PATTERN OF ACCUMULATION OF INORGANIC ELEMENTS IN SUNFLOWER (*HELIANTHUS ANNUUS* L.) PLANTS SUBJECTED TO SALT STRESS AND EXOGENOUS APPLICATION OF 5-AMINOLEVULINIC ACID

NUDRAT AISHA AKRAM[1] AND MUHAMMAD ASHRAF[1,2]

[1]Department of Botany, University of Agriculture, Faisalabad, Pakistan
[2]Second affiliation: Department of Botany and Microbiology,
King Saud University, Riyadh, Saudi Arabia

Abstract

Influence of a potential plant growth regulator, 5-aminolevulinic acid (5-ALA) on the pattern of accumulation of some key inorganic elements in salt-stressed sunflower plants was observed under greenhouse conditions. Two cultivars of sunflower viz., Hysun-33 and S-278 were grown under non-saline and saline (150 mM NaCl) regimes in sand culture. After two weeks of salt treatment, all plants were subjected to four (0 (no spray), 20, 50 and 80 mg L^{-1}) levels of 5-ALA as a foliar spray for 14 days. Shoot fresh and dry matter of both sunflower cultivars was markedly reduced due to salt stress. Of different inorganic ions, Na^+ and Cl^- in leaf, stem and root tissues increased markedly while, K^+, and Ca^{2+} in all these tissues reduced under the saline regime. However, salt stress did not alter the leaf, stem or root P as well as root K^+/Na^+ ratio. Foliar-applied ALA improved growth under normal (non-saline) and saline conditions, and 20 and 80 mg L^{-1} levels of 5-ALA were relatively more effective than the other levels used in this study. Of nutrient accumulation, 5-ALA altered only root Na^+ and K^+ and root K^+/Na^+ ratio e.g., root Na^+ was lower at 50 mg L^{-1}, while root K^+ and K^+/Na^+ ratio were higher at 80 mg L^{-1}. In contrast, accumulation of all other ions in plant organs remained unaffected. Overall, foliar-applied 5-ALA did not alter the accumulation of different nutrients in different plant parts except root Na^+, K^+ and K^+/Na^+ ratio in both sunflower cultivars.

Introduction

Inorganic elements play a key role in a variety of metabolic processes including osmotic regulation for the maintenance of turgor in plant cells (Greenway & Munns, 1980; Flowers, 1985; Moghaieb *et al*., 2004). High Na^+ and Cl^- concentrations reduced plant biomass and can cause adverse effects in almost all glycophytes (White & Broadley, 2001; Tester & Davenport, 2003). However, salt tolerant plants avoid plant tissue or organ injury by various means. A typical response is shoot damage due to accumulation of Na^+ at toxic level, while better root growth could be achieved due to excessive salt exclusion (Tester & Davenport, 2003; Munns *et al.,* 2006). Under saline conditions, plants may acquire Na^+ at the cost of K^+ and Ca^{2+}. Both K^+ and Ca^{2+} are essentially required for maintaining enzyme activities and membrane integrity, respectively, and thus have a key role in plant survival under saline environments (Bhandal & Malik, 1988; Lauchli, 1990; Alam, 1999). Ion homeostasis under saline conditions generally attributed to regulation of K^+/Na^+ ratio in the cytoplasm (Gaxiola *et al*., 1999). No pertinent entry system for Na^+ is known yet at molecular level, however, competition of this cation with K^+ for cellular influx may happen (Blumwald *et al*., 2000). However, the compartment of Na^+ into vacuoles eliminates Na^+ toxicity in the cytosol despite taking part in osmoregulation adjustment (Garg & Gupta, 1997;

Ramoliya & Pandey, 2002). Generally, in glycophytes, Na^+ and Cl^- in both shoot and root tissues increase, while K^+ and Ca^{2+} decrease consistently with a consistent increase in the salt level of the root growing medium. In addition, salt-tolerant cultivars greatly exclude Na^+ and maintain higher K^+ and lower Na^+/K^+ ratios than those of the sensitive cultivars in response to salinity stress (Chaudhary *et al.*, 1997).

5-aminolevulinic acid (5-ALA) biosynthesis is the first well-known step in tetrapyrrole biosynthesis, which is involved in the development of heme, chlorophyll, bilins, vitamin B and other specialized plant products (Garnick & Sassa, 1971). Exogenous application of 5-ALA is a novel strategy and has been considered as an effective means of minimizing the salt-induced adverse effects in a number of crops e.g., date palm (*Phoenix dactylifera*) (Youssef & Awad, 2008), oilseed rape (*Brassica rapa*) (Naeem *et al.*, 2010), potato (*Solanum tuberosum*) (Zhang *et al.*, 2006), Pakchoi (*Brassica campestris*) (Wang *et al.,* 2005) and spinach (*Spinacia oleracea*) (Nishihara *et al.*, 2003). ALA is known to regulate several key physiological processes associated with plant salt tolerance (Hotta *et al.*, 1997a,b) such as seed germination, reduced Na^+ uptake, altered light reactions, improved reactive oxygen species scavenging, enhanced photosynthetic assimilation and maintenance of nutrients status (Hotta *et al.*, 1997a, b; Tanaka & Kuramochi, 2001; Youssef & Awad, 2008). The present study was performed to assess the role of 5-ALA on the pattern of accumulation of some key inorganic nutrients subjected to saline regimes, as there is not much information available in the literature on the role of 5-ALA on accumulation of nutrients in plants supplied with 5-ALA.

Materials and Methods

Effect of foliar-applied 5-aminolevulinic acid (5-ALA) on growth and pattern of accumulation of different inorganic ions was examined in two sunflower cultivars viz., Hysun-33 and S-278 under greenhouse conditions. Achenes of the two sunflower cultivars were obtained from the National Agricultural Research Centre, Islamabad (NARC). A pot experiment was conducted under natural atmospheric conditions (mean day and night temp. 30.6 ± 5.1 and $18.3 \pm 7.6^{o}C$, respectively, RH, 31.06 ± 4.70, and the day length from 8 to 11 h) during February-May, 2009. Each pot contained 10 kg dry well washed river sand. Hoagland's nutrient solution (full strength) was supplied to plants for two weeks. After three weeks of plant growth, two NaCl levels, control and 150 mM (in full strength Hoagland's nutrient solution) were applied to rooting medium. Two liters of the salt treatment solution were applied to each pot after every week. During the week the sand in each pot was kept moist by adding 200 mL distilled water. Four levels of 5-ALA (MP Biomedical Inc., Mol. wt = 167.59) i.e., control (non spray), 20, 50 and 80 mg L^{-1}, prepared in 0.1% tween-20 solution were applied exogenously at the rate of 35 mL per pot. The treatment solutions were applied as a foliar spray to normal and NaCl-stressed plants of both sunflower cultivars. After two weeks of 5-ALA application, two plants per replicate were harvested and fresh weights of shoots recorded. Leaves, stems and roots were oven-dried at 65°C for one week. Then dried ground leaf, stem or root material (0.1 g) was digested with digestion mixture following Allen *et al.*, (1986). Na^+, K^+ and Ca^{2+} contents in the digests were appraised with a flame photometer (Jenway, PFP-7). For the determination of Cl^-, the ground leaf, stem or root material (100 mg) was extracted in 10 ml of distilled water at 80°C for 4 h. Chloride in the extracts was determined with a chloride analyzer (Model 926, Sherwood Scientific Ltd., Cambridge, UK). Phosphorus was determined spectrophotometrically (IRMECO, U2020, UV-Vis) following Jackson (1962).

Data analysis: Bartlett's test for analysis of variance of the data was performed for each dependant or independent variables computed using the MSTAT Computer Program (MSTAT Development Team, 1989).

Results

Shoot fresh and dry biomass of both sunflower cultivars (Hysun-33 and S-278) was reduced markedly under 150 mM NaCl (Fig. 1). However, foliar-applied 5-aminolevulinic acid (5-ALA) improved growth under both non-saline and saline conditions. Under non-saline conditions, the effective levels of 5-ALA were 20 and 80 mg L^{-1}. On the contrary, under saline regime, 50 mg L^{-1} 5-ALA caused an increase in shoot fresh and dry mass (Fig. 1). The cultivars response was almost the same for shoot fresh and dry weights (Table 1).

Saline stress effectively increased leaf, stem or root Na^+ in both sunflower cultivars (Fig. 2). Foliar-applied 5-ALA did not change the leaf or root Na^+ of both sunflower cultivars under saline or normal regimes. However, root Na^+ was significantly high at 20 and 50 mg L^{-1} of 5-ALA in both cultivars under saline or non-saline regimes, but variation in both cultivars with respect to leaf or root Na^+ was not apparent under the saline regime.

Addition of salt to the root growing medium caused a marked decrease in leaf, stem or root K^+ contents of both cultivars (Fig. 1, 2). Foliar-applied 5-ALA had no significant effect on leaf or stem K^+ of the non-stressed and stressed plants of both cultivars. The difference between the two cultivars was not discernable with respect to the above mentioned attributes. However, root K^+ accumulation increased considerably ($P \leq 0.01$) under non-saline and saline conditions. Of all foliar-applied 5-ALA levels, 80 mg L^{-1} was effective in enhancing K^+ content in the roots of both cultivars. Root Cl^- was significantly higher ($p \leq 0.05$) in cv. S-278 than that in the other cultivar.

Application of NaCl to the plant root zone significantly enhanced leaf, stem or root tissue Cl^- in the plants of both sunflower cultivars. Of both cultivars, cv. S-278 had greater leaf Cl^- than that in cv. Hysun-33. The influence of foliar applied 5-ALA with respect to leaf, stem or root Cl^- was almost unchanged. Chloride accumulation in both cultivars was different and of both cultivars, cv. S-278 was higher in stem Cl^- and cv. Hysun-33 in root Cl^- under saline conditions (Table 1; Fig. 2).

Saline stress had a non-significant effect on leaf, stem or root P of both sunflower cultivars. The P accumulation was the same in both cultivars, however, foliar-applied 5-ALA did not affect the leaf, stem or root P levels (Table 1; Fig. 2, 3).

Leaf Ca^{2+} in both cultivars remained unaffected due to rooting medium applied NaCl (Table 1; Fig. 3). However, stem and root Ca^{2+} accumulation decreased significantly in both sunflower cultivars under saline conditions. No change in leaf, stem or root Ca^{2+} was observed due to exogenous application of 5-ALA. Both cultivars did not differ in leaf, stem or root Ca^{2+} content under control as well as saline conditions.

K^+/Na^+ ratio in the roots of both sunflower cultivars remained unaffected under salt stress. Both cultivars had similar values of root K^+/Na^+ ratio under saline conditions (Table 1; Fig. 3). Foliar-applied 5-ALA caused a significant increase in root K^+/Na^+ ratio in the non-stressed and stressed sunflower plants. Of all 5-ALA levels used, 80 mg L^{-1} caused a slight increase in root K^+/Na^+ ratios in the salt stressed plants of both sunflower cultivars.

Table 1. Bartlett's test for analysis of variance (mean squares) of the data for shoot fresh and dry weights and accumulation of different inorganic nutrients in salt-stressed and non-stressed plants of two cultivars of sunflower (*Helianthus annuus* L.) subjected to foliar-applied varying levels of 5-aminolevulinic acid.

Source of variation	df	Shoot fresh weight	Shoot dry weight	Leaf Na^+	Stem Na^+	Root Na^+
Salinity (S)	1	8308.4***	180.6***	38.91**	357.5***	14.21ns
Cultivars (Cvs)	1	478.8ns	8.728ns	9.486ns	7.11ns	2.83ns
5-aminolevulinic acid (5-ALA)	3	75.79ns	4.343ns	5.456ns	4.31ns	13.02*
S x Cvs	1	194.97ns	5.644ns	2.764ns	0.019ns	0.577ns
S x 5-ALA	3	212.65ns	6.917ns	3.331ns	4.106ns	11.93ns
Cvs x 5-ALA	3	31.07ns	1.531ns	6.792ns	7.112ns	29.58ns
S x Cvs x 5-ALA	3	267.8ns	6.324ns	2.942ns	4.699ns	10.97ns
Error	48	129.2	3.949	3.701	8.226	12.73

	df	Leaf K^+	Stem K^+	Root K^+	Leaf Ca^{2+}	Stem Ca^{2+}
Salinity (S)	1	398.7***	240.9***	39.533**	1.00ns	100.8***
Cultivars (Cvs)	1	3.793ns	5.81ns	18.02*	42.25ns	8.74ns
5-aminolevulinic acid (5-ALA)	3	7.562ns	14.31ns	19.63**	20.93ns	12.59ns
S x Cvs	1	41.69*	6.688ns	0.042ns	5.06ns	14.38ns
S x 5-ALA	3	6.62ns	0.173ns	2.121ns	2.71ns	3.54ns
Cvs x 5-ALA	3	23.62ns	28.41*	1.187ns	12.79ns	3.51ns
S x Cvs x 5-ALA	3	12.68ns	2.803ns	1.676ns	18.94ns	1.44ns
Error	48	9.22	7.632	4.316	15.2	7.37

	df	Root Ca^{2+}	Leaf Cl^-	Stem Cl^-	Root Cl^-	Leaf P
Salinity (S)	1	70.85*	3751.5***	5095.6***	4440.7***	0.009ns
Cultivars (Cvs)	1	0.445ns	390.1***	1.068ns	134.3*	0.003ns
5-aminolevulinic acid (5-ALA)	3	5.746ns	12.455ns	14.8ns	40.02ns	0.005ns
S x Cvs	1	5.44ns	43.56ns	73.68ns	133.1*	0.006ns
S x 5-ALA	3	14.04ns	7.572ns	43.3ns	16.4ns	0.001ns
Cvs x 5-ALA	3	81.63***	6.64ns	3.35ns	1.84ns	0.004ns
S x Cvs x 5-ALA	3	21.82ns	10.24ns	22.56ns	6.64ns	0.001ns
Error	48	10.33	22.51	19.62	23.47	0.002

	df	Stem P	Root P	Root K^+/Na^+		
Salinity (S)	1	0.013ns	0.005ns	0.066ns		
Cultivars (Cvs)	1	0.007ns	0.001ns	0.157ns		
5-aminolevulinic acid (5-ALA)	3	0.022ns	0.003ns	0.878***		
S x Cvs	1	0.012ns	0.001ns	0.12ns		
S x 5-ALA	3	0.004ns	0.002ns	0.092ns		
Cvs x 5-ALA	3	0.003ns	0.002ns	0.0167ns		
S x Cvs x 5-ALA	3	0.004ns	0.002ns	0.251ns		
Error	48	0.008	0.002	0.1		

ns = Non-significant; *, ** and *** = Significant at 0.05, 0.01 and 0.001 levels, respectively
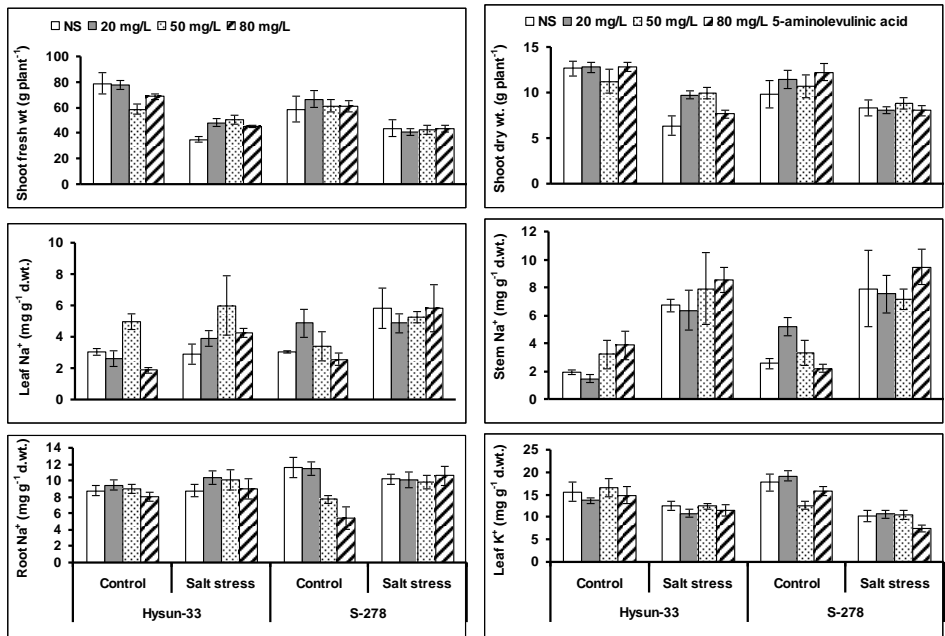
Fig. 1 Shoot fresh and dry weights and Na$^+$ and K$^+$ concentrations in different plant parts of two cultivars of sunflower (*Helianthus annuus* L.) subjected to foliar-applied varying levels of 5-aminolevulinic acid grown under non-saline and saline conditions (Mean ± S.E.; *n*= 4). NS, stands for No Spray.
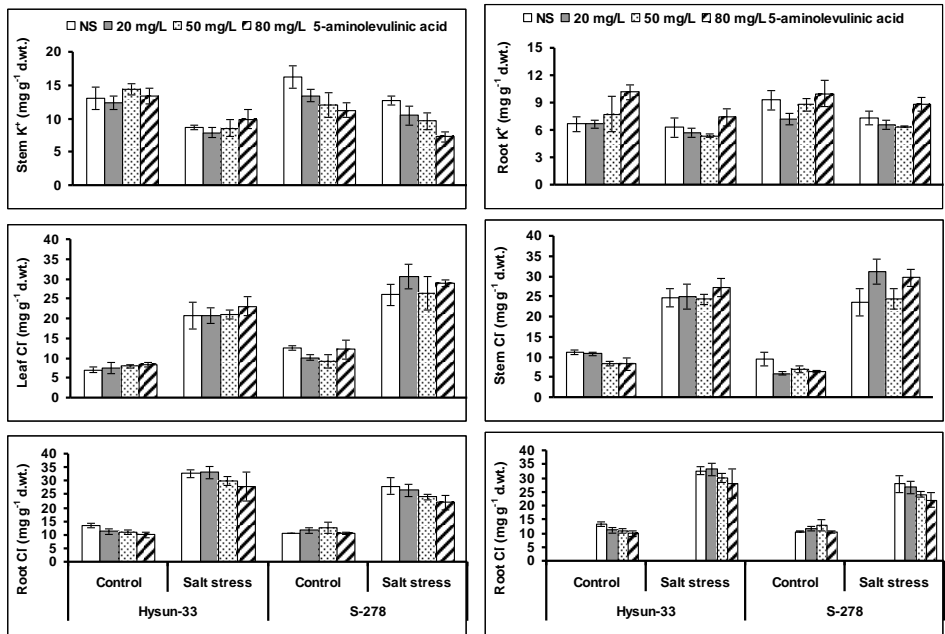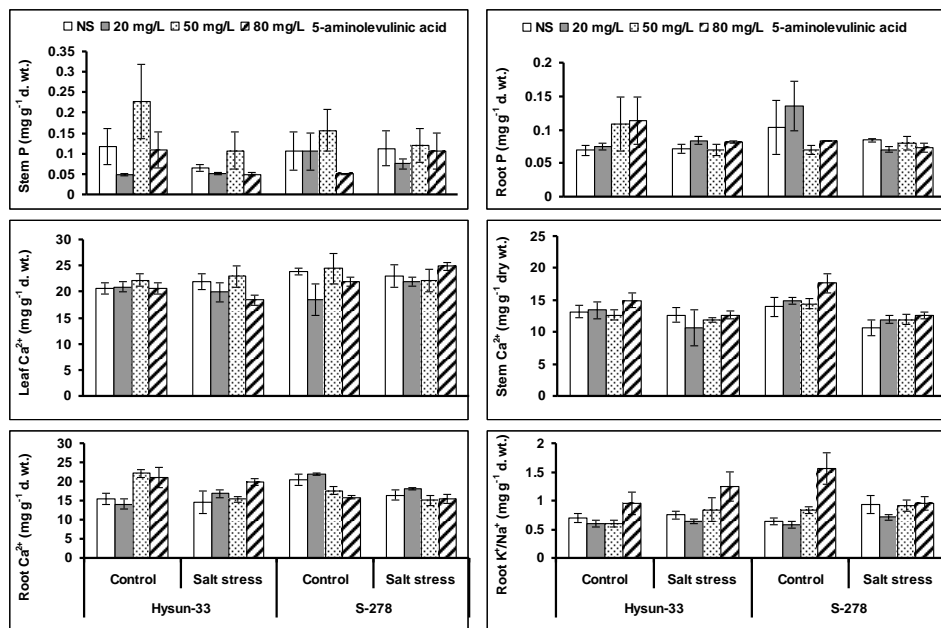

Fig. 2. K$^+$, Cl$^-$ and P in different plant organs of two cultivars of sunflower (*Helianthus annuus* L.) subjected to foliar-applied varying levels of 5-aminolevulinic acid grown under non-saline and saline conditions (Mean ± S.E.; *n*= 4). NS, stands for No Spray.

Fig. 3. Phosphorus, Ca^{2+} and K^+/Na^+ ratio in different plant organs of two cultivars of sunflower (*Helianthus annuus* L.) subjected to foliar-applied varying levels of 5-aminolevulinic acid grown under non-saline and saline conditions (Mean ± S.E.; *n*= 4). NS, stands for No Spray.

Discussion

Salt-induced growth reduction could be improved by a number of means including exogenous application of growth regulating substances (Ashraf & Foolad, 2007; Ashraf *et al*., 2008; Zhang *et al*., 2008). Aminolevulinic acid (5-ALA) has been found as one of the potential plant growth regulators (Mishra & Srivastava, 1983; Hotta *et al*., 1997b; Watanabe *et al*., 2000; Tanaka & Kuramochi, 2001). Foliar-applied 5-ALA improved growth of sunflower plants in the present study. Such type of 5-ALA-induced growth improvement has already been studied in date palm, potato, oilseed rape, pakchoi etc., (Youssef & Awad, 2008; Naeem *et al*., 2010). ALA has a diversity of agricultural applications not only as a growth promoting factor under salinity stress, but it can be used as herbicide or insecticide (Sasaki *et al*., 1998; Nishihara *et al*., 2001; Wang *et al*., 2005).

Pattern of ion accumulation is considered as one of the vital indicators of salt tolerance in most plants (Ashraf, 2004; Flowers & Flowers, 2005; Munns & Tester, 2008). Exclusion of Na^+ and/or Cl^- and maintenance of high K^+/Na^+ in plant tissues are key responses of salt tolerant plants of most species (Zheng *et al*., 2008; Munns & Tester, 2008). In the present study, salt stress caused a marked increase in Na^+ and Cl^- in sunflower leaf, stem or root tissues, whereas it decreased K^+ and Ca^{2+} in all these tissues. In the present study, 5-ALA application caused enhanced accumulation of K^+ and maintained high K^+/Na^+ ratio in the roots only. Salt-induced low K^+/Na^+ ratio has been reported to impair the activities of different enzymes and adversely affect various metabolic processes in plants (Tester & Davenport, 2003; Sudhir & Murthy, 2004; Munns & Tester, 2008). It has also been established by Zheng *et al*., (2008) while

examining the role of high K^+/Na^+ ratio in salt tolerance of two wheat cultivars and found that salt-tolerant cultivar maintained high K^+/Na^+ ratio accompanied with the maintenance of high activities of antioxidant enzymes, low electrolyte leakage, high malondialdehyde and soluble sugar contents. Generally, an appropriate level of K^+/Na^+ ratio has a key role in photosynthesis, activation of enzymes, cell osmoregulation, turgor maintenance, stomatal function, generation of antioxidants and protein synthesis (Shabala *et al*., 2003; Zheng *et al*., 2008; Akram & Ashraf, 2009; Akram *et al*., 2009).

Recently, Naeem *et al*., (2010) have found that foliar application of 5-ALA improved growth of shoots and roots of salt-stressed oilseed rape plants and accumulation of various macro- and micro-nutrients (N, P, K, S, Ca, Mg, and Fe). They suggested that ALA-induced growth improvement was associated with low uptake of Na^+. In the present study, foliar-applied 5-ALA altered only root Na^+ and K^+ and root K^+/Na^+ ratio e.g., root Na^+ was lower at 50 mg L^{-1}, while root K^+ and K^+/Na^+ ratio higher at 80 mg L^{-1}. In contrast, accumulation of all other ions in plant organs remained unaffected. In salt-stressed okra plants, Saleem *et al*., (2010) examined high leaf and root Na^+ and Cl^-, while low K^+ and Ca^{2+}. The relatively more reduction in growth in okra was found to be associated with higher accumulation of Na^+ and Cl^- in leaves and roots. Salt tolerance/resistance in a plant has been primarily attributed to its ability for salt exclusion. In view of a number of reports it is evident that plant ability to exclude Na^+ or Cl^- is one of the most important mechanisms associated with salt tolerance (Maas, 1993; Cromer *et al*., 1982; Chen *et al*., 2001, 2002; Ashraf, 2004).

In the present study, salt stress did not affect leaf Ca^{2+}, but it reduced stem and root Ca^{2+} in sunflower plants. Furthermore, foliar-applied 5-ALA did not affect the Ca^{2+} concentration in leaf, stem or root tissues of sunflower plants. In view of some reports it is evident that salt stress can alter Ca^{2+} concentration in plant cells, and an increase in Ca^{2+} concentration under salt stress can often improve plant growth (Knight *et al*., 1996; Kim *et al*., 2004), although the underlying mechanisms of role of Ca^{2+} in salt tolerance are not quite clear (Poovaiah & Reddy, 1993; Pandey *et al*., 2002).

In conclusion, foliar-applied 5-ALA was effective in improving growth under non-saline and saline conditions and its levels 20 and 80 mg L^{-1} were relatively more effective than the other levels used in the present study. Overall, foliar-applied 5-ALA did not affect the accumulation of different nutrients in different plant parts except root Na^+, K^+ and K^+/Na^+ ratio in both sunflower cultivars. ALA-induced improvement in sunflower growth under salt stress was associated with high root K^+ and K^+/Na^+ ratio.

Acknowledgement

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