiometer readings were maintained at -30 kPa at 30 cm and -20 kPa at 45 cm. A Class A pan evaporimeter fixed near the experimental field provided the evaporation data, which was recorded daily. No rainfall occurred during the entire experimental period. Trickle irrigation was scheduled daily at 100% of Epan to maintain soil moisture content near the field capacity (FC). Salinity and IAA treatments were started 20 days after sowing. Soil EC content was monitored weekly and once it reached to 10 dS m^{-1} it was maintained around this level. After the first week of salinity treatment, soil EC was 5.4 dS m^{-1} and in the second and third weeks it was 7.6 and 8.9 dS m^{-1} , respectively. After that it reached to 10 dS m^{-1} which was then maintained constant during the entire experimental period. The different treatments used were: (1) non-saline control (C), irrigation water alone; (2) salt treatment (S), 100 mM NaCl; (3) S + KP: 100 mM NaCl and 200 kg supplemental mono-potassium phosphate ha^{-1} ; (4) S + indoleacetic acid (IAA), 100 mM NaCl and 2 mM IAA; and (5) S + IAA+KP, 100 mM NaCl and 2 mM IAA plus 200 kg supplemental mono-potassium phosphate ha^{-1} . Salinity treatment was developed by adding appropriate amount of NaCl to irrigation water. The total volume of water applied from the initiation of the salt treatments until harvest was calculated to be 240 mm which was applied in 8 sequential irrigations. Plants were harvested 120 days after sowing. Supplemental mono-potassium phosphate was added to soil at the sowing time. IAA (Sigma) solution prepared in 0.1% Tween-20 (a surfactant) was applied to the plants at a rate of

150 to 500 mL plot^{-1} depending on plant age and growth once a week starting from day 10 after germination up to the maturity stage. The control (non-stressed) plants were sprayed with an equal amount of water containing only Tween-20 solution. At the sowing time, all plots were supplied with same amounts of total N (100 kg ha^{-1}), P_2O_5 (100 kg ha^{-1}), and K_2O (240 kg ha^{-1}) fertilizers. Second part of N (100 kg ha^{-1}) was applied to all plots at the beginning of grain filling stage. Hand weeding was done three times during the whole experimental period. Plants in one of four rows were used to determine different physiological attributes mentioned below at the beginning of the grain filling stage, and the plants in the other three rows were grown to maturity to appraise grain yield.

Grain yield determination

At harvest, 8 plants from each row within each replicate were harvested for measuring kernel yield. All maize cobs from the harvested plants were excised from the stalks, threshed, and weighed to record kernel weight. There was about 13% moisture content in the kernels at the time of threshing.

Leaf relative water content and electrolyte leakage

Leaf relative water content (RWC) was estimated following Yamasaki and Dillenburg (1999) and electrolyte leakage (EL) following Dionisio-Sese and Tobita (1998).

Chlorophyll and free proline determination

One plant from each replicate was used for determining chlorophyll and proline contents. A youngest fully expanded leaf was used for all these measurements. Chlorophyll concentrations were worked out using the equation of Strain and Svec (1966). Proline was determined according to Bates et al. (1973).

Protein content

Soluble protein content in the enzyme extracts was determined following Bradford (1976) using Bovine Serum Albumin fraction V as a standard.

Extraction and determination of activities of antioxidant enzymes

Catalase activity was determined following Kraus and Austin-Fletcher (1994) as the consumption of H_2O_2 at 405 nm. The SOD activity was determined by monitoring its ability to inhibit the photochemical reduction of NBT (Beauchamp and Fridovich, 1971).

The activity of POD was assayed according to the method of Chance and Maehly (1955). The POD activity was expressed as change in absorbance $\text{min}^{-1} \text{ mg}^{-1}$ protein. The activity of polyphenol oxidase (PPO) was determined following Zaubermann et al. (1991).

Determination of dry weight and inorganic nutrients

Shoots of three randomly selected plants per replicate were separated from the roots and dried in a forced air oven at $70 \text{ }^\circ\text{C}$ for two days to determine dry weights. The dried shoot samples were powdered using a pestle and mortar and stored in polyethylene bottles. The ground samples were ashed in a muffle furnace at $550 \text{ }^\circ\text{C}$ for 6 h. The white ash of each sample was added to 5 mL of 2 M hot HCl, filtered, and

Table 3. Concentrations (mmol kg⁻¹ d.wt) of sodium (Na⁺), potassium (K⁺), phosphorus (P) and calcium (Ca²⁺), and Na⁺/K⁺ ratio in the leaves of maize plants grown in saline soil with or without applied potassium and phosphorus, and sprayed with IAA.

Treatments	Na ⁺	K ⁺	P	Ca ²⁺	Na ⁺ /K ⁺ ratio
Control (C)	45 e	395 a	97 a	184 a	0.113 e
Salt (S)	386 a	254 e	58 e	112 d	1.519 a
S + KP	277 c	342 c	78 bc	169 c	0.809 c
S + IAA	246 d	321 d	74 cd	174 b	0.766 cd
S + IAA+KP	256 d	375 b	82 b	179 ab	0.682 d

Mean values followed by different letters within each column differ significantly at $P \leq 0.01$.

C: Control (non-saline); S: 100 mM NaCl; IAA: 2 mM indoleacetic acid; KP: 200 kg mono-potassium phosphate ha⁻¹ applied to soil.

Table 4. Antioxidant enzymes [(superoxide dismutase (SOD: Unit/mg protein/min), catalase (CAT: Unit x100/mg protein), peroxidase (POX: ΔA₄₇₀/min/mg protein) and polyphenol oxidase (PPO: Unit x100/mg protein)] in the leaves of maize plants grown in saline soil with or without applied potassium and phosphorus, and sprayed with IAA.

Treatments	SOD	CAT	POX	PPO
Control (C)	57 e	1.5 e	7.96 d	0.54 b
Salt (S)	143 b	2.45 bc	34.43 a	7.65 a
S + KP	30 f	1.66 e	9.60 d	0.62 b
S + IAA	217 a	2.79 b	28.10 b	0.42 b
S + IAA+KP	109 c	1.98 d	12.73 c	0.59 b

Mean values followed by different letters within each column differ significantly at $P \leq 0.01$.

C: Control (non-saline); S: 100 mM NaCl; IAA: 2 mM indoleacetic acid; KP: 200 kg mono-potassium phosphate ha⁻¹ applied to soil.

made up the final volume to 50 mL with distilled water. Sodium (Na), Ca, K, and P in the extracts were determined using an ICP (Chapman and Pratt 1982).

Statistical analysis

Two parallel experiments were conducted in the same growing season. Statistical analysis (ANOVA) indicated that there were no significant differences in measurements between the two different experiments. The data presented here are the means of the two experiments. One-way analysis of variance was performed on data of each attribute and the difference between the mean values was worked out using the LSD test at $P < 0.01$.

Conclusions

Taken together, although exogenous application of K + P or IAA proved to be effective in alleviating the adverse effects of saline stress on growth and kernel yield of maize plants, application of these two chemicals when applied in combination had a pronounced effect in terms of promoting growth and kernel yield of salt stressed maize plants. The exogenously applied inorganic nutrient- or auxin-induced growth promotion was found to be associated with reduced membrane permeability, enhanced photosynthetic pigment concentration, reduced leaf Na⁺/K⁺ ratio, and altered activities of some key antioxidant enzymes such as SOD and CAT under saline medium.

Acknowledgements

This research was financially supported by the Scientific and Technological Research Council of Turkey (TOVAG-104O528). The authors also wish to thank the University of Harran, University of Agriculture, Faisalabad (Pakistan) and University of Mugla (Turkey) for their support.

References

Akram MS, Ashraf M, Akram NA (2009) Effectiveness of potassium sulfate in mitigating salt-induced adverse effects on different physio-biochemical attributes in sunflower (*Helianthus annuus* L.). *Flora* 204: 471–483

Al-Karaki GN, Hammad R, Rusan M (2001) Response of two tomato cultivars differing in salt tolerance to inoculation with mycorrhizal fungi under salt stress. *Mycorrhiza* 11: 43–47

Ashraf M (2004) Some important physiological selection criteria for salt tolerance in plants. *Flora* 199: 361–376

Ashraf M (2009) Biotechnological approach of improving plant salt tolerance using antioxidants as markers. *Biotech Adv* 27: 84–93

Ashraf M, Athar HR, Harris PJC, Kwon TR (2008) Some prospective strategies for improving crop salt tolerance. *Adv Agron* 97: 45–110

Baker DB, Ray PM (1965) Direct and indirect effects of auxin on cell wall synthesis in oat coleoptile tissue. *Plant Physiol* 40: 345–352

Bates L S, Waldren RP, Teare LD (1973) Rapid determination of free proline for water-stress studies. *Plant Soil* 39: 205–207

Beauchamp C, Fridovich I (1971) Superoxide dismutase improved assays and an assay applicable to acrylamide gels. *Anal Biochem* 44: 276–287

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilising the principle of protein-dye binding. *Anal Biochem* 72: 248–254.

Chance B, Maehly C (1955) Assay of catalase and peroxidases. *Methods Enzy* 11: 764–775.

Chapman HD, Pratt PF (1982) *Methods of Plant Analysis. I: Methods of Analysis for Soils, Plants and Water*. Chapman Publishers, Riverside, CA, p. 170.

Cha-um S, Pokasombat Y, Kirdmanee C (2011) Remediation of salt-affected soil by gypsum and farmyard manure – Importance for the production of Jasmine rice. *Aust J Crop Sci* 5: 458–465

Chen C T, Li CC, Kao CH (1991) Senescence of rice leaves XXXI. Changes of chlorophyll, protein, and polyamine contents and ethylene production during senescence of a chlorophyll-deficient mutant. *J Plant Growth Reg* 10: 201–205

Cocucci MC, Rosa SD (1980) Effects of canavanine on IAA- and fusicoccin- stimulated cell enlargement, proton extrusion and potassium uptake in maize coleoptiles. *Physiol Plantarum* 48: 239–242

- Collado MB, Arturi M J, Aulicino MB, Molina MC (2010) Identification of salt tolerance in seedling of maize (*Zea mays* L.) with the cell membrane stability trait. *Inter Res J Plant Sci* 1: 126-132
- De Araújo SAM, Silveira JAG, Almeida TD, Rocha IMA, Morais DL, Viéga RA (2006) Salinity tolerance of halophyte *Atriplex nummularia* L. Grown under increasing NaCl levels. *R Bras Eng Agric Ambiental* 10: 848-854.
- Dionisio-Sese ML, Tobita S (1998) Antioxidant responses of rice seedlings to salinity stress. *Plant Sci* 135: 1-9.
- Egamberdieva D (2009) Alleviation of salt stress by plant growth regulators and IAA producing bacteria in wheat. *Acta Physiol Plant* 31: 861–864
- Farkhondeh R, Nabizadeh E, Jalilnezhad N (2012) Effect of salinity stress on proline content, membrane stability and water relations in two sugar beet cultivars. *International J Agri Sci.* 2: 385-392
- Fortmeier R, Schubert S (1995) Salt tolerance of maize (*Zea mays* L.): the role of sodium exclusion. *Plant Cell Env* 18: 1041-1047
- Gulnaz A, Iqbal J, Farooq S, Azam F (1999) Seed treatment with growth regulators and crop productivity. I. 2,4-D as an inducer of salinity-tolerance in wheat (*Triticum aestivum* L.). *Plant Soil* 210: 209–217.
- Guru Devi R, Pandiyarajan V, Gurusaravanan P (2012) Alleviating effect of IAA on salt stressed *Phaseolus mungo* (L.) with reference to growth and biochemical characteristics. *Rec Res Sci Tech* 4: 22-24.
- Haschke HP, Iuttge U (1973) IES-abhängiger K^+ - H^+ -Austausch Mechanismus und Streckungswachstum bei *Avena-Koleoptilen*. *Z Naturforsch* 28C: 555-558
- Iqbal M, Ashraf M (2007) Seed treatment with auxins modulates growth and ion partitioning in salt-stressed wheat plants. *J Integ Plant Biol* 49: 1003–1015
- Jamil A, Riaz S, Ashraf M, Foolad MR (2011) Gene expression profiling of plants under salt stress. *Crit Rev Plant Sci* 30: 435-458
- Kavitha PG, Miller A J, Mathew MK, Maathuis FJM (2012) Rice cultivars with differing salt tolerance contain similar cation channels in their root cells. *J Exp Bot* 63: 3289-3296
- Kaya C, Kirnak H, Higgs D (2001) Enhancement of growth and normal growth parameters by foliar application of potassium and phosphorus on tomato cultivars grown at high (NaCl) salinity. *J Plant Nutr* 24: 357–367
- Kaya C, Tuna AL, Okant AM (2010) Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. *Turk J Agr For* 34: 529-538
- Kaye Y, Golani Y, Singer Y, Leshem Y, Cohen G, Ercetin M, Gillaspay G, Levine A (2011) Inositol polyphosphate 5-phosphatase7 regulates production of reactive oxygen species and salt tolerance in Arabidopsis. *Plant Physiol* 157: 229-241
- Krasensky J, Jonak C (2012) Drought, salt, and temperature stress-induced metabolic rearrangements and regulatory networks. *J Exp Bot* 63: 1593-1608
- Kraus TE, Fletcher RA (1994) Paclobutrazol-induced protection of wheat seedlings from heat and paraquat injury. Is detoxification of active oxygen involved? *Plant Cell Physiol.* 35, 45-52.
- Mansour MMF (2012) Plasma membrane permeability as an indicator of salt tolerance in plants. *Biol Plant* DOI: 10.1007/s10535-012-0144-9
- Miranda L, Fischer DG, Ulrichs C (2010) Growth of cape gooseberry (*Physalis peruviana* L.) plants affected by salinity. *J Appl Bot Food Qual* 83: 175-181
- Munns R (1993) Physiological process limiting plant growth in saline soil: some dogmas and hypotheses. *Plant Cell Env* 16: 15-24
- Munns R, Tester M (2008) Mechanisms of salinity tolerance. *Annu Rev Plant Biol* 59: 651–681
- Naheed G, Shahbaz M, Latif CA, Rha ES (2007) Alleviation of the adverse effects of salt stress on rice (*Oryza sativa* L.) by phosphorus applied through rooting medium: growth and gas exchange characteristics. *Pak J Bot* 39: 729-737.
- Niu X, Bressan RAP, Hasegawa MJ, Pardo M (1995) Ion homeostasis in NaCl stress environments. *Plant Physiol* 109: 735-742
- Schubert S, Neubert A, Schierholt A, Sümer A, Zörb C (2009) Development of salt-resistant maize hybrids: The combination of physiological strategies using conventional breeding methods. *Plant Sci* 177: 196–202
- Singh JS, Pandey VC, Singh DP (2011) Efficient soil microorganisms: a new dimension for sustainable agriculture and environmental development. *Agric Ecosys Env* 140: 339–353
- Strain HH, Svec WA (1966) Extraction, separation, estimation and isolation of chlorophylls. In: Vernon, L. P. and Seely, G. R. eds. *The Chlorophylls*, Academic Press, New York, pp. 21-66.
- Synkova H, Semoradova S, Burketova L (2004) High content of endogenous cytokinins stimulates activity of enzymes and proteins involved in stress response in *Nicotiana tabacum*. *Plant Cell Tissue Organ Cult* 79: 169–179
- Taiz L, Zeiger E (2010) *Plant Physiology*, Sinauer Associates, Inc. Publishers, Sunderland, Massachusetts, p. 782.
- Tognetti VB, Müllenbock PER, Van Breusegem F (2012) Stress homeostasis—the redox and auxin perspective. *Plant Cell Environ* 35: 321–333.
- Yamasaki S, Dillenburg LC (1999) Measurements of leaf relative water content in *Araucaria angustifolia*. *R Bras Fisiol Veg* 11: 69-75
- Zauberman G, Ronen R, Akerman M, Weksler A, Rot I, Fuchs Y (1991) Postharvest retention of the red color of litchi fruit pericarp. *Sci Hort* 47: 89–97