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Responses of Glutamine Synthetase-Glutamate Synthase Cycle Enzymes in Tomato Leaves under Salinity Stress

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ABSTRACT

Reduction in leaf CO_2 assimilation rate is a common effect of salinity stress. In present study, 30 days old tomato plants were exposed to 0, 60 and 120 mM NaCl for 7 days. The results showed that stomatal conductance, CO_2 assimilation rate, NO_3^- content and NO_3^- metabolizing enzymes (NR & NiR) activities decreased in salinity stressed tomato leaves, which indicated that C-N crisis is dominant during salinity stress. Interestingly, some C-N rich compounds (glutamate, proline, glycine & serine) accumulated excessively in the leaves of salinity stressed plants. Results also showed that important C and N providing enzymes such as NADP-ICDH, protease and NADH-GDH activities were enhanced during salinity stress. In salinity stressed tomato leaves, GS/GOGAT cycle enzymes (GS, NADH-GOGAT & Fd-GOGAT) activities were also increased significantly. Finally, it is concluded that GS/GOGAT cycle plays an important role for proline synthesis in tomato leaves during salinity stress.

Key Words: CO_2 assimilation rate; Nitrate reductase; Nitrite reductase; NADH-GDH; Proline

INTRODUCTION

Salinity is an environmental challenge that severely limits plant growth and productivity worldwide (Hasegawa *et al.,* 2000; Ashrafuzzaman *et al*., 2003; Munns & Tester, 2008). The results from gas-exchange studies showed that salinity caused a significant decrease in stomatal conductance (g_s) (James *et al.,* 2002), and it is the initial and most profound cause of a decline of CO_2 assimilation rate (Moradi & Ismail, 2007; Lawlor & Tezara, 2009). In salinity stressed plant, ABA-mediated stomatal closure is evident (Damour *et al.,* 2010; Kim *et al.,* 2010), which limits C to the plant and enhanced photorespiration rate (Keys, 2006). Actually, photorespiration may cause a significant amount of assimilatory C loss from the plant (Zhu *et al.,* 2010). Therefore, CO_2 limiting condition is dominant in salinity stressed plant (Chaves *et al.,* 2009) and it is one of the main causes for leaf growth reductions under salinity stress (James *et al.,* 2008). Interestingly, C shortage salinity stressed plants produce some C-rich compounds like sorbitol, mannitol, pinnitol and trehalose that are well known stress tolerance metabolites in many plant species (Rathinasabapathi, 2000; Chen & Murata, 2002; Cortina & Culianez-Macia, 2005; Chen *et al.,* 2007).

In addition, ammonium and nitrate assimilation curtailed seriously during salinity stress (Hoai *et al.*, 2005). Therefore, N status in the plant is also significantly influenced by salinity. In aerobic soils, nitrate is the dominant species and it is converted to ammonium by the sequential action of two enzymes, nitrate reductase (NR) and nitrite reductase (NiR) (Dechorgnat *et al.,* 2011). In higher plants, NH_4^+ is mainly assimilated through the concerted action of glutamine synthetase (GS) and glutamate synthase (GOGAT). Salinity curtailed NO_3^- uptake by decreasing the activities of NR and NiR in plants and also decreased NH_4^+ assimilation seriously by influencing GS activity (Wang *et al.,* 2007). Recently, Debouba *et al.* (2006) reported that the activities of NR and GS were repressed in the tomato leaves, while NiR activity was decreased in both the leaves and roots. In addition to CO_2, salinity also limits N_2 to the plant. Therefore, it is evident that both C and N are limiting factor for the plant growth during salinity stress. Interestingly, proline (a C-N rich compound) accumulation increased several folds in salinity stressed peanut leaves (Hossain *et al.,* 20011) and it can serve as an adaptive mechanism to salt stress in higher plants (Kumara *et al.,* 2003; Chen *et al.,* 2007) including tomato. However, proline biosynthesis occurs predominantly from glutamate (Forde & Lea, 2007) and glutamate synthesis requires a C skeleton in the form of 2-oxoglutarate (Kusano *et al.,* 2011). Being an amino acid, glutamate synthesis also requires N_2. Interestingly, both elements (C & N) are in scarce condition during salinity stress. Therefore, we are interested to investigate the routes and ways of C and N supply for proline biosynthesis during salinity stress in tomato seedlings.

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MATERIALS AND METHODS

Plant material and growth condition: Seeds of tomato (*Lycopersicon esculentum*, Mill. cv. BINATomato-5) were surface-sterilized in 1% sodium hypochlorite for five min., followed by rinsing several times in distilled water. Then the seeds were sown in vermiculite wetted with distilled water. Five days after sowing, uniform seedlings were transplanted into trays containing half-strength Hoagland's nutrient solution and acclimated for 5 days. Subsequently, the seedlings were transferred to 3 L pots containing the same nutrient solution and 3 seedlings in each pot. Once a week pots were rinsed with tap water. Plants were grown in a greenhouse under natural conditions with day/night mean temperature of 29/25°C, relative humidity of 63/85%, average of 13 h photoperiod and an average maximum of photosynthetic photon flux density of 1570 µmol $m^{-2}s^{-1}$ measured at plant level. The greenhouse had no supplemental light system.

Salt treatment and harvest: In the present study, 30 days old seedlings were exposed to treatment solutions. To do this, the pots containing 3 seedlings were randomly assigned to 3 salt treatments: 0 (control), 60 and 120 mM NaCl (salt stress). Salt additions (at a rate of 30 mM per day, starting 24 h after transferring the plants to the pots) were split over time in an attempt to avoid sudden osmotic shock. All nutrient solutions were replaced every 4 days and kept aerated. The pH was checked daily and adjusted to 6.5 with 0.1 N NaOH or 0.1 N HCl when necessary. The amount of transpired water was checked daily by weighing the pots and was replaced with distilled water. Before harvesting, the gas exchange parameters were recorded by using LICOR-6400. Finally, plants were harvested at day 7 after treatment. After measuring leaf area using an LI-3000 leaf area metre (LI-COR, Inc. Lincoln, NE, USA), leaves were oven dried at 72°C for 24 h. Some fresh leaves and fresh roots were frozen in liquid nitrogen and kept at −20°C until used for biochemical analyses.

Determination of Na^+, K^+, NH_4^+ and NO_3^- ions: Dried leaf samples were ground into fine powder. After digestion, Na^+ and K^+ in the samples were analyzed by flame photometry. Ammonium (NH_4^+) was extracted from leaf at 4^0C with 0.3 mM H_2SO_4 and 0.5% (w/v) polyclar AT. Ammonium content was quantified according to the reaction of Berthelot modified by Weatherburn (1967). Nitrate (NO_3^-) was determined by the salicylic acid method (Cataldo *et al.,* 1975).

Determination of chlorophyll and amino acids in leaves: Chlorophyll was determined by the method of Arnon (1949). The absorbance of a sample was read at 652 nm after centrifugation. Total free amino acids were measured after hot water (90°C) extraction and reaction with ninhydrin according to Yemm and Cocking (1955) method. The free proline was determined by the Bates *et al.* (1973) method.

Enzyme Assays

Nitrate reductase: Frozen plant material was homogenized in chilled mortar and pestle with 100 mM potassium phosphate buffer (pH 7.4) containing 7.5 mM cysteine, 1 mM EDTA and 1.5% (w/v) casein. The homogenate was centrifuged at 30,000g for 15 min at 4°C. Nitrate reductase activity (NR) was determined according to the method as described by Debouba *et al.* (2006). The extract of 0.1 mL was incubated in a reaction mixture containing 0.5 mL of 0.1M potassium phosphate buffer (pH 7.4), 0.1 mL of 0.15 mM NADH, and 0.1 mL of 0.1 M KNO_3 at 30 1C for 30 min. Nitrate reductase (NR) was incubated with $MgCl_2$ 10 mM (for actual NR determination) or with excess of 15 mM EDTA (for maximum NR determination). The reaction was stopped by 0.2 mL of 1 M zinc acetate. Nitrite ions were assayed after diazotation with 1 mL of 5.8 mM sulfanilamide, 1.5 N HCl, and 1 mL of 0.8 mM N-naphthyl-ethylene-diamine-dichloride.

Nitrite reductase: Enzyme extracts were prepared as described above for nitrate reductase. Nitrite reductase was assayed using the method described by Losada and Paneque (1971). The extract of 0.1 mL was incubated in a solution containing 0.4 mL of 0.1 M potassium phosphate buffer (pH 7.4), 0.1 mL of 15 mM sodium nitrite, 0.2 mL of 5 mM methyl viologen, 0.2 mL of 86.15 mM sodium dithionite in a 190 mM $NaHCO_3$. The reaction was stopped by a violent agitation on vortex. Nitrite ions were assayed as described for NR assay.

Glutamine synthetase: Frozen samples were homogenized in a cold mortar and pestle with grinding medium containing 25 mM Tris-HCl buffer (pH 7.6), 1 mM MgCl2, 1 mM EDTA, 14 mM b-mercaptoethanol and 1% (w/v) polyvinylpyrrolidone (PVP). The homogenate was centrifuged at 25,000 g for 30 min at 4°C. GS activity was determined using hydroxylamine as substrate, and the formation of γ-glutamylhydroxamate (γ-GHM) was quantified with acidified ferric chloride (Wallsgrove *et al.,* 1979).

Glutamate synthase: Fd-GOGAT and NADH-GOGAT activities were measured as described by Suzuki *et al.* (2001). Glutamate synthase was extracted with 25 mM sodium sulfate buffer (pH 7.5), containing 14 mM β-mercaptoethanol and 1 mM dithiothreitol (DTT). Fd-GOGAT activity was determined in a reaction mixture containing 25 mM sodium sulfate buffer (pH 7.5), 100 mM glutamine, 100 mM 2-oxoglutarate, 3.9 mM methyl viologen and 190 mM sodium dithionite in 180 mM $NaHCO_3$. NADH-GOGAT was assayed using the same reaction mixture, except that methyl viologen and sodium dithionite were replaced by 1.4 mM NADH.

Glutamate dehydrogenase: Frozen samples were homogenized in a cold mortar and pestle with 100 mM Tris-HCl (pH 7.5), 14 mM b-mercaptoethanol and 1% (w/v) PVP. The extract was centrifuged at 12,000 g for 15 min at 4°C. GDH aminating activity was determined by following

the absorbance changes at 340 nm (Masclaux-Daubresse *et al.*, 2006).

Protease assay: The total proteolytic activity in leaves of control and salt-treated seedlings were determined using casein hydrolysis assay of Kunitz (1947) but optimized according to the plant materials. Absorbance of the released-azo-dye- was measured at 340 nm and one unit of activity was defined as the activity producing an increase of 0.01 units of absorbance during 1 h incubation.

Protein content: Soluble protein content was quantified using Coomassie Brilliant blue (Bradford, 1976) with bovine serum albumin as a protein standard.

Statistical analyses: All the experimental data obtained in this study were calculated as percent of those found for the control plants. A completely randomized design was used with four replicates per treatment and the data presented in this study represent the mean of them. Data were analyzed by the SAS (statistical analysis system) method and means were compared by the least significant difference (LSD) test at the 0.05 level of confidence.

RESULTS

Effects of salinity on leaf growth: Effects of salinity on leaf growth of tomato seedlings were studied based on leaf area and leaf dry matter yield. In compared to the control, leaf area was decreased by 21% and 40% at 60 and 120 mM NaCl salinity levels, respectively (Table I). Leaf dry matter yield reduction was followed the similar pattern. That is, leaf dry matter yield was decreased by 18% and 36% over the control after 7 days exposure to 60 and 120 mM NaCl salinity levels, respectively.

Chlorophyll content and seedling growth: As shown in Table I, chlorophyll content was significantly decreased in salinity-stressed tomato seedlings. Chlorophyll content was decreased by 11% and 24% at 60 and 120 mM NaCl salinity, respectively. The growth of tomato seedlings was also monitored by measuring the dry weight of whole plants (Table I). The plant biomass was clearly affected by salinity stress. As compared to the control, the reduction was 21.5% and 40% at 60 and 120 mM NaCl, respectively.

Effects of salinity on gas exchange parameters in tomato leaves: Leaf gas exchange in tomato was very sensitive to salinity stress (Table I). Table II showed that CO_2 assimilation rate (*A*) and stomatal conductance (g_s) were significantly decreased at 60 and 120 mM NaCl salinity levels and both were greater at 120 mM NaCl salinity. The intercellular CO_2 concentration (C_i) of the leaves declined significantly, whereas CO_2 concentration in the ambient atmosphere surrounding the leaf (C_a) was un-changed at 60 and 120 mM NaCl salinity levels.

Na^+, K^+, NH_4^+ and NO_3^- content: Sodium (Na^+) ion was rapidly accumulated in the leaves of tomato seedlings after 7 days exposure to NaCl stress (Table III). In contrast, K^+ was decreased significantly in the leaves and hence, declined the K^+/Na^+ ratio (data not shown). There was a significant decrease of NO_3^- content in the leaves, representing 65%

Table I: Leaf area, leaf dry weight, shoot dry weight and leaf chlorophyll content of tomato seedlings in control (0 mM NaCl) and saline (60, 120 mM NaCl) treatments. Three plants per replicate were sampled for measurements. Means within a column that do not have a common letter are significantly different by LSD $_{0.05}$; test

Treatment mM NaCl	Leaf area (cm^2 $plant^{-1}$)	Leaf DW (g $plant^{-1}$)	Chlorophyll (mg g^{-1}FW)	Shoot DW (g $plant^{-1}$)
0	15.41a	4.83a	1.58a	11.51a
60	12.23b	3.78ab	1.41b	9.15b
120	9.20c	3.00b	1.20b	6.92c

Table II: Gas exchange parameters of a tomato cultivar grown in nutrient solution containing 0. 60 and 120 mM NaCl for 7 days. Means within a column that do not have a common letter are significantly different by LSD $_{0.05}$ test

Treatment mM NaCl	A (µmol $m^{-2}s^{-1}$)	g_s (mmol $m^{-2}s^{-1}$)	C_i (µL L^{-1})	C_a
0	12.2a	270a	260a	400a
60	8.5b	228b	265a	399a
120	4.9c	151c	180b	400a

A = CO_2 assimilation rate; g_s = stomatal conductance; C_i = intercellular CO_2 concentration; C_a= CO_2 concentration in the ambient atmosphere surrounding the leaf

Table III: Effects of salinity stress on Na^+, K^+, NO_3^- and NH_4^+ content in tomato leaves. Thirty days old seedlings were exposed to nutrient solution containing 0, 60 and 120 mM NaCl for 7 days. Means within a column that do not have a common letter are significantly different by LSD $_{0.05}$; test

Treatment mM NaCl	µmol g^{-1} DW			
	Na^+	K^+	NO_3^-	NH_4^+
0	250c	960a	450a	20b
60	745b	700b	295b	23b
120	1605a	500c	180c	51a

Table IV: Effects of salinity stress on amino acids and total soluble protein content in tomato leaves. Thirty days old seedlings were exposed to treatment solution for 7 days. Means within a row that do not have a common letter are significantly different by LSD $_{0.05}$; test

Parameters	Salinity levels (mM)		
	0	60	120
Glycine	0.14c	0.22b	0.34a
Serine	1.37c	3.56b	4.87a
Alanine	0.98c	1.21b	2.48a
Glutamate	2.64c	3.63b	4.65a
Proline	1.54c	16.37b	34.35a
Arginine	0.53c	0.80b	1.26a
Total amino acid	12.10c	50.87b	63.48a
Soluble protein	1.958a	1.456b	1.121c

and 40% of the control at 60 and 120 mM NaCl salinity levels, respectively. Unexpectedly, NH_4^+content was 2.5 times greater than the control in the leaves of 120 mM NaCl treated seedlings (Table III).

Different amino acids content: Glycine, serine and alanine are regarded as photorespiratory amino acids and their contents were significantly increased in salinity-stressed plants (Table IV). At 60 mM NaCl salinity level, glycine, serine and alanine content increased by 157%, 260% and 123%, respectively whilst the estimated values at 120 mM NaCl salinity level were 243%, 355% and 253%. The results indicated that high salinity enhanced the yields of photorespiratory amino acids in the leaves of tomato seedlings. Salinity also increased other amino acids level in the leaves. Glutamate content was increased by 137% and 176% at 60 and 120 mM NaCl, respectively when compared to the control. However, proline content changed abruptly at high salinity stress (Table IV). Arginine and the total amino acids also increased significantly under salinity stress.

Soluble protein content and proteolytic activity under salinity stress: Soluble protein content was decreased significantly under salinity stress (Table IV). In fact, treatment with 60 mM NaCl decreased soluble protein content by 25.64% and it was 42.75% with the treatment of 120 mM NaCl. The proteolytic activity in the leaves of salt-treated seedlings was significantly higher than that of the control (Fig. 1).

Activities of NR and NiR in roots and leaves: Salinity stress decreased the activities of nitrate reductase (NR) in the roots of tomato seedlings following 7 d treatment with different levels of NaCl salt (Fig. 2a). After 1 week of treatment, nitrite reductase (NiR) activity also decreased in the roots irrespective of NaCl supply (Fig. 2c). In case of leaves, NR activity also decreased to 35% following 7 d treatment of tomato seedlings by 120 mM NaCl (Fig. 2b), whereas it was 10% for NiR activity (Fig. 2d).

Activities of GS, Fd-GOGAT and NADH-GOGAT in tomato leaves: In the leaf extract, activities of both GS and Fd-dependent glutamate synthase (Fd-GOGAT) significantly increased at 60 mM NaCl salinity level as compared to the control (Figs. 3a & 3b). Based on the activities of GS, there was no significant difference between 60 and 120 mM NaCl salinity levels. But a significant difference was found in the acitivity of Fd-GOGAT. In contrast, NADH-dependent glutamate synthase (NADH-GOGAT) activity was unchanged due to salinity stress (Fig. 3c).

Activities of NADP-ICDH and NADH-GDH in tomato leaves: In higher plants, NADP-dependent isocitrate dehydrogenase (NADPH-ICDH) provides C-skeletons in the form of 2-oxoglutarate, which requires for both the activities of GS/GOGAT cycle enzymes and/or the activity of NADH-GDH to synthesize glutamate. Our results showed that NADPH-ICDH activity increased significantly

Fig. 1: Effects of NaCl treatments (0, 60 and 120 mM) for 7 days on protease activity in the leaves of tomato plants. Vertical bars represent standard error of 4 replications

LSD test at 0.05 level of confidence

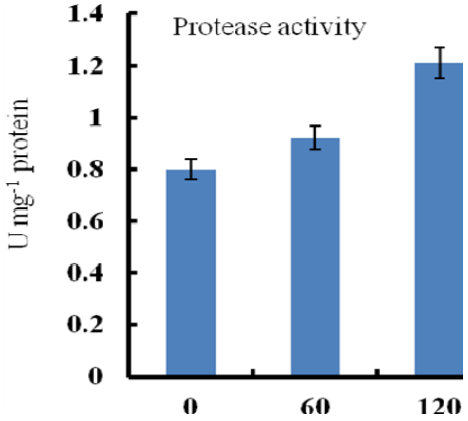
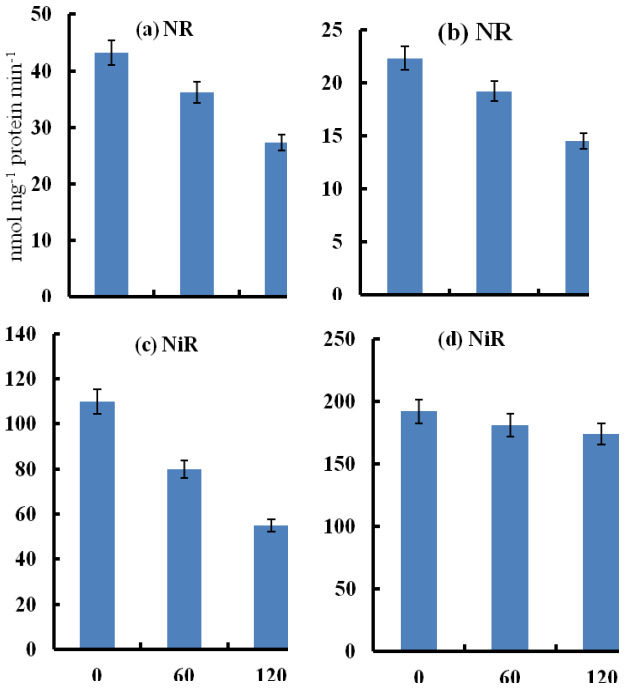


Fig. 2: Effects of salinity on the activities of nitrate reductase in the roots (a) and in the leaves (b) and nitrite reductase in the roots (c) and in the leaves (d)

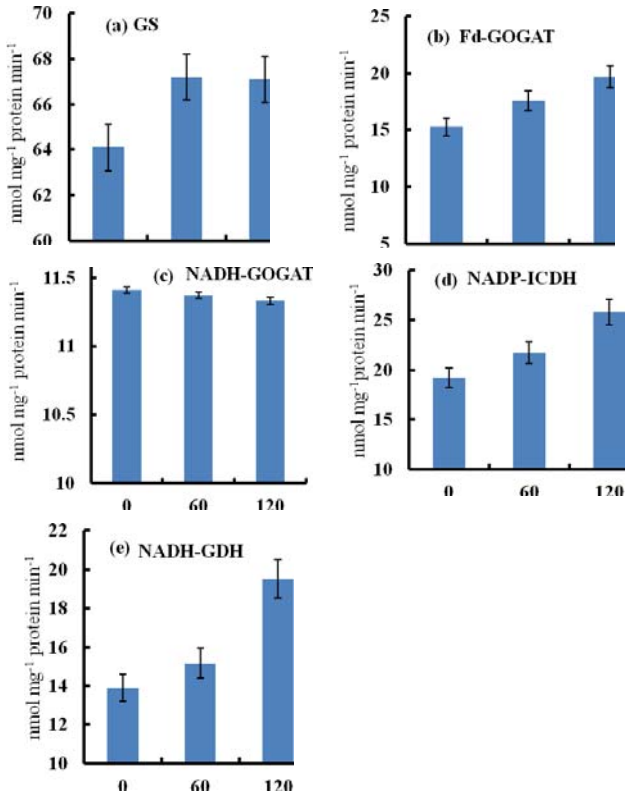
Plants were grown under salinity stress (60 and 120 mM NaCl) and non-stress (0 mM NaCl; control) for 7d. Bars represent standard error of 4 replications. LSD test at 0.05 level of confidence



under salinity stress (Fig. 3d). The aminating activity of glutamate dehydrogenase (NADH-GDH) was also determined in the leaves of the control and salinity-stressed seedlings. The NADH-GDH activity was 1.3 and 2.5 folds higher than that of the control when seedlings were treated with 60 and 120 mM NaCl, respectively (Fig. 3e).

Fig. 3: Effects of different salinity levels on the activities of GS (a), Fd-GOGAT (b), NADH-GOGAT (c), NADP-ICDH (d) and NADH-GDH (e) in the leaves of tomato seedlings

One month old seedlings were grown under salinity stress (60 and 120 mM NaCl) and non-stress (0 mM NaCl; control) condition for 7d. Bars represent standard error of 4 replications. LSD test at 0.05 level of confidence



DISCUSSION

Being an osmolyte, K^+ plays an important role for osmoregulation, turgor maintenance, cell expansion, stomatal function and photosynthesis activation (Buschmann *et al.,* 2000; Hasegawa *et al.,* 2000; Shabala, 2003). Generally, salinity stress inhibits K^+ uptake in many glycophytic plants (Gouia *et al.,* 1994; Shabala, 2000; Tarakcioglu & Inal, 2002; Uddin *et al.,* 2011). Our data also showed that salinity stress led to the decrease in K^+ with the concomitant increase in Na^+ content in tomato leaves (Table IV). It is reasonable since salinity enhanced Na^+ uptake via non-selective cation channels in plants (Kader & Lindberg, 2005) and suppressed K^+ uptake by altering the selectivity of HKT (the K^+-selective transporter protein) for Na^+ over K^+ (Rubio *et al.,* 1995; Garciadeblas *et al.,* 2004). This ionic imbalance might be associated with the cause of growth inhibition during salinity stress.

Salinity stress also limits CO_2 uptake by closing stomata (James *et al.,* 2002; Zhang *et al.,* 2006) that results in decreased carbon reduction by Calvin cycle and subsequently plant growth (Lawlor & Cornic, 2002; Lawlor & Tezara, 2009). In the present study, a significant decrease of CO_2 assimilation (*A*) rate (Table II) in synchronous with stomatal conductance (Table II) was found in the leaves of tomato seedlings after 7days exposure to different levels of salinity. The degree of CO_2 assimilation (*A*) rate reduction was positively associated (R^2=0.92) with the salinity levels and it was reflected in leaf area and leaf dry matter yield as well (Table I). Actually, photosynthesis (*A*) is limited by reduced intercellular CO_2 concentrations (C_i) due to stomatal closure (Kaiser, 1987; Cornic & Briantais, 1991; Quick *et al.,* 1992). Since reduced values of C_i lead to increase oxygenation of RUBP by RUBISCO (Wingler *et al.,* 1999; Zeng *et al.,* 2010), photorespiration is likely to increase in tomato leaves under salinity stress in the current investigation. At 25°C and current atmospheric CO_2, ~ 30% of the carbohydrate formed in C_3 photosynthesis is lost via photorespiration (Zhu *et al.,* 2010). The photorespiration and gas-exchange studies in the current investigation confirmed that C crisis is dominant in salinized seedlings, which may lead to poor growth of tomato seedlings.

Interestingly, C-N rich compound, proline was accumulated several folds in the leaves of tomato seedlings after 7 days exposure to NaCl stress (Table IV). Again, the magnitude of proline accumulation is positively (R^2=0.96, $P \leq 0.05$) associated to the concentration of NaCl in the culture solution. These results are consistent with the findings of some previous investigations (Sairam *et al.,* 2002; Yokota, 2003; Sumithra *et al.,* 2006; Hossain *et al.,* 2011). Generally, a substantial supply of glutamate is required for high rate of proline synthesis (Lutts *et al.,* 1999). Our results showed that glutamate and proline content were significantly increased in salinity treated tomato leaves (Table IV), indicating that salinity-induced C shortage tomato plants possibly get C as glutamate for proline synthesis. The enzyme NADP-ICDH is considered to play a primary role in 2-oxoglutarate synthesis (Lancien *et al.,* 1999; Hodges *et al.,* 2003; Abiko *et al.,* 2005), which functions as C-skeleton for glutamate. Our results showed that NADP-ICDH activity significantly increased after imposition of salinity stress (Fig. 3d).

To investigate the N source for glutamate as well as proline, we studied the activities of NR and NiR in the roots of tomato seedlings under salinity stress. We observed that both enzymes activities reduced drastically under salinity stress (Figs. 2a-d). In addition, Cl^- ions inhibit NO_3^- uptake (Deane-Drummond, 1986) resulted in the low content of NO_3^- in tomato leaves under salinity stress (Table III). In contrast, NH_4^+ content in the leaves of salinity stressed plants was greater than that of the control (Table III). The results suggested that the flux of NH_4^+ in leaves, particularly at 120 mM NaCl may not be originated from nitrate reduction. Recently, it is reported that the photorespiratory NH_4^+ release by the oxidative decarboxylation of glycine exceeds by about 10-fold the primary nitrate reduction in the vegetative leaves of tobacco (Masclaux-Daubresse *et al.,*

2006). However, a large amount of NH_4^+ is produced as a result of protein hydrolysis in the senescing leaves (Kant *et al.,* 2011). Our gas exchange study confirmed that salinity enhanced photorespiration by decreasing Ci level (Table II) and thus contributed in the flux of NH_4^+ in tomato leaves under salinity stress (Table III). Again, the protease activity (Fig. 1) in synchronous with the greater decrease in soluble protein (Table IV) and leaf chlorophyll (Table I) of salinity treated tomato seedlings could be argued in favor for high NH_4^+ content (Table III). The NH_4^+ is toxic for plant's cell. Therefore, the photorespiatory NH_4^+ is generally re-assimilated by GS/GOGAT cycle and produces glutamate (Lea & Miflin, 1974; Tabuchi *et al.,* 2007). Our results showed that the activities of GS and Fd-GOGAT at 60 and 120 mM NaCl salinity levels were statistically insignificant but significantly higher than the control. Interestingly, glutamate content was 137% and 176% over the control at 60 and 120 mM NaCl salinity levels, respectively. It meant that the GS/GOGAT cycle enzymes contributed partly to form glutamate by detoxifying NH_4^+. The results also revealed that other metabolic routes of NH_4^+ assimilation might be involved for glutamate synthesis, particularly at 120 mM NaCl salinity level. Therefore, we studied the aminating activity of NADH–GDH, which was significantly higher in the leaves of salinity treated seedlings than that of the control. The activation of aminating GDH pathway by NaCl suggests that GDH may be involved *in vivo* in NH_4^+ detoxification and in the replenishment of glutamate pool, which is highly required to produce proline. This is consistent with some previous reports (Santa-Cruz *et al.,* 1999; Chandra *et al.,* 2001; Debouba *et al.,* 2006).

Taken all together, it may be concluded that NADP-ICDH contributed C-skeletons in the form of 2-OG, and photorespiration and protein degradation collectively supply N in the form of NH_4^+, which efficiently aminated by GDH and forms glutamate, the precursor of proline. However, the GS/GOGAT cycle plays an important complementary role in supplying glutamate for proline synthesis during salinity stress in the present study.

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