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Full Length Research Paper

Humic acid application alleviate salinity stress of bean (*Phaseolus vulgaris* L.) plants decreasing membrane leakage

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The effect of varying humic acid supplies (0, 0.05 and 0.1% w/w) on some agro-physiological properties and ionic balance of bean plants in different salt source and doses were investigated. Plants were treated with eight salt sources [sodium chloride (NaCl), sodium sulphate (Na_2SO_4), calcium chloride ($CaCl_2$), calcium sulphate ($CaSO_4$), potassium chloride (KCl), potassium sulphate (K_2SO_4), magnesium chloride ($MgCl_2$) and magnesium sulphate ($MgSO_4$)] in four different concentrations (0, 30, 60, and 120 mM doses) for 60 days in a growth media. The highest salt doses; 120 mM of NaCl, $CaCl_2$, $MgCl_2$ and KCl_2 at no humic acid applications caused plant death, but no plant death was obtained in humic acid application (0.05 and 0.1%) doses in all of the salt types and doses except for $CaCl_2$. Total chlorophyll and nitrate contents of plants decreased with increasing salt doses, but were negatively related to humic acid application doses. Proline contents of plant were increased with increasing salt doses and the highest value was obtained for NaCl application. The effects of salt concentrations in nitrogen and phosphorus content of plants were significant. Humic acid added to saline soil significantly improved the variables affected by high salinity and also increased plant nitrate, nitrogen and phosphorus, reduced soil electricity conductivity, proline and electrolyte leakage of plant, enhanced plant root and shoot dry weight by allowing nutrients and water to be released to the plant as needed. The result suggested that humic acid have great potential in alleviating salinity stress on plant growth and growth parameter in saline soils of arid and semi-arid areas. This humic acid appeared to be highly effective for soil conditioners in vegetable growth, to improve crop tolerance and growth saline conditions.

Key words: Arid soil, electrolyte leakage, leaf area, nitrate, proline, total chlorophyll, salt stress.

INTRODUCTION

Salinity is a major abiotic stress, reducing the yield of wide variety of crops all over the world (Tester and Davenport, 2003; Ashraf and Foolad, 2007). Worldwide, 100 million ha or 5% of the arable land is adversely affected by high salt concentration which reduces crop growth and yield (Heuer, 1994; Ghassemi et al., 1995). 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 may occur when there is irregular irrigation, inadequate drainage, wrong fertilizer application and it extremely increases particularly in protected cultivation (Tekinel and Çevik, 1983; George et al., 1997; Wang et al., 2003). Plants growing in saline media come across generally with major drawbacks. The first is the increase in the osmotic stress due to high salt concentration of soil solution that decreases water potential of soil. The second is the increase in concentration of sodium (Na) and chloride (Cl), exhibiting tissue accumulation of Na and Cl, and inhibition of mineral nutrients uptake (Marschner, 1995). For overcoming the negative effect of salinity, the addition of supplemental organic matter (Walker and Bernal, 2004, 2008), different source of nitrogen (Frechilla et al., 2001), calcium (Tuna et al., 2007)

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and potassium (Türkmen et al., 2000) to growth media as an ameliorative agent could be necessary. The general effect of soil salinity on plants is called a physiological drought effect. High salt content decrease the osmotic potential of soil water, this reduces the availability of soil water for plants (Aşik et al., 2009). Therefore, due to insufficient rainfall and irrigation water in arid areas, can be detrimental to plant growth and development (Huang and Gao, 1999; Wang et al., 2003). Thus, the agricultural areas affected by salt need amendments such as determination of the most suitable salt tolerant plant species (Abrol et al., 1988) or an alternative way is the use of high water holding capacity and organic - inorganic groups as a possible solution for conserving irrigation and rainwater in such arid and semi-arid region in order to reduce the effects of salinity (Lynch and Lauchli, 1985).

Kulikova et al. (2005) and Xudan (1986) also indicated that humic substances might show anti-stress effects under abiotic stress conditions such as unfavourable temperature, salinity, pH, etc. The major functional groups of humic substance include carboxyl, phenolic hydroxyl, alcoholic hydroxyl, ketone and quinoid (Russo and Berlyn, 1990). Humic substances are well known as stimulators of plant germination and growth (Dell'Amico et al., 1994; Garcia et al., 1992). In particular, they increase membrane permeability, facilitate transport of essential elements within roots and favor respiration. As indicated by Vaughan (1985), humic substances act in a very similar way to growth hormones. The mechanism of humic acid in promoting plant growth may enhance the uptake of nutrients and reduce the uptake of some toxic elements. However, increasing cell membrane permeability, oxygen uptake, respiration, photosynthesis, phosphate uptake and root cell elongation of plant growth factors have been proposed by some authors to explain positive effect of humic acid (Vaughan, 1974; Cacco and Dell Agnolla, 1984; Russo and Berlyn, 1990; Masciandaro et al., 2002). On the other hand, humic acid has beneficial effects on nutrient uptake by plants and was particularly important for transportation and availability of micro nutrient (Bohme and Thi, 1997). *Phaseolus vulgaris* L., the common bean is an important source of protein and other nutrients in many developing countries (CIAT, 1992). Of the 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 ha with an annual production of 17.5 million tons (FAO, 2002). Approximately, 20 to 30% of bean production area in the Middle East, 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 due to limited water uptake (Lauchli, 1984).

Plants overcome this difficulty by increasing the concentration of proline accumulation in plants exposed to salt; water stress has been correlated in many species with their adaptation to osmotic stress. Complex molecular responses including the accumulation of compatible solutes, the production of stress proteins, and the expression of different sets of genes are part of the plant signaling and defense system against salinity (Hasegawa et al., 2000; Sairam and Tyagi, 2004). It is well known that, one of the most common responses to water deficit and saline environments is the accumulation of proline which acts as a compatible solute, an osmoprotectant, and a protective agent for cytosolic enzymes and cellular organelles (Taylor, 1996; Demir and Oztürk, 2003; Turan and Aydın, 2005; Jimenez-Bremont et al., 2006). Additionally, proline is a nitrogen source available for the recovery from stress and for restoration of growth (Trotel et al., 1996). Salt-induced proline accumulation is often a late response, appearing only when cell injury is evident and elevated levels of proline are maintained long enough after stressed tissues return to normal osmotic conditions (Trotel et al., 1996). The objective of this study was to investigate mitigation effect of humic acid on growth, inorganic ions, proline and nitrate contents of bean plants under different salinity stress condition.

MATERIALS AND METHODS

Plant material and growth conditions

Bean plants (*P. vulgaris* L.) were grown under the following controlled greenhouse conditions with 25 to 30/10°C day/night temperatures, 30 to 40% relative humidity in Erzurum (Turkey). Day length was approximately 14 h during the experimental period. The soil samples were taken from the 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 the analyses of the 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 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. 2 kg soils were transferred to polyethylene pots (20 cm diameter and 15 cm depth). Salt concentrations were initiated 45 days before the sowing time. Eight salt sources (NaCl, Na_2SO_4, KCl, K_2SO_4, $CaCl_2$, $CaSO_4$, $MgCl_2$, $MgSO_4$), four concentrations (0, 30, 60 and 120 mM) and three doses of humic acid (control, 0.05 and 0.01% w/w) were applied to the soil. Humic acid, K-humate (Proxin 85) contained 75% humic and fulvic acid and 20% water soluble K_2O. After the incubation period (45 days salt and humic acid application), soil samples were taken from each pot, then electricity conductivity (EC) was measured (Figure 1). Seeds were sown in pots. The number of plants per pot was adjusted to three, 15 days after the germination. Solution of basal fertilizers including 100 mg kg^{-1} N, 30 mg kg^{-1} P and 130 mg kg^{-1} K were 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 been 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

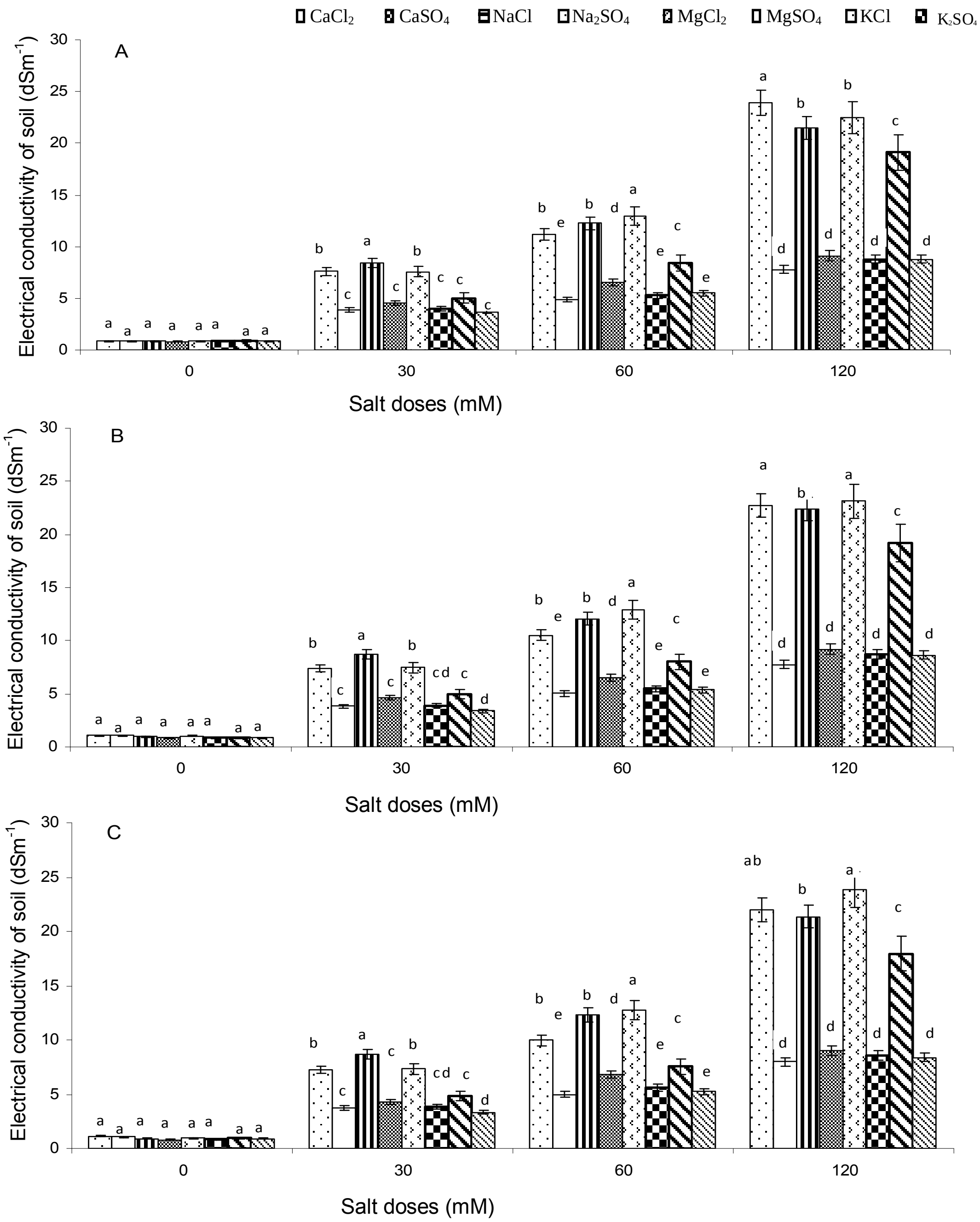

Figure 1. Effects of humic acid (HA) on soil electric conductivity at different salt concentrations (A: no HA application, B: 0.05% HA application and C: 0.1% HA application).

and electricity conductivity (EC) were measured. Each pot (three plants) was considered as one replicate with three pots per treatment per salt. The experiment design was completely randomized block design.

Growth parameters

60 days after sowing, tree plants from each replicate were harvested, and data on plant shoot and root dry weight were collected and dried at 70°C for 48 h.

Leaf area measurement

Leaf area of plant was determined with CI-202 portable leaf area meter.

Plant analysis

In order to determine the mineral contents of leaves, plant samples were collected from fully expanded leaves at 60 days and oven dried at 68°C for 48 h. It was then ground and passed through a 1 mm size sieve. Total nitrogen was determined using the micro-Kjeldahl method. The tissues sampled of plant were oven-dried at 68°C for 48 h and ground. Potassium (K) and phosphorus (P) were determined after the wet digestion of dried and ground sub-samples using a HNO_3-$HClO_4$ acid mixture (4:1 v/v) (AOAC 922.02, 2005). Phosphorus in the extraction solution was measured spectro-photometrically using the indophenol-blue and ascorbic acid method (AOAC, 2005) and a UV/VIS Aqumat Spectrophotometer (Thermo Electron Spectroscopy LTD, Cambridge, UK). Potassium analysis was determined by atomic absorption spectrometry using a Perkin–Elmer 360 Atomic Absorption Spectrophotometer (Perkin-Elmer, Waltham, Massachusetts, USA) (AOAC, 2005).

The nitrate content in the leaves was estimated as described by Agbaria et al. (1996). 100 mg fresh weight of leaf samples were extracted for 60 min in deionized water at 45°C. After centrifugation at 6000 x *g* for 15 min, 200 µl of the supernatant were incubated at ambient temperature (about 24°C) with 0.8 ml 5% salicylic acid in concentrated sulphuric acid for 20 min. After 12 min, the samples were cooled to ambient temperature and the coloration was measured spectrophotometrically at 410 nm by a UV/VIS Aqumat Spectrophotometer (Thermo Electron Spectroscopy Limited, Cambridge, UK). The nitrate content was determined using a standard curve established with the solution of KNO_3. The proline content was determined using the method of Bates et al. (1973). Proline was extracted from leaf samples of 100 mg fresh weight with 2 ml of 40% methanol. 1 ml extract was mixed with 1 ml of a mixture of glacial acetic acid and orthophosphoric acid (6 M) (3:2 v/v) and 25 ml ninhydrin. After 1 h incubation at 100°C, the tubes were cooled and 5 ml toluene was added.

The absorbance of the upper phase was spectrophotometrically determined at 528 nm by a UV/VIS Aqumat Spectrophotometer (Thermo Electron Spectroscopy Limted, Cambridge, UK). The proline concentration was determined using a standard curve. For the chlorophyll analysis, fresh leaf samples were cleaned with deionized water to remove any surface contamination. Chlorophyll extraction was carried out on fresh fully expanded leaf material. 1 g leaf sample was ground in 90% acetone using a pestle and mortar. The absorbance was measured with a UV/VIS Aqumat Spectro-photometer (Thermo Electron Spectroscopy Limited, Cambridge, UK) and chlorophyll concentrations were calculated using the equation proposed by Strain and Svec (1966):

$$\text{Chl a (mg l}^{-1}) = 12.7\,A_{663} - 2.69\,A_{645} \quad (1)$$

$$\text{Chl b (mg l}^{-1}) = 22.9\,A_{645} - 4.68\,A_{663} \quad (2)$$

Where, A663 and A645 represent absorbance values read at 663 and 645 nm wavelengths, respectively.

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:

$$\text{Electrolyte leakage (\%)} = \frac{Lt}{Lo} \times 100$$

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 SPSS 13.0 statistical software (SPSS Inc., 2004).

RESULTS AND DISCUSSION

Electric conductivity (EC) of the soil treated with different salt and humic acid (HA) application was measured after 45 days incubation period. EC value in the soil rose with increasing salt concentrations and the highest increasing rate of EC were determined at the highest doses (120 mM) of NaCl, KCl, $MgCl_2$ and $CaCl_2$ at non treatment of HA application, respectively (Figure 1). On the other hand, the EC value of the soil were lower in 0.05 and 0.1% HA application doses compared to the non treatment of HA (Figure 1). Alleviated effects of HA at 0.1% application doses were 9.10, 1.0, 2.0, 3.0, 1.0, 7.0 and 6.0% for $CaCl_2$, $CaSO_4$, NaCl, $NaSO_4$, $MgCl_2$, $MgSO_4$, KCl_2 and K_2SO_4 when compared to the non treatment of HA application, respectively.

Salt type and doses affected plant shoot and dry matter (Figure 2). As the salt dose increased, plants shoot and root dry matter decreased for all types of salts; and shoot growth was more inhibited by NaCl than root growth. Previous studies carried out with cotton and *Prosopis alba* (Meloni et al., 2001, 2004) and with soybean and alfalfa (Bernstein and Ogata, 1966; Kant et al., 1994), corn (Turan and Aydin, 2005) also showed that shoot growth was more inhibited by NaCl than root growth. Humic acid (HA) application to the soil was ameliorated to the adverse effects of salinity on the shoot and root dry matter. The highest salt doses (120 mM) of NaCl, $CaCl_2$, $MgCl_2$ and KCl_2 at no HA applications caused plant death, but no plant death was obtained in HA application (0.05 and 0.1%) doses of all the salt types and doses except for $CaCl_2$. The reduction of shoot and root dry matter was higher in Cl salinity (NaCl > $MgCl_2$ > KCl) at 120 mM than SO_4 salinity (Na_2SO_4 > $MgSO_4$ > K_2SO_4) in HA application (0.05 and 0.1%) doses. When HA was applied to the soil at 0.05 and 0.1% under salinity stress, shoot and root dry matter reduction remained. This ameliorative effect of HA at 0.05 and 0.1% doses on shoot and root dry matter was observed over the 100% in NaCl, $MgCl_2$ and KCl treatment, because plants death did not occur. Plant shoot and root dried due to the increased HA doses (from 0 to 0.1% doses) under $CaSO_4$, Na_2SO_4, Mg_2SO_4, and K_2SO_4 salinity stress; these increasing ratio was 8.6 to 35.5, 10 to 30, 21.5 to 55.4 and 18.4 to 13.2% for shoot dry weight, and 25 to 111, 3.1 to 75, 7.4 to 20.4 and 26.2 to 57.4% for root dry, respectively. This effect may be attributed to the fact that HA became absorbed many times their weight of water which diluted the salt effect and store it for relatively long period of time.

HA were found to promote soil water holding capacity and reduce watering requirements for plants (Orzolek, 1993; Hynes and Naidu, 1998). Some studies reported that HA can be used as a growth regulator to regulate

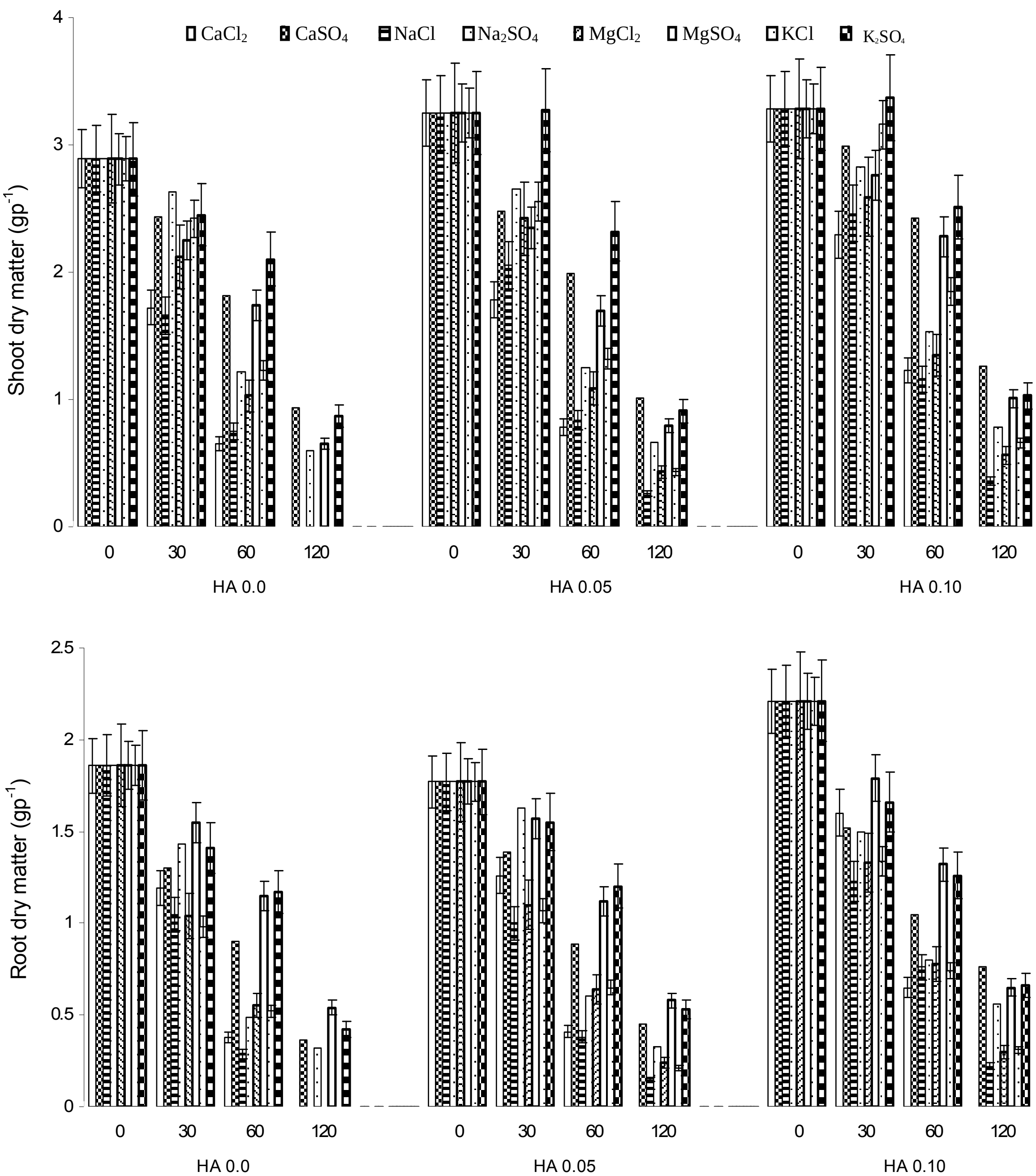

Figure 2. Effects of humic acid (HA) application on plant shoot and root dry matter under different salt growth concentrations (HA 0.0: no HA application, HA 0.05: 0.05% HA application, HA 0.10: 0.1% HA application).

hormone level, improve plant growth and enhance stress tolerance (Piccolo et al., 1992). HA may stimulate shoot and root growth, and improve resistance to environmental stress in plant, but the physiological mechanism has not been well established (Delfine et al., 2005). Masciandaro et al. (2002), Pilanali and Kaplan (2003) and Türkmen et al. (2005) suggested that HA application mitigate the salinity effect in strawberry, maize and pepper seedlings in salty condition. Statistical analysis indicated a significant effect of salinity sources, concentration and HA application on the proline content (PC) and electrolyte leakage (EL) in plants (Figures 3 and 4). Both PC and EL were increased in the leaves of bean plants grown at high salinity condition compared to the control. The highest salt concentration (120 mM) of NaCl, $CaCl_2$, $MgCl_2$ and KCl at no HA applications treatment caused plant death; thus, the 120 mM application was not considered in the evaluation of plant PC and EL. The highest PC and EL amount was observed with NaCl of 60 mM concentration at no HA application treatment and the lowest was

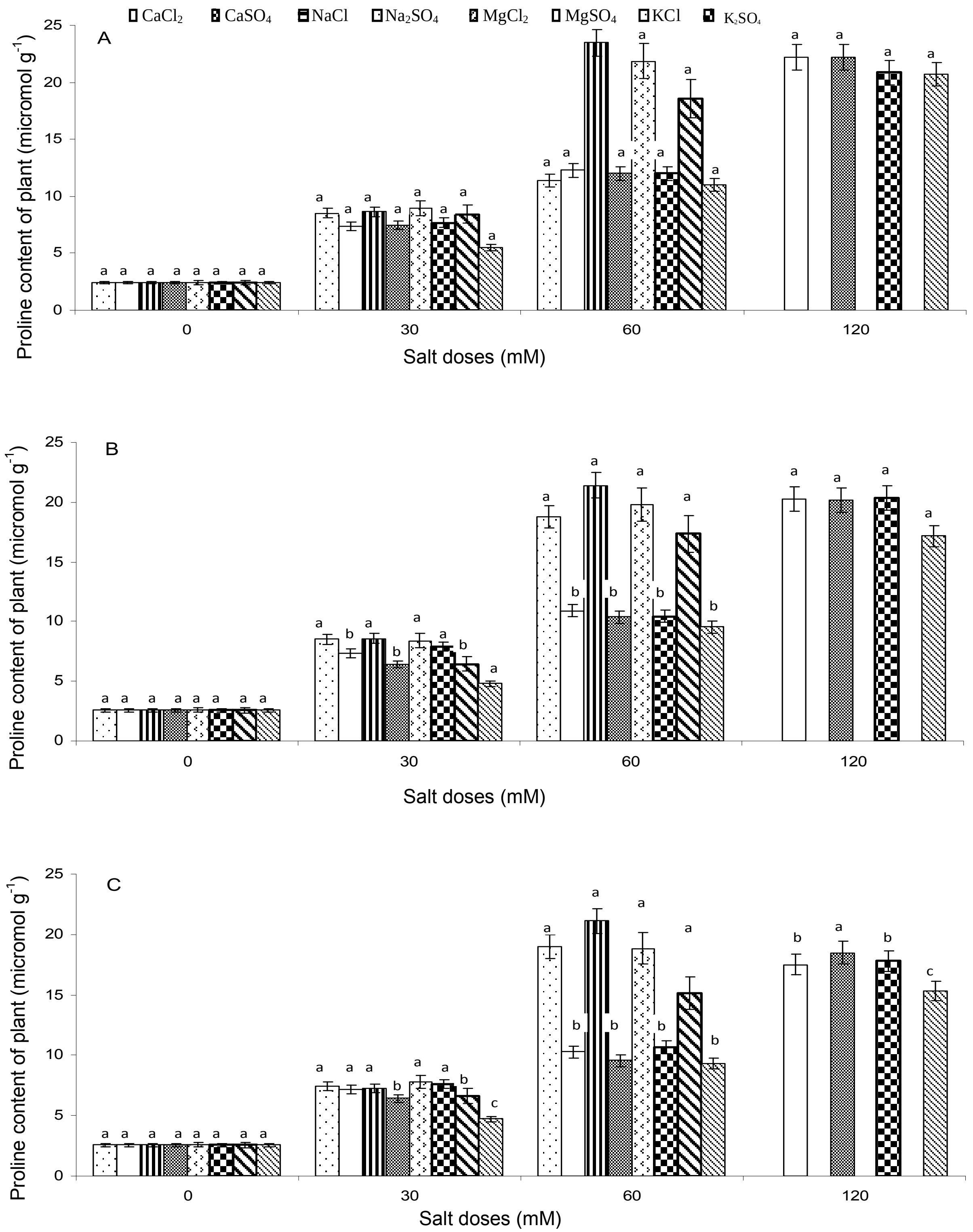

Figure 3. Effects of humic acid (HA) application on plant proline content under different salt growth concentrations (A: no HA application, B: 0.05% HA application, C: 0.1 % HA application).

observed with 30 mM K_2SO_4 at 0.1% HA application treatment. The increase in PC and EL of plant positively correlated to the level of salt but negatively related to HA application concentration. HA application at 0.05 and 0.1% concentration for the highest salt stress condition (120 mM) decreased plant leaves PC at the rate of 8.7 to

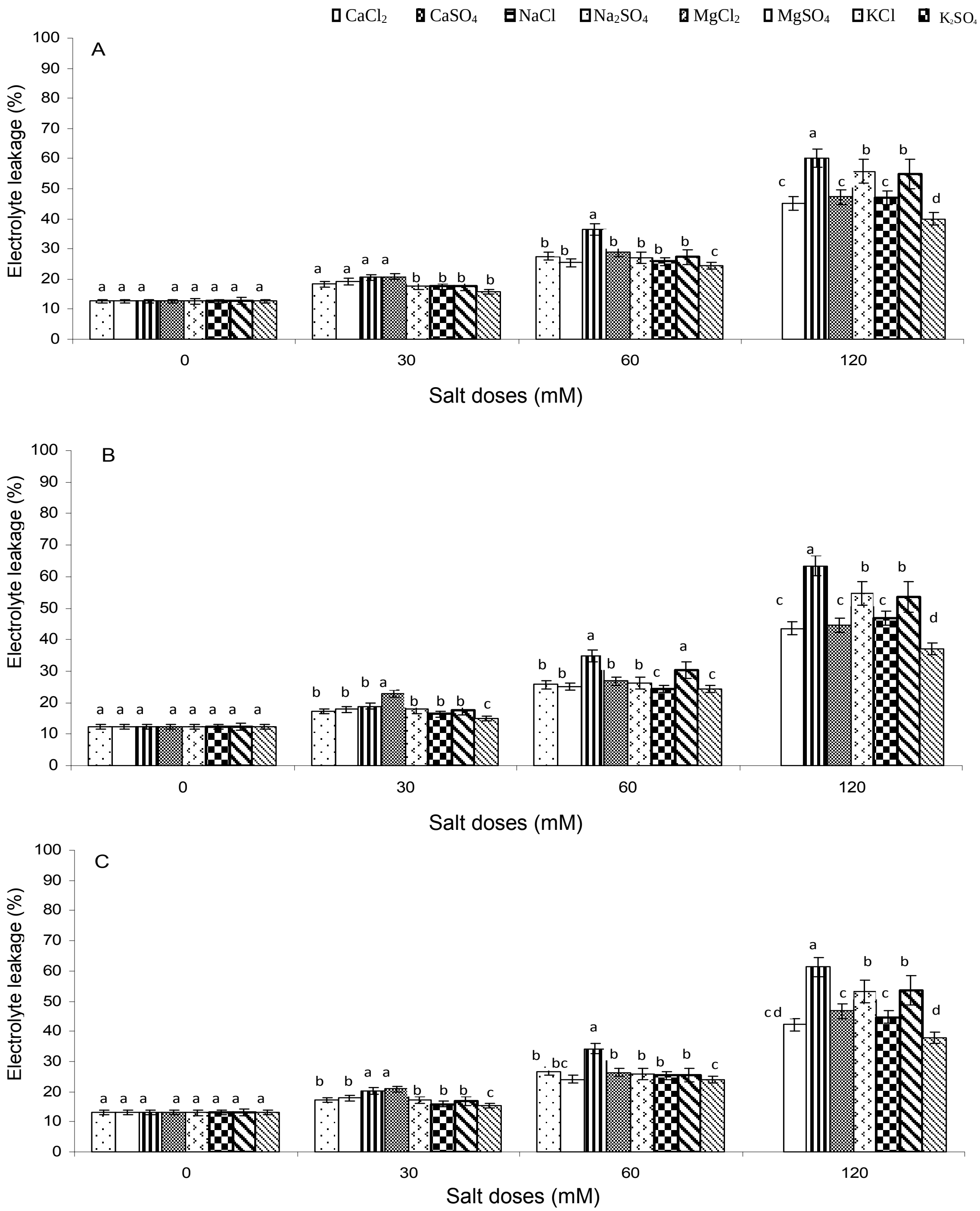

Figure 4. Effects of humic acid (HA) application on plant electrolyte leakage under different salt growth concentrations (A: no HA application, B: 0.05% HA application and C: 0.1% HA application).

21.0, 9.1 to 16.7, 1.0 to 14.6 and 17.2 to 26.1% for $CaSO_4$, $NaSO_4$, Mg_2SO_4 and K_2SO_4 when compared to no HA application treatment with highest salt stress condition (120 mM), respectively.

Similarly, EL decreasing rate of plant leaves was 14.3 to 4.5, 1.2 to 5.9, 1.0 to 4.7 and 4.9 to 5.1% for $CaSO_4$, $NaSO_4$, Mg_2SO_4 and K_2SO_4 when compared to no HA application treatment with highest salt stress condition (120 mM), respectively. But, supplied HA ameliorated this leakage partly and decreased proline content, but the values were still higher compared to Lutts et al. (1996), Inal et al. (1997), Villora et al. (2000), Kaya et al. (2002) and Turan and Aydin (2005) who reported that high salt concentration increased the membrane permeability, and

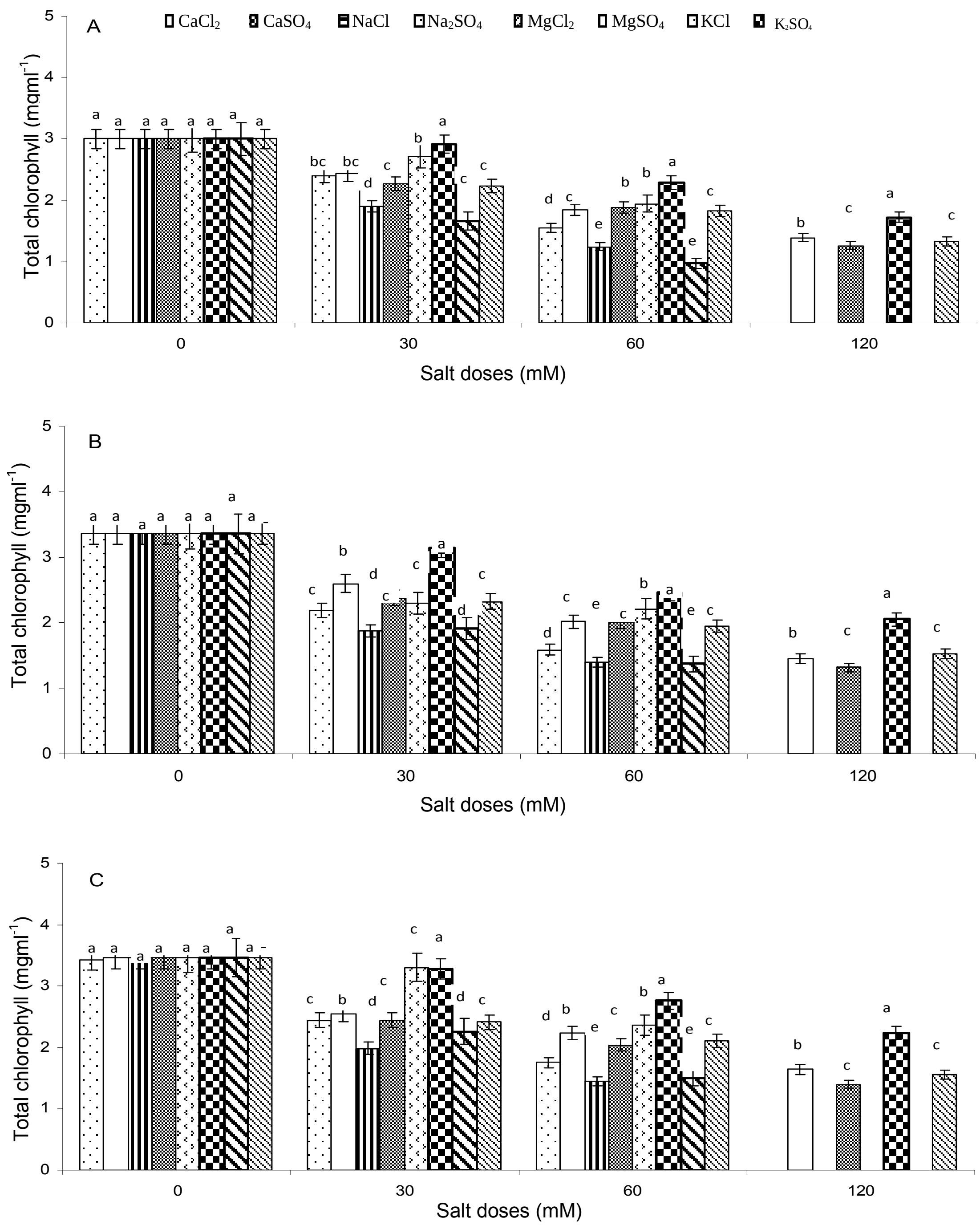

Figure 5. Effects of humic acid (HA) application on total chlorophyll content of plant under different salt growth concentrations (A: no HA application, B: 0.05% HA application and C: 0.1% HA application).

proline content of rice (*Oryza sativa* L.), tomato (*Lycopesicon esculantum* L.), zucchini (*Cucurbita pepo* L. var. Moshota), strawberry (*Fragaria ananassa* L.) and corn (*Zea mays* L.), respectively. Leaf total chlorophyll content (LTCC) decreased with increasing salt concentration for all of the salt sources but negatively related to HA application concentration (Figure 5). HA application at 0.05 and 0.1% concentration for the highest salt stress condition (120 mM) increased LTCC at the rate of 5.0 to 17.9, 4.8 to 11.1, 19.2 to 29.7 and 14.3 to 17.3% for $CaSO_4$, $NaSO_4$, Mg_2SO_4 and K_2SO_4 when compared to no HA application with highest salt stress treatment (120 mM), respectively. Decrease in chlorophyll concentration in salinized plants could be attributed to increasing activity of chlorophyll-degrading enzyme chloroplast (Reddy and Vora, 1986). Ion accumulation in leaves also

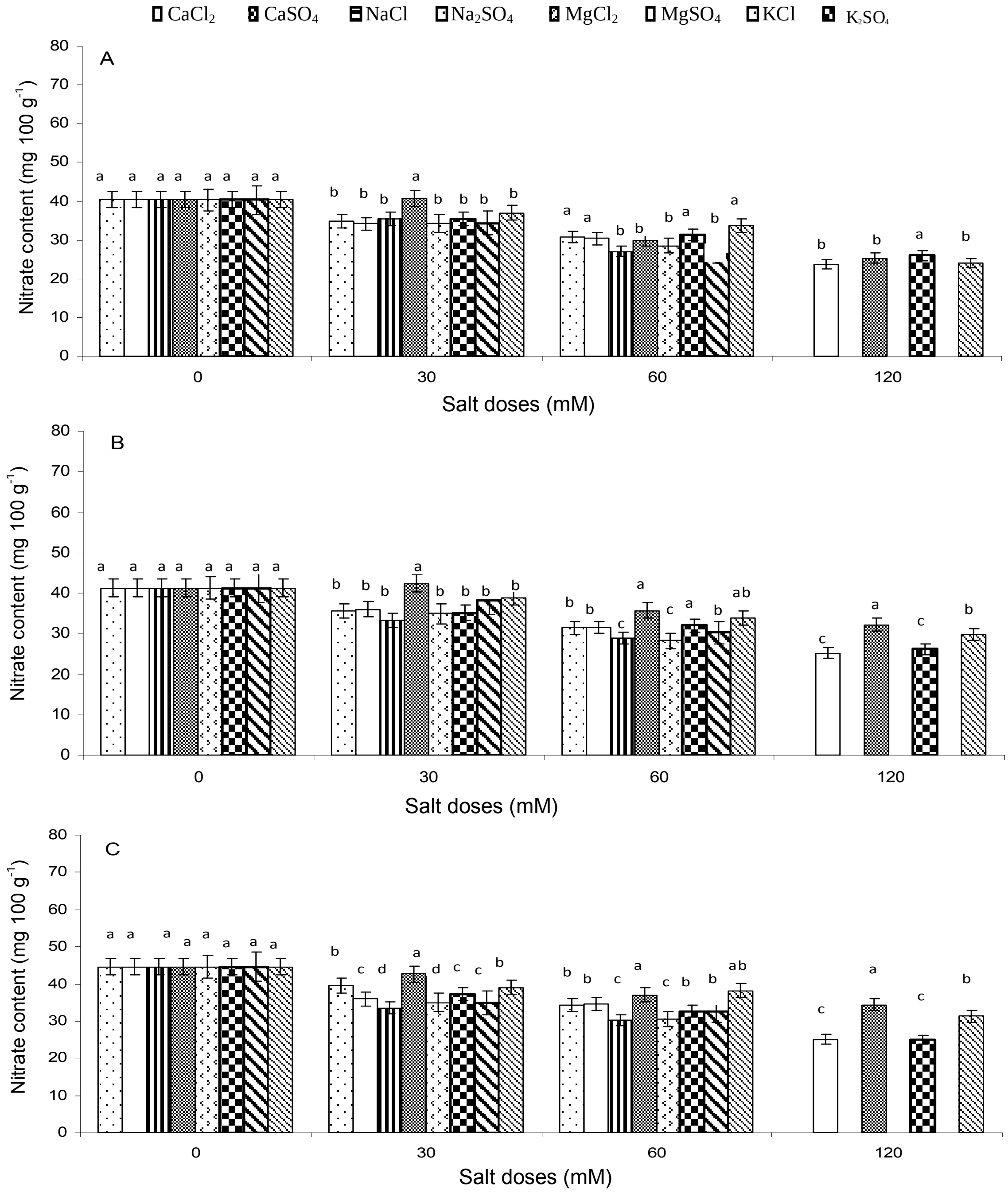

Figure 6. Effects of humic acid (HA) application on nitrate content of plant under different salt growth concentrations (A: no HA application, B: 0.05% HA application and C: 0.1% HA application).

adversely affected chlorophyll concentration (Yeo and Flovers, 1983). Plant nitrate content (PNC) and leaf area (LA) decrease with increasing concentration for all the salt treatments but increase with increasing HA application concentration. The highest PNC and LA of plants were observed with K_2SO_4 salt source with 0.1% HA application concentration and the lowest value was obtained by NaCl at no HA application treatment (Figures 6 and 7).

HA application at 0.05 and 0.1% concentration at the highest salt stress condition (120 mM) increased PNC at rate of 6.0 to 13.9, 16.0 to 20.5, 5.0 to 10.0 and 1.0 to 7.9% for $CaSO_4$, $NaSO_4$, Mg_2SO_4 and K_2SO_4 when compared to no HA application with highest salt stress treatment (120 mM), respectively. Similarly, LA increased rate was 29.1 to 67.0, 4.8 to 18.2, 25.9 to 96.7 and 23.9

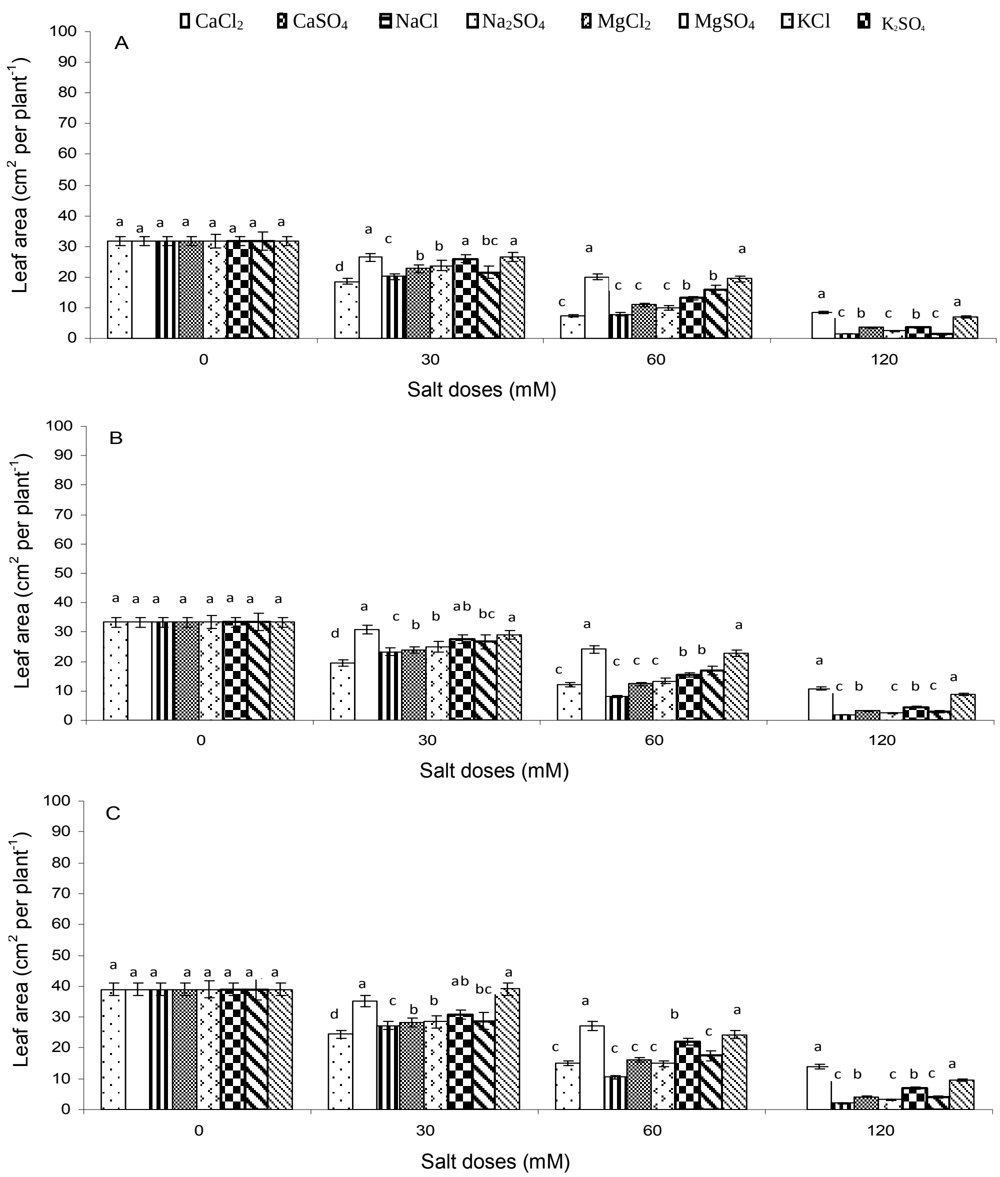

Figure 7. Effects of humic acid (HA) application on plant leaf area under different salt growth concentrations (A: no HA application, B: 0.05% HA application and C: 0.1% HA application).

to 32.9% for $CaSO_4$, $NaSO_4$, Mg_2SO_4 and K_2SO_4, respectively. At increased chlorine salt of Na^+, Ca^{++} and Mg^{++}, plant nitrate content drastically decreased especially with NaCl treatments. It has been reported that nitrate uptake was reduced by salinity (Botella et al., 1994; Turan and Aydin, 2005). Nitrogen (N) and phosphorus (P) content of bean plants was decreased seven and four fold for shoot, four and two fold for root grown at high salinity condition (120 mM) compared to no HA application. On the other hand, HA application at 0.05 and 0.1% concentration increase N and P at rate of 8.0 to 6.0, 6.0 to 5.0, 9.0 to 6.5 and 14.0 to 9.0% for $CaSO_4$, $NaSO_4$, Mg_2SO_4, KCl and K_2SO_4 when compared to no HA application with highest salt stress treatment (120 mM), respectively. The highest salt concentration (120 mM) of NaCl, $CaCl_2$, $MgCl_2$ and KCl at no HA application treatment causes plant death so that, 120 mM application was not considered in the evaluation of plant macro nutrient content. The highest N and P increase were obtained with the 0.1% HA application at all salt types both root and shoot part of plants (Tables 1 and 2). This positive effect of HA on the N and P uptake was higher in the shoot part of plant than the root part of plant.

The highest decreases occurred in N and P contents of plant parts when NaCl, KCl, $CaCl_2$ and $MgCl_2$ were applied. Responses of plants to chemical variations in soil varied drastically. Owing to salt and its concentration in soil, plants respond to salts by changing nutrients in the

Table 1. Effects of humic acid (HA) application on N and P contents increasing ratio of shoot part of plant under growth different salt †.

Salt dose (mM)	$CaCl_2$	$CaSO_4$	NaCl	Na_2SO_4	$MgCl_2$	$MgSO_4$	KCl	K_2SO_4
				Shoot N increasing ratio (%)				
0.05% Humic acid application								
0	14.71±2.07[a]	13.72± 0.06[a]	14.71 ± 1.09[a]	13.72 ± 2.15[a]	14.71 ± 1.21[a]	13.72 ± 1.75[a]	14.71 ±1.45[a]	13.72± 1.23[a]
30	1.29± 0.15[b]	3.53± 1.22[b]	12.50 ± 1.12[b]	10.43 ±2.14[b]	9.40 ±0.92[b]	6.49 ±0.38[b]	10.33± 1.12[b]	5.98± 0.91[b]
60	0.38±0.05[c]	3.21± 0.86[c]	ND	4.73±0.14[c]	0.44± 0.08[c]	5.80 ± 1.02[c]	9.76± 0.98[b]	5.16± 0.50[c]
120	ND	3.13± 0.72[c]	ND	ND	ND	3.80± 0.13d	ND	4.28± 0.22[c]
0.1% humic acid application								
0	29.41±2.11[a]	30.85± 2.45[a]	29.41 ± 2.33[a]	30.85 ± 2.50[a]	29.41± 2.55[a]	30.85 ± 3.00[a]	29.41 ±2.12[a]	30.85± 3.10[a]
30	8.23± 1.88[b]	7.42± 1.04[b]	17.31 ± 1.88[b]	12.61 ±1.30[b]	16.67 ±1.34[b]	14.56± 2.54[b]	12.19± 1.22[b]	12.26± 1.50[b]
60	4.60±0.78[c]	6.88± 0.60[c]	3.16 ± 0.22[c]	4.31 ±0.65[c]	5.73± 0.94[c]	8.78 ±1.22[c]	10.74± 1.55[c]	9.83± 1.10[c]
120	ND	5.88± 0.75[c]	ND	3.64 ± 0.44[c]	ND	4.97± 0.96d	ND	5.95± 1.27d
				Shoot P increasing ratio (%)				
0.05% humic acid application								
0	6.67±1.10[a]	0.65± 1.44[a]	6.67 ± 1.19[a]	6.87 ±1.22[a]	6.67± 1.13[a]	7.28 ± 1.54[a]	6.67 ±1.22[a]	7.28± 1.93[a]
30	3.69± 0.95[b]	2.15± 0.74[b]	1.55 ± 0.82[b]	5.71± 1.00[b]	3.41 ±0.55[b]	7.38± 1.13[b]	1.81± 0.98[b]	2.25± 0.91[b]
60	ND	0.30± 0.05[c]	0.90± 0.02[b]	ND	1.83± 0.12[c]	4.57 ±0.38[c]	1.50± 0.52[b]	2.17± 0.62[b]
120	ND	0.29± 0.02[c]	ND	ND	ND	0.31 ± 1.02d	ND	2.48± 0.59[b]
0.1% humic acid application								
0	6.03±1.57[a]	6.28± 1.25[a]	6.03 ± 1.09[a]	7.28 ± 1.35[a]	6.03± 1.26[a]	7.38± 1.59[a]	6.03 ±1.27[a]	6.28± 1.25[a]
30	4.00± 1.00[b]	4.29± 1.10[b]	1.20± 0.30[b]	5.65 ±1.10[b]	5.26 ±0.92[b]	4.92± 0.76[b]	0.90± 0.05[b]	3.10± 0.80[b]
60	0.87±0.28[c]	2.35± 0.62[c]	0.62 ± 0.12[c]	4.11 ±0.99[b]	2.13± 0.82[c]	2.78 ±0.35[c]	0.30± 0.02[c]	1.86± 0.27[c]
120	ND	ND	ND	3.94 ± 0.95[b]	ND	2.28 ± 0.44[c]	ND	1.61± 0.10[c]

†Values are means ± SE at 0.5% level of three replications with plant samples, ND: not determined.

the cell and accumulating proline. The highest salt stress occurred in the NaCl application and in this case, because of unbalanced nutrient uptake, plants tend to resist stress conditions by accumulating proline and the highest reaction occurred in NaCl application. Recent literature has shown that HA could be used as a growth regulator to regulate hormone levels, improve plant growth and enhance stress tolerance (Serenella et al., 2002). Studies indicated that HA was in general not only beneficial to shoot and root growth but also nutrient uptake of vegetable crops (Padem et al., 1997; Aydin et al., 1999; Akinremi et al., 2000; Dursun et al., 2002; Cimrin and Yilmaz, 2005).

Saline soils and irrigations constitute a serious production problem for vegetable crops as saline conditions are known to suppress plant growth. This study demonstrated that, salinity stress induced lowered root and shoot biomass production, chlorophyll, nitrate and macro element content of plant. The assessment of the effect of

Table 2. Effects of humic acid (HA) application on N and P contents increasing ratio of root part of plant under growth different salt concentrations †.

Salt dose (mM)	$CaCl_2$	$CaSO_4$	NaCl	Na_2SO_4	$MgCl_2$	$MgSO_4$	KCl	K_2SO_4
Root N increasing ratio (%)								
0.05% Humic acid application								
0	7.49 ± 1.29[a]	8.31 ± 2.05[a]	9.12 ± 1.35[a]	7.31 ± 2.09[a]	8.09 ± 1.90[a]	8.31 ± 1.55[a]	6.18 ± 2.05[a]	7.31 ± 2.03[a]
30	0.69 ± 0.10[b]	2.99 ± 0.10[b]	5.25 ± 1.02[b]	6.47 ± 1.05[b]	5.17 ± 0.92[b]	4.02 ±1.12[b]	4.10 ± 0.88[b]	3.71 ± 0.81[b]
60	0.20 ± 0.05[c]	2.19 ± 0.22[b]	ND	2.93 ± 1.05[c]	0.24 ± 0.08[c]	3.59 ± 1.02[b]	2.34 ± 0.32[c]	3.20 ± 0.60[b]
120	ND	1.94 ± 0.08[c]	ND	ND	ND	2.36 ± 0.38[c]	ND	2.11 ± 0.12[c]
0.1% humic acid application								
0	15.59 ± 1.22[a]	19.13 ± 2.05[a]	18.24 ± 1.69[a]	19.13 ± 1.65[a]	16.18 ± 1.66[a]	19.13 ± 1.84[a]	12.35 ± 1.57[a]	19.13 ± 1.95[a]
30	4.36 ± 0.78[b]	4.27 ± 0.50[b]	7.27 ± 1.12[b]	7.82 ± 1.10[b]	9.17 ± 0.42[b]	5.44 ± 0.35[b]	5.12 ± 0.82[b]	6.09 ± 1.00[b]
60	2.44 ± 0.38[c]	4.60 ± 0.44[b]	1.33 ± 0.37[c]	2.67 ± 0.39[c]	3.15 ± 0.52[c]	4.94 ± 1.16[b]	4.51 ± 0.32[b]	3.69 ± 0.27[c]
120	ND	3.65 ± 1.02[c]	ND	2.25 ± 1.25[c]	ND	2.83 ± 0.79[c]	ND	3.66 ± 1.10[c]
Root P increasing ratio (%)								
0.05% humic acid application								
0	3.53 ± 0.77[a]	3.40 ± 0.26[a]	4.13 ± 0.49[a]	4.26 ± 0.54[a]	3.67 ± 0.54[a]	3.41 ± 0.62[a]	3.80 ± 0.85[a]	3.41 ± 0.73[a]
30	1.96 ± 0.15[b]	1.33 ± 0.16[b]	0.65 ± 0.12[b]	3.54 ± 0.96[b]	2.01 ± 0.18[b]	2.58 ± 0.13[b]	0.76 ± 0.18[b]	1.40 ± 0.11[b]
60	ND	0.18 ± 0.42[c]	0.38 ± 0.08[b]	ND	1.87 ± 0.42[b]	2.04 ± 0.38[b]	0.63 ± 0.42[b]	1.05 ± 0.40[b]
120	ND	0.12 ± 0.22[c]	ND	ND	ND	0.19 ± 1.02[c]	ND	0.54 ± 1.12[c]
0.1% humic acid application								
0	3.20 ± 0.47[a]	3.41 ± 0.85[a]	3.74 ± 0.79[a]	3.41 ± 0.95[a]	3.32 ± 0.96[a]	3.41 ± 0.94[a]	3.53 ± 0.57[a]	3.41 ± 0.75[a]
30	2.12 ± 0.68[b]	2.66 ± 0.10[b]	0.50 ± 0.37[b]	0.90 ± 0.10[b]	2.89 ± 0.42[b]	2.588 ± 0.09[b]	0.38 ± 0.12[b]	1.00 ± 0.10[b]
60	0.46 ± 0.38[c]	1.46 ± 1.02[c]	0.26 ± 0.12[c]	0.55 ± 0.39[c]	1.17 ± 0.12[c]	1.69 ± 0.35[c]	0.13 ± 0.32[c]	0.66 ± 0.27[c]
120	ND	ND	ND	0.12 ± 1.25d	ND	0.45 ± 1.16d	ND	0.42 ± 0.10[c]

†Values are means ± SE at 0.5% level of three replications with soil samples; ND: not determined.

salinity on the growth parameters by different salt sources and concentration enabled the conclusion that ‘all of the considered parameters were affected by salinity. 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, KCl, $CaCl_2$ and $MgCl_2$ salt concentration in the soil solution, plant yield and growth parameters have higher decreasing rate than the SO_4 salts in the soil solution. This can be achieved to some extent by the application of HA soil amendments. HA added to saline soil significantly improved the variables affected by high salinity and also increased plant N and P, reduced soil salinity, enhanced plant growth by allowing the nutrients to be incorporated into the HA matrix and release it to plant as needed. These may compensate osmotically for relative reduction in ion uptake except for N and P and in turn this reduction may explain reduced toxicity. In conclusion, it was found that soil salinity level, type and HA application concentrations were important for taking benefit from HA under salinity stress condition. The addition of HA could offer a 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 and economical aspect of these materials under natural field condition.

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