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Full Length Research Paper

Hydrogel substrate alleviates salt stress with increase antioxidant enzymes activity of bean (*Phaseolus vulgaris* L.) under salinity stress

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The effect of varying hydrogel (0, 0.05 and 0.1% w/w) supply on major antioxidant enzyme (super oxide dismutase, SOD; catalase, CAT and peroxidase, POD) activity, membrane permeability and salt tolerance index (STI) of bean plants in different salt source and doses stress were investigated. Plants were treated with eight salt sources (NaCl, Na_2SO_4, $CaCl_2$, $CaSO_4$, KCl, K_2SO_4, $MgCl_2$, and MgSO4) and four concentrations (0, 30, 60, and 120 mM doses) for 60 days in a growth media. Salt type, doses and hydrogel (HG) affected soil electrical conductivity. Soil salinity affected the parameters considered and changed the antioxidant activity of plants. Different salt concentrations negatively affected STI of plants. High salt concentration caused substantial increase leakage of plant. CAT antioxidant enzyme activities of plants decreased with increasing salt doses, and the lowest value was obtained for NaCl application. SOD and POD enzyme activity of plant were increased with increasing salt doses, and the highest value was obtained for NaCl application. HG added to saline soil significantly improved the variables affected by high salinity and also reduced soil electricity conductivity, electrolyte leakage of plant, enhanced STI and caused decrease SOD and POD enzyme activity. The result suggested that HG have great potential for use in alleviating salinity stress on plant growth and growth parameter, in saline soils of arid and semi-arid areas. This HG appears to be highly effective for use as soil conditioners in vegetable growing, to improve crop tolerance and growth saline conditions. It is intended to confirm the results of these studies by field trials.

Key words: Bean, salinity, enzyme activity, hydro gel

INTRODUCTION

Salinity is one of the major environmental stresses which is among the most limiting factors to plant growth and productivity. Among these, high salinity is the most harmful (Hamdia et al., 2004). Worldwide, 100 million ha or 5% of the arable land is adversely affected by high salt concentration which reduces crop growth and yield (Ghassemi et al., 1995; Heuer, 2003). The restriction of plant growth and productivity due to salinity is especially acute in arid and semi-arid regions around the world (Kuznetsov and Shevyakova, 1997). Salinity effects on plants are complex.

Under salt stress, plants have adapted to osmotic and ionic stress. The deleterious consequences of high salt concentrations on plant cells include hyperosmotic shock and ionic imbalance (Zhu et al., 1997) . Moreover, salinity causes physiological and biochemical changes in plants. In the plants, these changes appear, depending on the effects of ions and solutes in the root zone on water activity in the cell and physiological and biochemical functions of the cell, reducing turgor, limiting photosynthesis and increasing ion deficiency due to inadequate transport mechanism (Hasegawa et al., 1986). Plant survival and growth under salinity condition are dependent on adaptations to reestablish ionic balance. Much effort has been devoted toward understanding the adaptive mechanism to salt tolerance (Zhu et al., 1997; Borsani et al., 2001).

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Salt stressed plants accumulate various molecules found in organic matter such as proline, glucose, glycine betaine etc. in the cell membrane for osmoregulation to occur thereby protecting enzyme activity (Munns and Termaat, 1986). However, levels of antioxidant enzyme activitiy and antioxidant concentrations are frequently used as indicators of oxidative stress in plants (Mittler, 2002). Several studies have demonstrated that generation of reactive oxygen species (ROS), such as the superoxide radical (O_2), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2), alter antioxidant enzymes. Antioxidants are induced in plants in response to stressors such as salinity (Bor et al., 2003). A ROS causes oxidative damage to biomolecules such as lipids and proteins and eventually leads to cell death. To protect against oxidative stress, plant cells produce both antioxidant enzymes such as superoxide dismutase (SOD), peroxidase (POD) and catalase (CAT), and non-enzymatic antioxidants such as ascorbate, glutathione and "- tocopherol (Mittler, 2002) . Ascorbate peroxidase (APX) is part of the scavenging cycle and catalyzes the reaction of ascorbic acid with H_2O_2, while glutathione reductase (GR) catalyzes the regeneration of ascorbic acid.

Hydrogels, which were developed to increase water holding capacity of amended media, have been used to aid plant establishment and growth in dry soils. Plants grown in substrates amended with hydrophilic polymers were slower to wilt than those in unamended medium (Baassari et al., 1986; Chen et al., 2001). There is other evidence showing that the presence of polymers prolongs the survival of plants and increases water use efficiency and dry matter production during periods of drought and salinity condition. Recently, the effects of application of hydrogel on crops grown in substrates have been investigated under saline conditions. Hydrogel amendment improved emergence of wheat (Saleh and Hussein, 1987), germination of maize pollen (El-Sayed and Kirkwood, 1992), and salt tolerance and growth of certain horticultural crops, including barley, tomato, cucumber and lettuce. In most of this studies, cross-linked water insoluble but swellable hydrogels, example, poly (ethylene oxide) hydrogel (Szmidt and Graham, 1990), polyacrilamide hydrogel (Awad et al., 1986) and cross-linked poly (ethylene oxide-co- polyuıethane hydrogel (El-Sayed and Kikrkwood, 1992) were usually used under saline conditions. However, little is known about the effect of hydrogel on bean growth under salt stress.

Phaseolus vulgaris L., the common bean, is in an important source of protein and other nutrients in many developing countries (CIAT, 1992) . Of over 30 different *Phaseolus* species of American origin, none is as important worldwide. In Eastern Africa and Latin America, common bean is cultivated on 14 millions hectares with an annual production of 17.5 millions tons (FAO web, 2002). Approximately 20 to 30% of bean production area in the Middle East, and 5 to 10% in Latin America and 1.4 to 2% in Turkey are affected by soil salinity (CIAT, 1992; DPT, 2001).

The common bean is extremely sensitive to salinity, and suffers from yield losses at soil salinity due to limited water uptake. Limited soil water content restricts plant growth under drought and salinity conditions. Plants overcome this difficulty by increasing concentration of compounds in cells. Various studies were made, whether proline accumulation could be used as an indicator in salt tolerance of plants (Chen et al., 2001; Turan and Aydın, 2005).

The objective of this study was to evaluate the efficiency of a hydrogel in delaying the effect of water deprivation with increasing antioxidant enzyme and decreasing membrane permeability in salt sensitive bean plant. We hypothesized that hydrogel amendment would increase substrate water content and therefore reduce or at least delay the effects of salt stress and drought stress on plant growth and physiology.

MATERIALS AND METHODS

Plant material and growth conditions

Bean plants (*Phaseolus vulgaris* L.) were grown under the controlled greenhouse conditions with 25 to 30 C, 30 to 40% relative humidity, and 10 C night temperatures in Erzurum (Turkey). Day length was approximately14 h during the experimental period. The soil samples were taken from depth of 0 to 15 cm from agricultural fields in Erzurum province (39° 55' N, 41º 61' E) of Turkey, dried indoors until it could be crumbled to pass through 4 mm for pots experiment and 2 mm sieves for analyses of physicochemical properties. The soil was classified as Aridisol according to the USA taxonomy (Soil Survey Staff, 1992) with parent materials mostly consisting of volcanic, marn and lacustrin transported material. The soil had loamy texture (35.5% sand, 34.7 % silt, and 29.8% clay), 0,62% $CaCO_3$, 385.2 mmol kg^{-1} P_2O_5, 455.3 mmol kg^{-1} K_2O, 7.20 pH (H_2O) and 0.85 dS m^{-1} electrical conductivity. Two kg soils were transferred to polyethylene pots (20 cm diameter and 15 cm depth). Salt concentrations were initiated 45 days before sowing time. Eight salt sources (NaCl, Na_2SO_4, KCl, K_2SO_4, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$), four concentrations (To=0, T_1=30, T_2=60 and T_3=120 mM) and three doses of hydrogel (control, 0.05% and 0.1% w/w) were applied to soil. Hydrogel; the polymer used was Stockosorb K 410 (Stockhausen, Krefeld, Germany), a highly cross-linked polyacrilamide with about 40% of the amide group hydrolysed to carboxylic groups. After incubation period (45 days salt and Hydrogel application), soil samples were taken from each pot, then electrical conductivity (EC) was measured (Table 1). Seeds were sown in pots. The number of plants per pot was adjusted to three, 15 days after germination. Solution of basal fertilizers including 100 mg kg^{-1} N, 30 mg kg^{-1} P, 130 mg kg^{-1} K was given once a week at 10 days after sowing time. During the growth period, plants were regularly irrigated with pure water. Soil water content was carefully controlled. When 70% of useful water in the soil had consumed, pure water was applied to the soil and leakage from the pots was not allowed. After 105 days of salt treatment, the plants were harvested (60 days in growth media), measured, and analyzed. Soil samples were taken from the plant rhizosphere area from each pot, then pH and electricity conductivity (EC) were measured. Each pot (three plants) was considered as one replicate with three pots per treatment per salt. The experimental design was completely randomized block

Table 1. EC of the soil medium after application of different salt and hydrogel doses to soil at the end of 45 day incubation period.

Salt Doses mM	$CaCl_2$	$CaSO_4$	NaCl	Na_2SO_4	$MgCl_2$	$MgSO_4$	KCl	K_2SO_4
Electrical conductivity (dS m^{-1})								
Non treatment hydrogel								
T_0	0.84±0.04 d	0.84± 0.03 d	0.84 ± 0.08 d*	0.84 ± 0.04 d	0.83 ± 0.03 d	0.84 ± 0.03 d	0.84 ±0.01 d	0.84± 0.01 d
T_1	5.8± 0.01 c	5.20± 0.03c	6.20 ± 0.03 c	5.90 ±0.05 c	6.00± 0.05 c	6.00± 0.07 c	6.10± 0.04 bc	5.80± 0.02 c
T_2	9.90±0.02 ab	9.00± 0.09 b	10.90 ± 0.06 b	10.30 ±0.05 b	10.60 ±0.03 b	10.20 ±0.06 b	9.90± 0.04 b	10.20± 0.06 b
T_3	17.60±0.06 a	17.20± 0.10 a	18.40 ± 0.10 a	17.70 ± 0.09 a	18.20 ± 0.03 a	17.50 ± 0.07 a	18.30± 0.08 a	17.60± 0.05a
0.05% Hydrogel application								
T_0	0.76±0.05 d	0.73± 0.05 d	0.80 ± 0.08 d	0.74 ± 0.4 d	0.78 ± 0.04 d	0.75 ± 0.03 d	0.80 ±0.03 d	0.70± 0.01 d
T_1	4.2± 0.02 c	4.10± 0.06 c	6.00 ± 0.03 c	5.25 ±0.06 c	5.40± 0.04 c	4.00± 0.08 c	5.00± 0.04 c	3.80± 0.04 c
T_2	8.30±0.03 ab	8.00± 0.06 b	9.60 ± 0.06 b	8.70 ±0.08 b	9.00 ±0.09 b	8.15 ±0.10 b	8.75± 0.06b	7.70± 0.08 b
T_3	15.10±0.09 a	11.80± 0.10 a	16.20 ± 0.10 a	14.70 ± 0.09 a	15.90 ± 0.11 a	15.30 ± 0.12 a	15.10± 0.10 a	17.60± 0.10a
0.1 % Hydrogel application								
T_0	0.69±0.05 d	0.61± 0.05 d	0.77 ± 0.08 d	0.66 ± 0.04 d	0.72± 0.04 d	0.70 ± 0.03 d	0.75 ±0.04 d	0.60± 0.03 d
T_1	3.7± 0.03 c	3.90± 0.06 c	5.10 ± 0.03 c	3.90 ±0.07 c	3.60± 0.06 c	3.60± 0.06 c	4.55± 0.05 bc	2.85± 0.04 c
T_2	6.30±0.04 ab	5.85± 0.08 b	8.60 ± 0.06 b	5.32 ±0.09 b	6.60 ±0.08 b	6.60 ±0.09b	7.00± 0.10 b	5.40± 0.08 b
T_3	13.10±0.08 a	10.85± 0.10 a	14.50 ± 0.10 a	10.66 ± 0.10 a	13.80 ± 0.10 a	9.64 ± 0.12 a	13.30± 0.10 a	8.60± 0.10a

Values are means ± SE, at 1% level, of three replications with soil samples. To=0, T_1=30, T_2=60 and T_3=120 Mm.

design.

Enzymes analysis

Enzymes extraction

All operations were done at 4ºC. Cells (500 mg) of plant leaves were homogenised in a mortar with 3 ml of 50 mM phosphate buffer, pH 7. Homogenates were filtered through two layers of Miracloth and the filtrate was centrifuged at 15 000 × g for 15 min, at 4ºC. The resulting supernatant was stored at - 80ºC. For antioxidant enzyme assays, frozen cell samples were ground to a fine powder with liquid nitrogen and extracted with ice-cold 0.1 mM phosphate buffer, pH 7.8, containing 1 mM ethylenediaminetetraaceticacid (EDTA), 1 mM phenylmethanesulphonylfluoride (PMSF) and 0.5% polyvinylpyrrolidone (PVP). CAT, POX and SOD enzyme activities in the apoplastic fractions were measured spectrophotometrically. The CAT activity was measured by monitoring the decrease in absorbance at 240 nm in 50 mM phosphate buffer (pH 7.5) containing 20 mM H_2O_2. One unit of CAT activity was defined as the amount of enzyme that used 1 µmol H_2O_2/ min. The POX activity was measured by monitoring the increase in absorbance at 470 nm in 50 mM phosphate buffer (pH 5.5*)* containing 1 mM guaiacol and 0.5 mM H_2O_2. One unit of POX activity was defined as the amount of enzyme that caused an "increase in absorbance of 0.01/min. The SOD activity in apoplastic fractions was estimated by recording the- decrease in optical density of nitro-blue tetrazolium dye by the enzyme (Dhindsa et al., 1981). Three milliliter of the reaction mixture contained, 2 µM riboflavine, 13 mM methionine, 75 µM nitroblue tetrazolium chloride (NBT), 0.1 mM EDTA, 50 mM phosphate buffer pH 7.8), 50 mM sodium carbonate and 0.I ml the apoplastic fraction, Reaction was started by adding 60 µL from 100 µM riboflavine solution and placing the tubes under two 30 W fluorescent lamps for 15 min. A complete reaction mixture without enzyme, which gave the maximal color, served as control. Reaction was stopped by switching off the light and putting the tubes into dark, a non-irradiated complete reaction mixture served as a blank. The absorbance was recorded at 560 nm, and one unit of enzyme activity was taken as that amount of enzyme, which reduced the absorbance reading to 50% in comparison with tubes lacking enzyme (Sairam and Srivastava, 2002).

Electrolyte leakage

Electrolyte leakage was assessed as described by Lutts et al. (1996) using five young leaf discs for each treatment. Samples were washed three times with deionized water to remove surface-adhered electrolytes. Leaf discs were

placed in closed vials containing 10 ml of deionized water and incubated at 25 C on a rotary shaker for 24 h; subsequently electrical conductivity of the solution (Lt) was determined. Samples were then autoclaved at 120 C for 20 min and the last electrical conductivity (Lo) was obtained after equilibration at 25 C. The electrolyte leakage was defined as follows:

Electrolyte leakage (%) = (Lt/Lo) x100

Statistical analysis

Each pot was considered as a replicate and all of the treatments were repeated three times. A two-way analysis of variance (ANOVA) was performed using (SAS) statistical software (SAS, 1982) program.

RESULTS AND DISCUSSION

EC of the soil treated with different salt and hydrogel (HG) application was measured, after 45 day incubation period. EC value in the soil rose with increasing salt concentrations and the highest increasing rate in EC were determined at the highest doses (120 mM) of NaCl, KCl, $MgCl_2$ and $CaCl_2$, at non treatment HG application, respectively (Table 1) . On the other hand, EC value of the soil was lower in 0.05% and 0.1% HG application doses compared to the no- treatment HG (Table 1).

To understand the protective action of antioxidants against salinity stress, bean plants were treated with HG application followed by measurement of the level of antioxidant activity. Statistical analysis indicated a significant effect of salinity sources, doses and HG application on the antioxidant enzyme activity, electrolyte leakage (EL) and salt tolerance index (STI) of plants. As the salt dose increased, CAT enzyme activity of plants decreased for all types of salts and activity was more inhibited by NaCl than the other treatment. But, HG application treatment caused to increase the CAT activity of plant (Figure 1).

Even under optimal conditions many metabolic processes produce ROS. The production of toxic oxygen derivatives is increased as a result of all types of abiotic or biotic stresses. Plants possess efficient systems for scavenging active oxygen species that protect them from destructive oxidative reactions (Foyer et al., 1994). As part of this system, antioxidant enzymes are key elements in the defence mechanisms. Many changes have been observed in the activities of antioxidant enzymes in plants under salt stress. The activity of antioxidant enzymes has been reported to increase under saline conditions in the case of salt-tolerant wheat shoot (Meneguzzo et al., 1999) and pea (Hernandez et al., 1999), but decreases in wheat roots (Meneguzzo et al., 1999), or is unaffected as in the case of SOD in cucumber (Lechno et al., 1997). Increases in GPX, APX, DHAR and GR activity in the leaves after 10-day treatment were observed in the present experiment under salt stress, which was consistent with the results of Lechno et al. (1997) who have described an increase in GR activity in cucumber. However, decreases in CAT activity after treatment in the present experiment were not consistent with the results of Lechno et al. (1997) who have observed an increase in CAT activity and no effect on SOD activity in cucumber. However, the effects of salt stress on the antioxidant enzymes are very complex and depend on the treatment time, plant species and genotypes. In the present experiment the difference in effects of salt stress on the antioxidant enzymes activity has been also observed with treatment time and genotype.

Increasing salinity stress significantly increased enzyme activity, including POD and SOD, of bean leaves compared to the control in the experiment. Application of HG under salinity stress decreased enzyme activity with increasing salinity stress. It is interesting to note that though a significant interaction was found, yet treatment with HG application tended to reduce the salinity stress effect on the activity of the two enzymes. POD and SOD enzyme activity was found to be increased in the leaves of bean plants grown at high salinity condition compared to the control. The highest POD and SOD enzyme activity values were observed from $CaCl_2$ and NaCl, respectively. However, HG applications treatment ameliorated salt effect and decreased this enzyme activity (Figures 2 and 3).

The activity of antioxidant enzymes was reported to increase under salinity in wheat shoot (Meneguzzo et al., 1999; Sairam and Srivastava, 2002) and pea (Hernandez et al., 1999). Most of the results of the study conducted here show a correlation between the resistance to NaCl stress and more effective antioxidative system. The observed increase in SOD activity (Figure 2) could increase the ability of the seedlings to scavenge O_2 - radicals, which could cause membrane damage. At higher NaCl concentration (120 mM) it seems that such resistance to oxidative stress may be overcome leading to growth reductions (Pandey and Agarwal, 2002). Increase in POD activity is supposed to be an adaptive trait possibly helping to overcome the damage to the tissue metabolism by reducing toxic levels of H_2O_2 produced during cell metabolism and protection against oxidative stress (Sudhakar et al., 2001; Bor et al., 2003). Similarly in the present study, the salt induced enhancement of POD activity (Figure 3) may suggest its effective scavenging mechanism to remove H_2O_2 and imparting tolerance against NaCl oxidative stress. The POX and PPO are the two major enzymes responsible for oxidation of phenolic compounds. It seems possible that oxido-reductases POD and PPO may play an important role as defense against salt stress.

The highest EL amount was observed with NaCl 60 (T_3) mM doses at no HG application treatment, and the lowest was determined 30 mM K_2SO_4 at 0.1% HG application treatment. The increasing in EL of plant positively

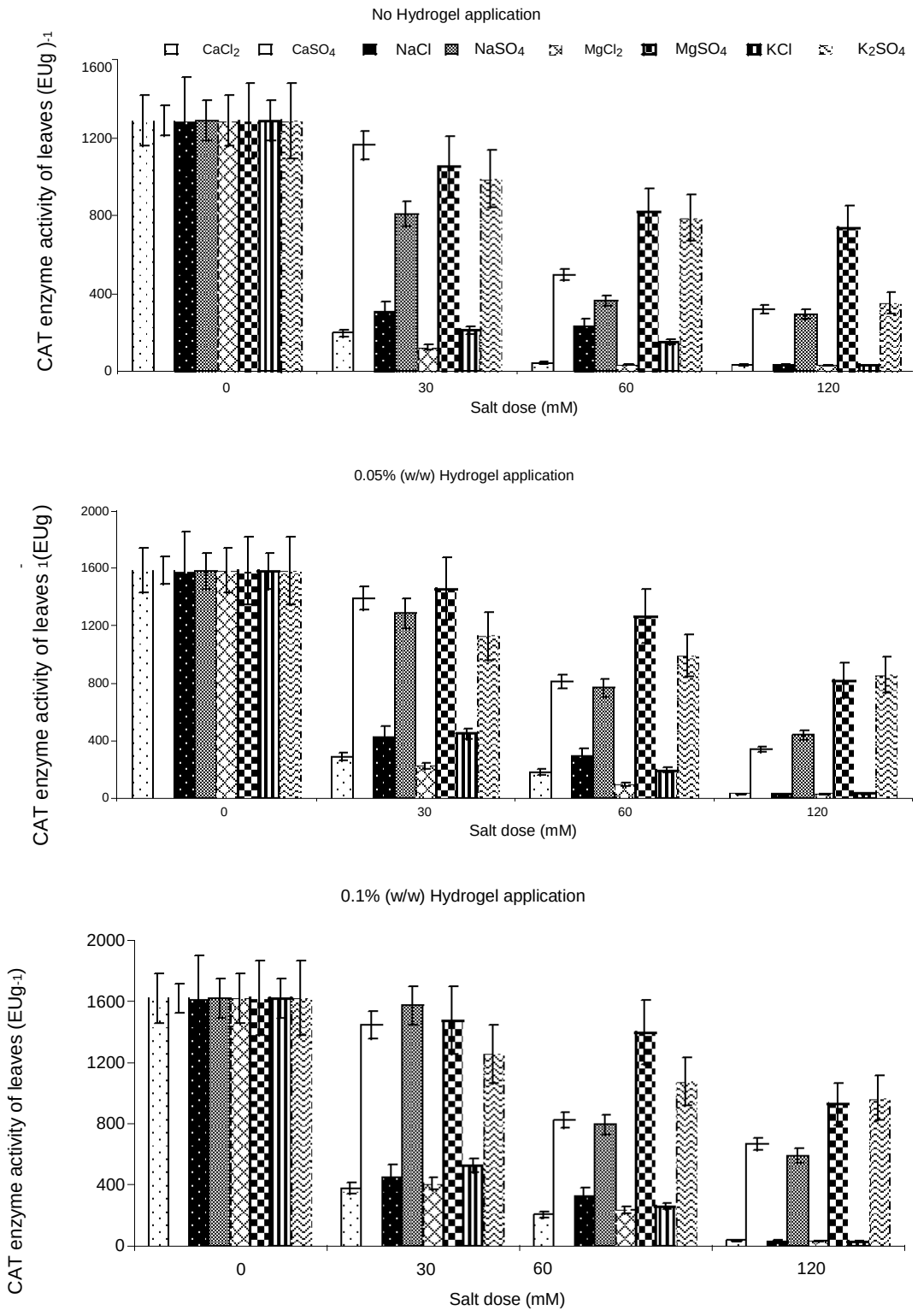

Figure 1. Effects of application of different salt concentrations and HG on plant Catalase (CAT) enzyme activity.

correlated to the level of salt but negatively related to HG application doses (Figure 4). But, supplied HG ameliorated this leakage partly, but the values were still higher compared to by Lutts et al. (1996), Villora et al. (2000), and Turan and Aydın (2005) who reported that high salt concentration increased the membrane

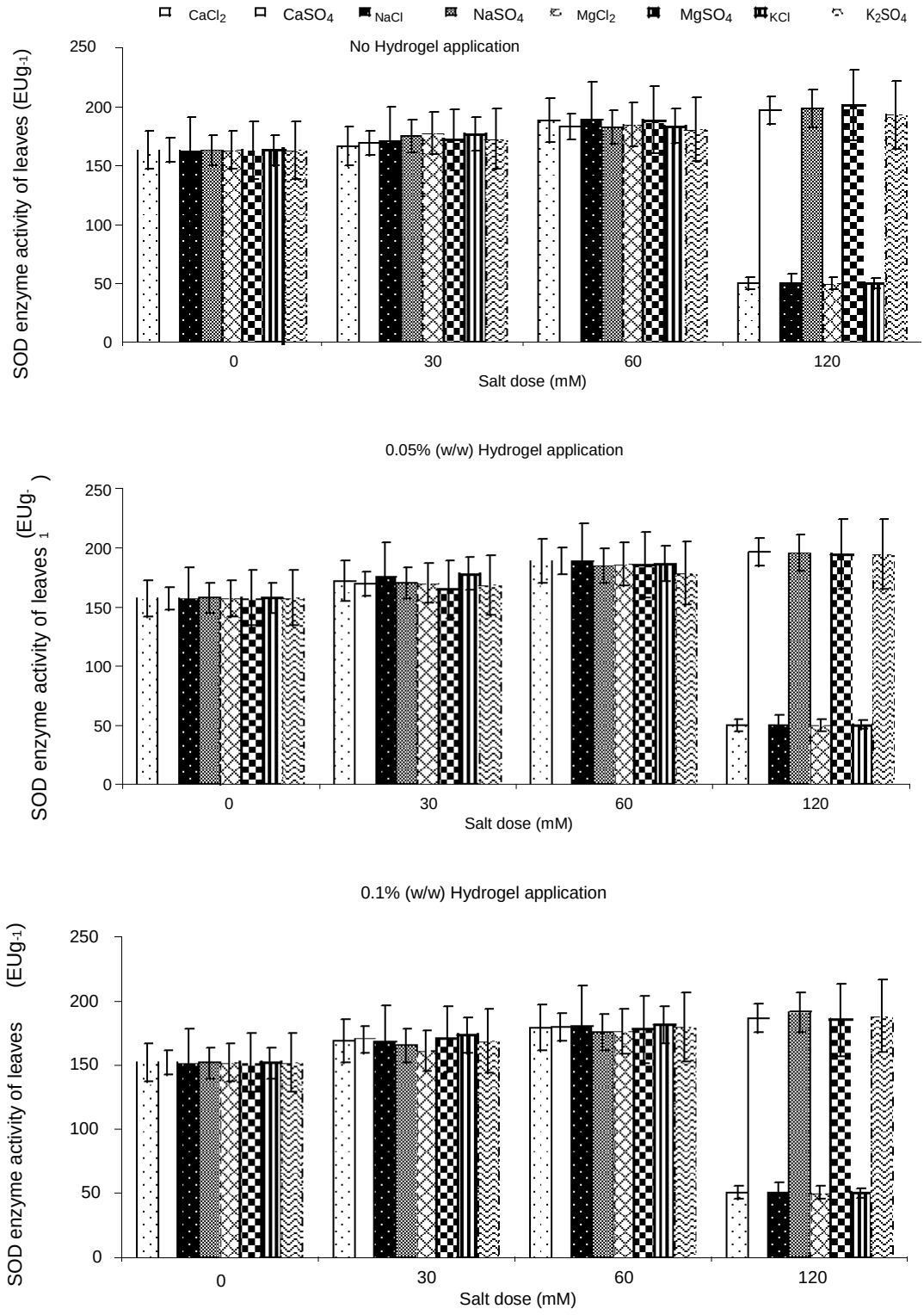

Figure 2. Effects of application of different salt concentrations and HG on plant superoxide dismutase (SOD) enzyme activity.

permeability, and proline content of rice varieties, *Lycopesicon esculentum L*., *Cucurbita pepo L. var*. moshota, and corn, respectively.

Salt tolerance index (STI) decreased with increasing salt doses for all of the salt sources. The highest STI was observed with 0.1 HG and no salt treatment (T_1) mM

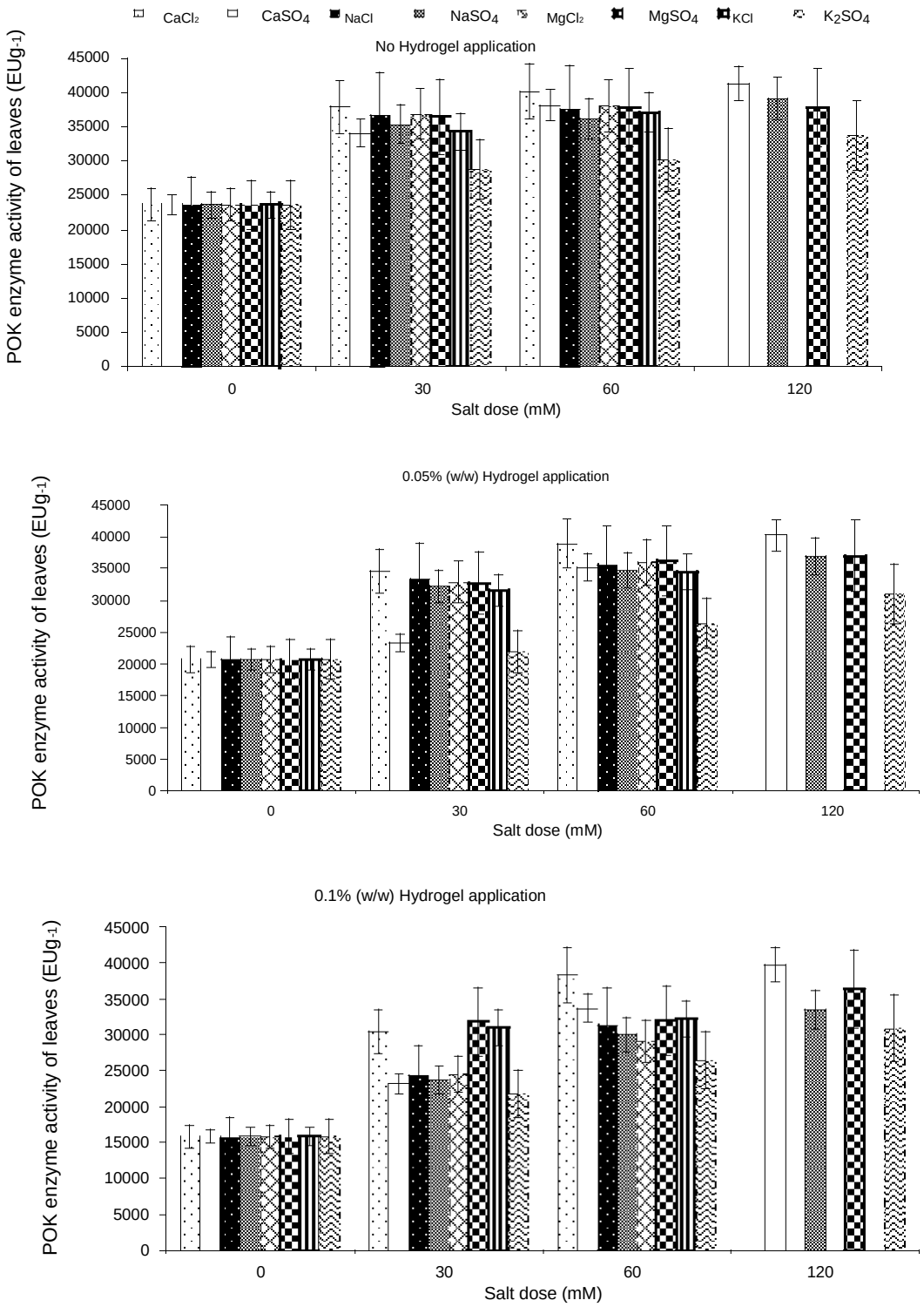

Figure 3. Effects of application of different salt concentrations and HG on plant peroxidase (POX) enzyme activity.

doses and the lowest the 120 mM NaCl and no HG application treatment (Figure 5) . However, HG applications treatment ameliorated salt effect and increased STI of plant.

Saline soils and saline irrigations constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth. The present study demonstrates salinity stress induced lower

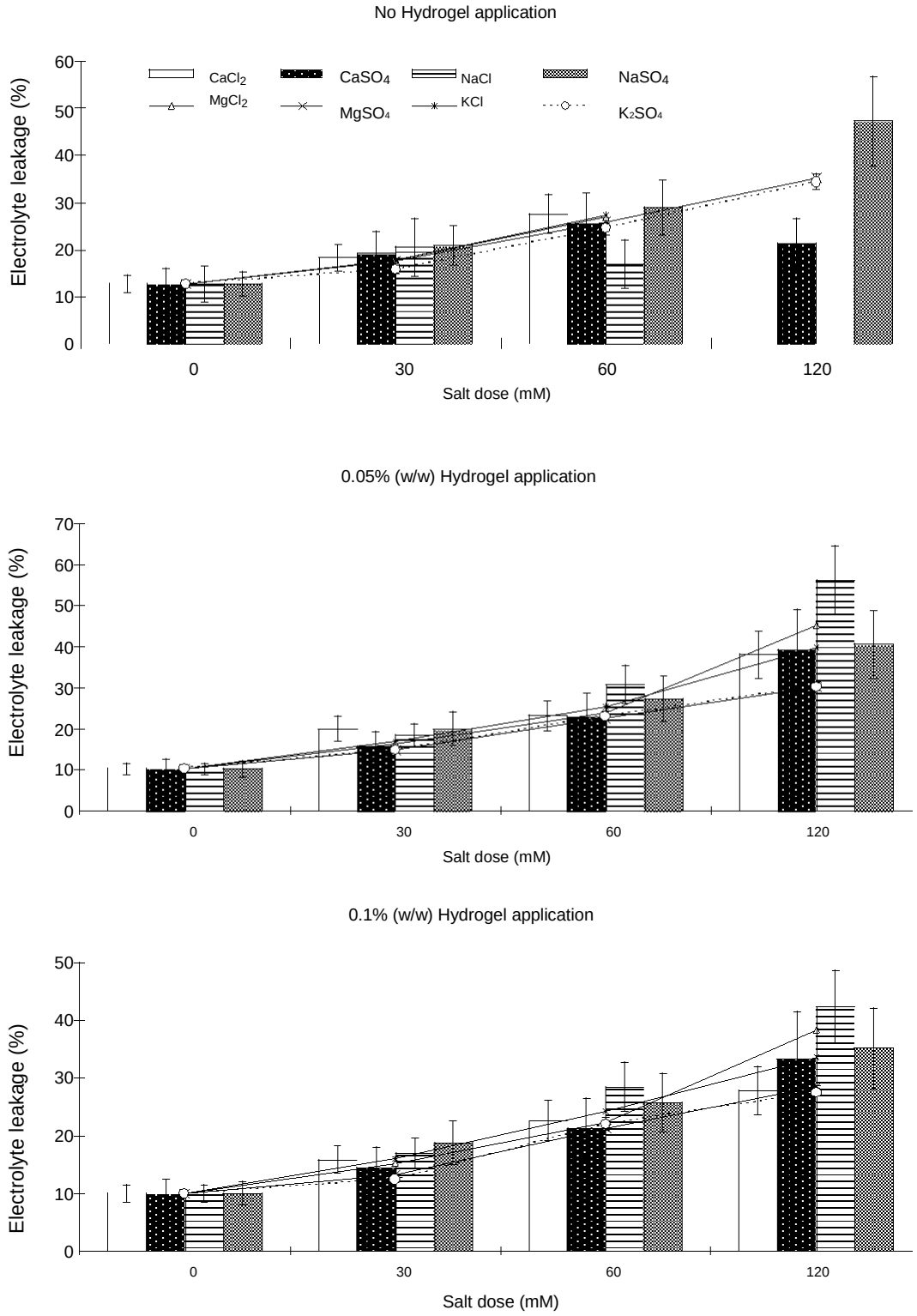

Figure 4. Effects of application of different salt concentrations and HG on plant electrolyte leakage.

biomass production and high SOD and POD enzyme activity of plant. The assessment of the effect of salinity on the growth parameters by different salt sources and doses enabled us to conclude that all of the considered parameters were affected by salinity. Ion balance in the soil solution is very important for plant growth and tolerance of salinity. Plant SOD and POD activity, electrolyte leakage and STI were negatively related to

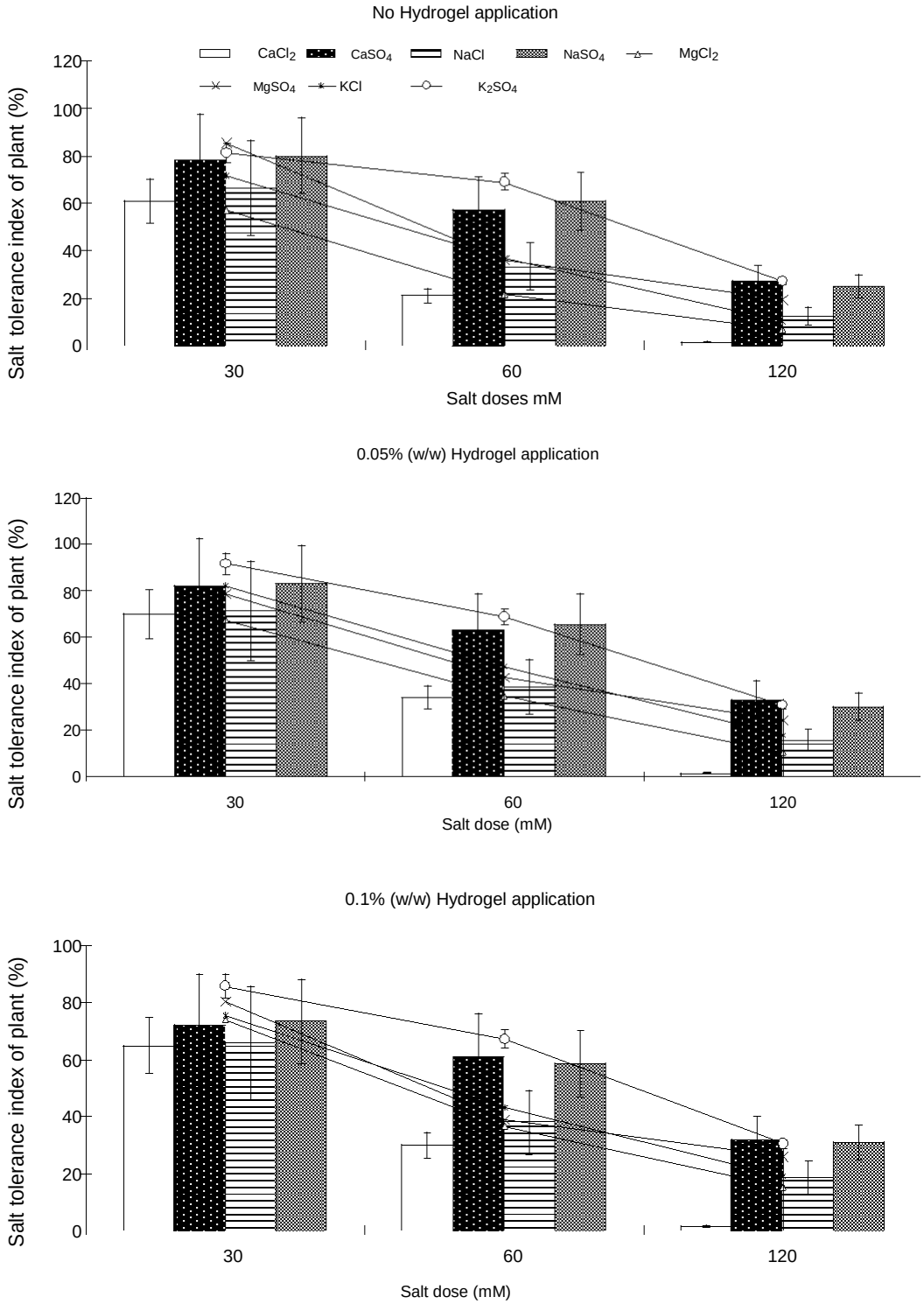

Figure 5. Effects of application of different salt concentrations and HG on salt tolerance index.

salt doses, but CAT enzyme activity of plants was positively correlated to the level of salt doses. The under stress condition, bean plants have evolved complex mechanisms allowing for adaptation to osmotic and ionic stress caused by high salinity. In the presence of $NaCl_2$ and KCl, $CaCl_2$ and $MgCl_2$ salt concentration in the soil solution, bean plant showed higher decrease rate, plant yield and growth parameters than the SO_4 salts in the soil solution. This can be achieved, to some extent, by the application of hydrophilic polymer soil amendments.

HG added to saline soil significantly improved the variables affected by high salinity and also decreased plant antioxidant enzyme activity, reduced soil salinity, enhanced plant growth by allowing nutrients, incorporated into the HG matrix, to release to plant as needed.

Conclusion

Our data indicate that salt induced an oxidative stress in *Phaseolus* vulgar cells, despite the concomitant decrease in antioxidant enzymes and increase in the STI of plant. This increase could reflect a defence response to the cellular damage provoked by NaCl treatment. Moreover, this decreased in antioxidant activities, which was not strong enough to eliminate all the deleterious effects provoked by salt, only alleviated the impact of stress, thus allowing cell growth to occur.

The addition of HG could offer an economical and simple application to salt sensitive plant of bean production problems in aridisol caused by high salinity but further studies are required in order to determine the efficiency of these materials under natural field condition.

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