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Effect of Sodium Nitro-Prusside (SNP) Preatreatment on Ammonia Assimilating Enzymes of Salt Stressed Tomato Leaves (*Lycopersicon esculentum*)

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THE EFFECTS of treatment with 100mM NaCl on photosynthetic activity, protein and proline contents , activities of key nitrogen metabolism enzymes, nitrate reductase (NR) and nitrite reductase (NiR), ammonia assimilating enzymes: Glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (NADH-GDH) activities and finally ammonia and nitrate contents were investigated in 40-day old leaves of tomato plants. There was a decline in total protein (TP) and insoluble protein (InsP) fractions accompanied with a significant increase in proline and soluble protein contents in response to NaCl – stress. Under salinized conditions, there was a significant inhibition in all tested leaf gas exchange parameters, stomatal conductance (g_s), internal CO_2 concentration (Ci) and hence, CO_2 assimilation (A). Also, a significant inhibition of NR & NiR enzymes and a strongly decrease in nitrate content were observed. In contrast, ammonia assimilating enzymes (GS, GOGAT, NADH-GDH and NAD-GDH) activities were obviously increased in NaCl – salinized tomato leaves, this accompanied with a significant increase in ammonia content. Soaking tomato seeds in 10µM sodium nitroprusside (SNP) for 8h were elevated-to some extent- all the studied parameters and there was an improvement in TP, proline, all gas exchange parameters, NR & NiR activities and nitrate content. While ammonia assimilating enzymes (GS, GOGAT, NADH-GDH and NAD-GDH) activities and ammonia content were significantly decreased compared to NaCl - salinized tomato leaves.

Keywords: Salt stress, Sodium nitroprosside (SNP), Nitrogen metabolism enzymes, Ammonia assimilating enzymes, Tomato plants.

Introduction

Salt stress has important effects upon plant growth and development. It causes the plant tissue to absorb water difficultly, breeds physiological disorder, and destroys cell structure, resulting in the decrease in yield and quality of crops. It is known that salt stress inhibits photosynthetic machinery (Jamil et al., 2007 and Stoeva & Kaymakanova, 2008). Hossain et al. (2012) suggested that decrease of internal CO_2 led to enhancement of photorespiration and production of H_2O_2. Zeng et al. (2010) conclude that oxygenation of ribulose 1,5 diphosphate and generation of ROS will be obtained under salt stress, in which O_2 is considered as H-acceptor from $NAPH_2$, instead of CO_2 and generation of H_2O_2.

Nitrogen is an essential plant macronutrient, and its availability has a major influence on the growth and development of plants. The most available form of this element in higher plants is nitrate (NO^{3-}), which is the most important source of N in the majority of agricultural soils. Compared with other inorganic sources of nitrogen, nitrate must be reduced to ammonium before its incorporation into organic compounds (Nasholm et al., 2009). Nitrate is first reduced by nitrate reductase (NR) to nitrite in the cytosol, and the resulting nitrite is in turn reduced to ammonium by nitrite reductase (NiR) in the plastids/chloroplasts. Ammonium must be rapidly assimilated as it is toxic to plant cells. These processes are carried out by two highly regulated pathways. Ammonium can be directly incorporated into glutamate by the aminating reaction of

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glutamate dehydrogenase (NADH-GDH). The resulting N is assimilated into glutamine by glutamine synthetase (GS), which is subsequently converted to glutamate using 2-oxoglutarate by glutamate synthase (GOGAT). The assimilation of ammonium into glutamine and glutamate is vital for plant growth because these two amino acids are precursors for the synthesis of the other amino acids, as well as almost all nitrogenous compounds (Kusano et al., 2011).

Interestingly, proline (a C-N rich compound) accumulation increased several folds in salinity stressed peanut leaves (Hossain et al., 2012) and it can serve as an adaptive mechanism to salt stress in higher plants (Kumara et al., 2003 and Chen et al., 2007) including tomato.

However, proline biosynthesis occurs predominantly from glutamate (Forde & Lea, 2007) and glutamate synthesis requires a Carbon skeleton in the form of 2-oxoglutarate (Kusano et al., 2011). Nitric oxide is a highly reactive molecule and the fact of being a free radical allows it to scavenge other reactive intermediates and end chain-propagated reactions. The rapid reaction between $O_2^{\bullet-}$ and NO to form the powerful oxidant peroxynitrite ($ONOO^-$) has been suggested as a deleterious mechanism (Leshem, 2000). However, it was also reported that, that in systems where the toxicity comes predominantly from peroxides, these compounds are much more toxic than NO and $ONOO^-$, making NO a protective agent against them (Wink et al., 1993).

Although it is now clear that NO is an essential signal required to mediate ABA-induced stomatal closure, there is little information on whether these signaling molecules mediate ABA-inhibition of stomatal opening (Schroeder et al., 2001). Sakihama et al. (2003) reported that NO promoted stomatal opening in *Vicia faba*. However, these data contradict those reported by Garcia-Mata & Lamattina (2001) from the same species, where NO was found to induce stomatal closure. These discrepancies could possibly be accounted for the relative concentrations of the NO donor (SNP) used. Desikan et al. (2003) reported that NO (administered *via* SNP) in the range of 10–200µM causes stomatal closure whilst at higher concentrations of SNP (0.5–2mM), stomata remain open. The physiological relevance for this phenomenon is not known, particularly as endogenous NO concentrations in and around guard cells have not yet been determined.

The aim of this study was to throw a beam of light on the role of SNP to improve the nitrogen metabolism in tomato plants grown under salt stress.

Materials and Methods

Tomato seeds (super strain B) were selected for uniformity of size, shape and color. Prior to germination, seeds were surface sterilized by soaking for two minutes in 4% (v/v) sodium hypochlorite, then washed several times with distilled water. The sterilized seeds were soaked in 10µM sodium nitroprusside (SNP) for 8h. The soaked seeds in distilled water were taken as control. Then, germinated seeds were transferred to plastic pots (15cm in diameter, 20cm length with a hole at the bottom) filled with fixed amount of mixture of previously acid-washed quartz sand and clay soil in a ratio of 2:1 w/w. The pots were placed under natural environmental conditions (photoperiod, 16L/8D light/dark; temperature, 27 ±2ºC light/23±2ºC dark; light intensity PPFD, 23µmol M-2 S-1) with 80% water holding capacity and irrigated by 1/10 strength modified Hoagland solution (Epstein, 1972) every two-days interval with distilled water. After twenty-one days from the beginning of the experiment, the pots were divided into four sets and irrigated as following: Set I, soaked seeds in distilled water and irrigated with 1/10 strength modified Hoagland solution described as control; Set II, soaked seeds in distilled water and irrigated with 1/10 strength modified Hoagland solution supplemented with 100mM NaCl described as NaCl-salinized treatment; Set III, soaked seeds in 10µM SNP for 8h and irrigated with 1/10 strength modified Hoagland solution described as SNP treated; Set IV, soaked seeds with 10µM SNP for 8h and irrigated with 1/10 strength modified Hoagland solution supplemented with 100mM NaCl described as SNP+NaCl. At the end of experimental period (40 days) from sowing, plants were taken carefully from the pots, washed thoroughly from adhering soil particles, leaves were taken for estimation of photosynthetic activity, enzymes assay and chemical analyses.

Determination of gas exchange parameters

Before harvesting, the gas exchange parameters (stomatal conductance (gs), internal CO_2 concentration (Ci) and CO_2 assimilation (A) were recorded using LICOR6400.

Determination of proline and protein fractions

The method described by El-Sharkawi & Michel (1977) was used for extraction and the acid ninhydrin method described by Bates et al. (1973) was carried out for quantitative estimation of proline. Total and soluble protein fractions were extracted according to the method described by Rausch (1981) quantitively and were estimated by Hartree (1972).

Determination of nitrate

Nitrate was determined according to the method of Johnson & Ulrich (1950). The color developed was measured at 420nm using spectrophotometer (JENWAY, 6305, UK) with reference to known concentrations of nitrate as potassium nitrate KNO^{-}_{3}.

Determination of free ammonia

This was made after the procedures described by Solorzano (1969), the developed color was measured at 630nm using (JENWAY, 6305 Uk/ UV) visible spectrophotometer with reference to a standard curve prepared from ammonium sulphate.

Enzyme assay

Nitrate reductase

Nitrate reductase activity (NR) was determined according to the method described by Debouba et al. (2006).

Nitrite reductase

Nitrite reductase was assayed using the method described by Losada & Paneque (1971).

Glutamine synthetase

Glutamine synthetase (GS) was determined according to the method described by Wallsgrove et al. (1979).

Glutamate synthase

NADH-GOGAT activities were measured as described by Suzuki et al. (2001).

Glutamate dehydrogenase

GDH aminating activity was determined by following the absorbance changes at 340nm (Masclaux-Daubresse et al., 2006).

Statistical analysis

All data were expressed as means of triplicate samples. Comparisons of means were performed using SPSS 20.0 software. The level of significant was ≤0.05. Replicates of all treatments were determined and results are given as mean. Data for all attributes were subjected to one-way analysis of variance and the mean values were compared with the least significance difference (LSD).

Results

Exposure of tomato plants to salinized nutrient media resulted in a significant decline in total protein (TP) and insoluble protein (InsP) fractions whereas these fractions were significantly increased compared to SNP-treated or untreated controls (Table 1). In addition, there was significant increase in proline in the NaCl – salinized leaves compared to SNP-untreated and treated controls. The data showed that soaking in SNP resulted in a decline of proline content in NaCl-stressed leaves compared also to those in absence of SNP (Table 2).

TABLE 1. Changes in protein fractions (TP and SP) (mg g^{-1}d.m.) of leaves of tomato plants as a result of presoaking of seeds for 8h either in water, 10µM sodium nitroproside (SNP) followed by irrigation with 1/10 Hoagland solution alone or supplemented with 100mMNaCl.

Treatments	SP	InSP	TP	SP/TP%
Control	51.76±4.17[a]	96.49±7.78[a]	148.25±11.96[a]	35±2.82[a]
NaCl	78.65±5.70[b]	15.88±1.15[b]	94.53±6.85[b]	83±6.01[b]
SNP	46.55±3.98[a]	109.04±9.32[a]	155.59±13.30[a]	30±2.56[a]
SNP+NaCl	63.41±4.92[c]	49.50±3.84[c]	112.91±8.75[c]	56±4.34[c]
P	0.012*	0.002*	0.0032*	0.001*
LSD	6.5	15.0	16.0	14.5

TABLE 2. Changes in gas exchange parameters (stomatal conductance (gs), internal CO_2 concentration (Ci) and CO_2 assimilation (A)) of leaves of tomato plants as a result of presoaking of seeds for 8h either in water, 10µM sodium nitroproside (SNP) followed by irrigation with 1/10 Hoagland solution alone or supplemented with 100mMNaCl.

Treatments	A	g_s	C_i
Control	14.6±1.18[a]	285±22.98[a]	256±20.65[a]
NaCl	3.3±0.24[b]	126±9.13[b]	105±7.61[b]
SNP	14.4±1.23[a]	268±22.91[a]	250±21.37[a]
SNP+NaCl	9.8±0.76[c]	177±13.72[c]	216±16.74[c]
P	0.022*	0.004*	0.021*
LSD	3.1	25.0	30.0

TABLE 3. Changes in accumulative NO^-_3 (µmoL No_3g^{-1}d.m.), accumulative NH^+_4 (m mol NH^+_4 g^{-1}d.m.) and proline content (µg g^{-1}d.m.) of leaves of tomato plants as a result of presoaking of seeds for 8h either in water, 10µM sodium nitroproside (SNP) followed by irrigation with 1/10 Hoagland solution alone or supplemented with 100 mM NaCl.

Treatments	NO^{3-}	NH^{4+}	Proline
Control	21.06±1.70[a]	1.86±0.15[a]	116±9.35[a]
NaCl	2.90±0.21[b]	7.53±0.55[b]	4224±306.09[b]
SNP	33.99±2.91[c]	1.35±0.12[a]	139±11.88[a]
SNP+NaCl	8.68±0.67[d]	2.45±0.19[a]	1320±102.33[c]
P	0.001*	0.006*	0.001*
LSD	5.1	1.6	50.0

It is clearly shown that all tested leaf gas exchange parameters of 100mM NaCl-grown tomato plants were significantly decreased compared to the untreated plants. On the other hand, preatreatment of seeds with 10µM-SNP and irrigated with salinized rooting medium (SNP+NaCl) significantly increased leaf gas exchange parameters compared to those grown in NaCl-salinized nutrient (Table 2).

There was a significant suppression of foliar NO^-_3 content in response to NaCl stress compared to SNP-treated and untreated tomato plants. Moreover, soaking tomato seeds in 10µM SNP resulted in a significant NO^-_3 accumulation compared to control. Conversely to the trend of NO^-_3 accumulation, foliar NH^+_4 content was significantly increased due to NaCl stress conditions. The NH^+_4 content in NaCl-stressed leaves was 4.1 fold of that in the control. The corresponding value in SNP-NaCl treated leaves was 1.8 fold as that in the SNP-treated plants (Table 3).

Salinization the growth medium of tomato plants with 100mM NaCl significantly suppressed folair NRA and NiRA compared to SNP-treated and untreated plants. The decrease of NRA and NiRA in NaCl-stressed leaves was reached to 88% and 71%, respectively compared to control (Fig 1). Conversely, there was a significant enhancement of foliar NRA and NiRA in SNP-treated compared to control plants. The foliar NRA and NiRA of SNP-pretreated plants was 1.4-and 1.8-fold of control plants. Moreover, SNP-pretreatment could shift of the inhibitory effect of NaCl stress on both NRA and NiRA. The NAR and NiRA in the salinized SNP-pretreated leaves were 3.8 and 2.5 fold of those in the NaCl-stressed plants (Fig. 1). It is clearly shown that, there was a significant increase of foliar NH^{4+}-assimilating enzyme activites in SNP-treated or untreated compared to control plants. The GS and GOGAT activities in NaCl-treated tomato leaves were 3.2 and 3.5 fold, respectively as those in the control plants (Fig. 2 and 3). The corresponding values in salinized - SNP-pretreated leaves were 2.3 and 2.3 fold, respectively as those in the SNP-treated leaves. Similarly, the NADH-GDH and NAD-GDH activities were 4.7 and 12.3 fold in NaCl-stressed foliar tomato plants as those in the control, respectively. In salinized-SNP-pretreated plants these values were 2.0 and 6.3 fold, respectively as those in the SNP-treated plants (Fig. 2 and 3).

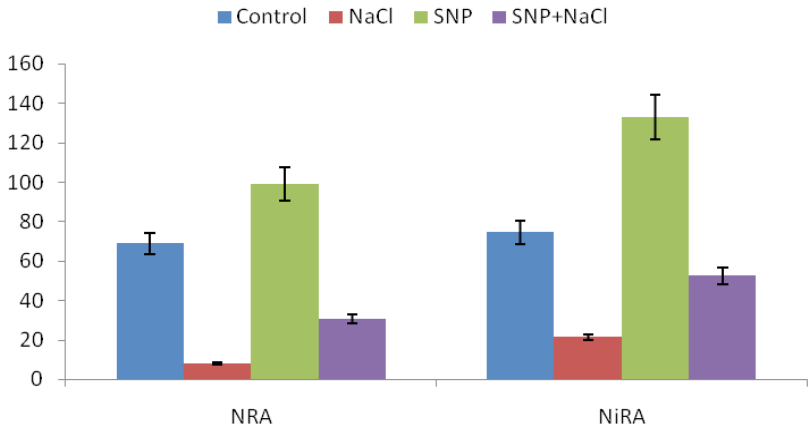

Fig. 1. Changes in nitrate reductase (NRA) activity (µmol $NO_2^-g^{-1}$ d.m. $30min^{-1}$) and nitrite reductase (NIRA) (µmol $NO_2^-g^{-1}$ d.m. $30min^{-1}$) activity of leaves of tomato plants as a result of presoaking of seeds for 8h either in water, 10µM sodium nitroproside (SNP) followed by irrigation with 1/10 Hoagland solution alone or supplemented with 100mM NaCl.

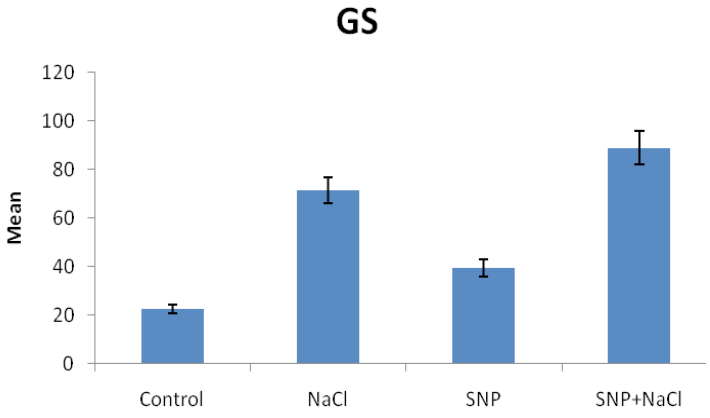

Fig. 2. Changes in glutamine synthetase (GS) of leaves of tomato plants as a result of presoaking of seeds for 8h either in water, 10µM sodium nitroproside (SNP) followed by irrigation with 1/10 Hoagland solution alone or supplemented with 100mM NaCl.

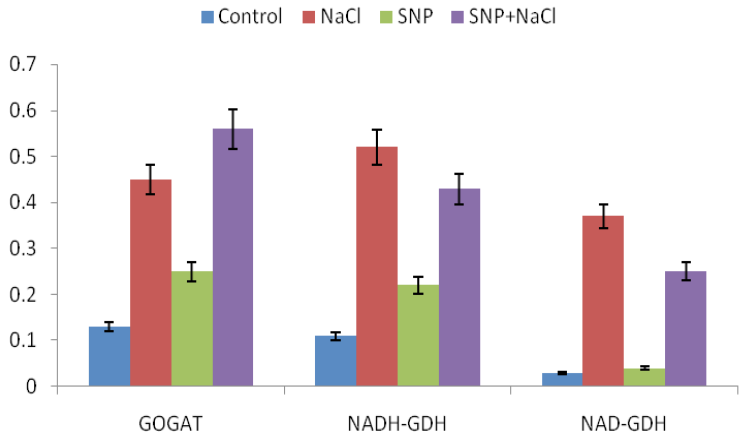

Fig.3. Changes in glutamate synthetase (GOGAT) and glutamate dehydrogenase (NADH-GDH, NAD-GDH) of leaves of tomato plants as a result of presoaking of seeds for 8h either in water, 10µM sodium nitroproside (SNP) followed by irrigation with 1/10 Hoagland solution alone or supplemented with 100mM NaCl.

Discussion

Total protein (TP) and insoluble protein (InsP) fractions were significantly decreaed in NaCl-stressed leaves (Table 1), this was accompanied with an increase of SP, SP/TP and proline contents (Table 3). These results are consistent with the findings of other authors (Sairam et al., 2002; Yokota, 2003; Sumithra et al., 2006 and Hossain et al., 2012). In contrast, Hossain et al. (2012) have reported that SP content in tomato plants was markedly decreased under salt stress. Generally, the increase of SP and proline could reflect the enhancement of protein hydrolysis or inhibition of amino acids incorporation to protein and that might play a role as osmoregulatory and/or protective agents of the oxidative stress (Rontein et al., 2002 and Yazici et al., 2007). Amini & Ehsanpour (2005) concluded that under salt stress the proteolytic activity, in tomato leaves, was significantly increased, compared to controls.

It is well shown that salt stress resulted in an increase of stomatal clousure led to a significant decrease of stomatal conductance (g_s), internal CO_2 concentration (Ci) and hence CO_2 assimilation (A) (Table 2). The decrease of internal CO_2 concentration enhance photorespiration processes (Hossain et al., 2012) and oxygenation of ribulose 1,5 biphosphate (Zeng et al., 2010) as well as $NADPH_2$ (Hodges et al., 2003). Therefore, oxygen might be an acceptor of H^+ from $NADPH_2$ instead of CO_2 resulting in a generation of H_2O_2. Moreover, the enhancement of photorespiration led to increase of H_2O_2 production and hence oxidative stress. On the other hand, application of biomolecule SNP with salinized nutrient solution of tomato plants significantly increased the photosynthetic activity, compared to NaCl-stressed ones resulting in a decline of H_2O_2 accumulation and improve the growth (Ezzat et al., 2015). These observations might attribute to reaction of SNP with generated ROS (Leshem, 2000 and Wendehenne et al., 2001), therefore shift of, to some extent, the inhibitory effect of ROS on photosynthetic activity.

Data in Table 3 observed that, salinity stress resulted in a significant decrease in foliar NO^{-3} content and both NRA and NiRA (Fig. 1) of tomato plants. These observations were reported in many glycophytes (Saber et al., 1995, Abd El-Baki, 2000; Surabhi et al., 2008; Hossain et al., 2012 and Gao et al., 2013). There are several possibilities could be explained the suppression of NO^{-3} accumulation and NO^{-3} reduction, in this study, firstly, the inhibitory effect of NaCl stress on the plasma membrane integrity due to the generation of ROS (Ezzat et al., 2015) resulting in a disturbance of NO^{-3}-transporter ions such as K^+ and Ca^{2+} as well as blocking NO^{-3} channels (Debouba, 2006 and Wahid et al., 2007). Moreover, the decrease of NRA and NiRA activities could be attributed to suppression of their inducer (NO^{-3}). Zhou et al. (2004), Debouba et al. (2006) and Dluzniewska et al. (2007) have been reported that NO^{-3} is an inducer of NRA and NiRA activities. Secondly, the breakdown of enzyme protein that is induced by generated ROS. Lillo (2004) have reported that ROS caused a destructive effect on protein by oxidative reactions and increased proteolytic activity. Carillo et al. (2005) concluded that the decrease of NR activity in wheat plants under NaCl salinity was related to the low of NR protein. Hossain et al. (2012) stated that increased proteolytic enzymes activity in the leaves of NaCl-stressed tomato plants was accompanied with a marked decreased in NO^{-3} content and NR activity. Thirdly, the inhibitory effect of NaCl stress on PSII activity (Ezzat et al., 2015) might be resulted in a decrease of available C-seckleton and H-donor for NO^{-3} reduction. Jamil et al. (2007) reported that NaCl inhibits photosynthetic activity in several plants.

In this study, pretreatment with SNP caused a significant increase of foliar NO^{-3} content (Table 3), NAR and NiRA of tomato plants (Fig. 1) grown under NaCl stress. These observations might indicate that SNP trigger several changes linked to the maintenance of metabolic processes and activation of defense mechanisms for plant NO^{-3} requirement and NO^{-3} reduction in tomato leaves under saline conditions. Neill et al. (2003) have reported that nitric oxide (NO) react with oxygen or superoxide $(O_2)^-$ results in generation of NOX compounds (NO^{-2}, N_2O) which simply hydrolyzed to NO^{-2} and NO^{-3}. These observations could be explained the increase of foliar NO^{-3} accumulation, NRA and NiRA of SNP-pretreated tomato seeds grown under non saline or saline conditions. Lamotte et al. (2004) concluded that SNP directly react with lipid radicals to stop the propagation of lipid peroxidation. Gaber (2012) showed that SNP significantly decrease H_2O_2 and MDA content in tomato plants grown under NaCl stress. He

reported that SNP stimulates a defense mechanism for protection of plasma membrane integrity. In this connection, the increase of foliar NO^{-3} content and both NRA and NiRA of NaCl-stressed tomato plants in presence of SNP might be related to improvement of plasma membrane integrity and therefore increase of NO^{-3} uptake and induction of NO^{-3} assimilating enzymes. Moreover, the protection of photosynthetic activity of NaCl-stressed plants in presence of SNP could led to increase the production of $NAPH_2$ which act as H-donor for NRA and NiR, thus increase their activities.

Many authors (Gouia et al., 1994; Hoai et al., 2005; Hossain et al., 2012 and Gao et al., 2013) suggested that salinity stress resulted in a marked increase of NH^{+4} accumulation. In accordance with these views, noteworthy, there was a significant accumulation in foliar NH^{+4} content (Table 3) of tomato plants in response to $NaCl^{-}$ stress. These finding were accompanied with a significant decrease of internal CO_2 concentration (Table 2).

Nasholm et al. (2009) reported that NH^{+4} released during photorespiration was exceeded by 10-fold the NO^{-3} reduction in tobacco leaves.

Hossain et al. (2012) concluded that enhancement of photorespiration by decreasing internal CO_2 concentrations (Ci) could attribute in the increase of NH^{+4} accumulation in tomato plants under salt stress. While, Kant et al. (2011) suggested that an increase of NH^{+4} accumulation under salinity might be related to protein hydrolysis in the senescing leaves. Thus, the increase of foliar NH^{+4} accumulation, in this study, could be attributed to the increase of photorespiration due to the decrease of internal CO_2 concentrations (Ci).

SNP pretreatment significantly suppressed the foliar NH^{+4} accumulation compared to NaCl-stressed plants (Table 3). These results might reveal to enhancement of GS/GOGAT cycle. Miflin & Habash (2002) reported that ammonia is rapidly assimilated into organic N via the GS/GOGAT cycle or via the GDH alternative pathway. In this study, NaCl stress was related to a significant increase in the activities of GS, GOGAT, NAD- GDH and NADH-GDH (Fig. 2). These results might reveal the detoxification of NH^{+4} by enhancement of ammonia assimilating enzymes to glutamic acid. Therefore, the increase of folair proline accumulation, in this study, (Table 3), might be related to increase the supply of its precursor (glutamic acid) during NH^{+4} assimilation.

Some reports have suggested that GS/GOGAT and GDH pathways are activated by salt stress and are the major route of ammonium assimilation (Zhou et al., 2004 and Surabhi et al., 2008). In this connection, there was a significant increase of GS, GOGAT, NAD- GDH and NADH-GDH in foliar NaCl- salinized tomato plants. The activation of GS/GOGAT and GDH activity suggests that these enzymes might play important roles in scavenging excessive endogenous ammonium and the replenishment of the glutamate pool in tomato plants. It is clearly demonstrated that, SNP-pretreated tomato seeds grown under NaCl stress resulted in a significant decrease of ammonia assimilating enzyme activities compared to NaCl-treated alone. These results may be reflect the role of SNP on reduction of ammonia accumulation (Table 3) and diminish the detoxification of both NH^{+4} and NaCl in the leaves of tomato plants.

Conclusion

This study concluded that pre-treatment of tomato seeds with 10µM sodium nitroprusside (SNP), soaked for 8h, improve the drastic effect of salinity (100mM NaCl) on photosynthetic activity, protein and proline contents, activities of key nitrogen metabolism enzymes, nitrate reductase (NR) and nitrite reductase (NiR), ammonia assimilating enzymes: Glutamine synthetase (GS), glutamate synthase (GOGAT) and glutamate dehydrogenase (NADH-GDH) activities and finally ammonia and nitrate contents.

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تاثير المعالجه بصوديوم نيتروبروسيد على انزيمات تمثيل الامونيا في اوراق نبات الطماطم الوا قع تحت الاجهاد الملحي.

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يهدف البحث إلى دراسة تأثير اجهاد الملوحة بوجود تركيز 100ملى مول كلوريد الصوديوم على نشاط عملية البناء الضوئى – محتوي البروتين – البرولين وكذالك نشاط النزيمات المرتبطة بالعمليات الايضية للنيتروجين مثل انزيمى النترات ريدكتيز والنيتريت ريدكتيز (NR-NIR) وايضا نشاط انزيمات الأمونيا مثل جلوتامين سينستيز- جلوتاميت سينسيز وجلوتاميت ديهيدروجينيز (GS, GOGAT and NADH-GDH) وكذلك دراسة محتوى الأمونيا والنترات فى أوراق نبات الطماطم المزروع لمدة 40 يوم فى الملح السابق ذكر تركيزه فتبين لنا وجود نقص ملحوظ فى المحتوى البروتينى الكلى و البروتينات الغير ذائبة مصاحب بزيادة فى محتوى البرولين والبروتينات الذائبة و وجد ايضا تثبيط فى عملية تبادل الغازات فى ورقة نبات الطماطم – معدل الثغور– تركيز ثانى اكسيد الكربون – تمثيل ثانى اكسيد الكربون و النتائج توضح نقص انزيمى NR-NIR وبالعكس يوجد ارتفاع ملحوظ فى انزيمات تمثيل الأمونيا (GS, GOGAT, NADH-GDH and NAD-GDH) فى وجود كلوريد الصوديوم وذلك مصاحب بزيادة فى محتوى الأمونيا ولكن عند نقع بذور الطماطم فى تركيز 10 ميكرو مول من صوديوم نيتروبروسيد قبل الزراعة ولمدة 8 ساعات يلاحظ وجود ارتفاع فى مستوى القياسات المختارة وتحسن واضح فى البروتين الكلى والبرولين وأيضا معدل تبادل الغازات ونشاط انزيمى (NR-NIR) ومحتوى النترات بينما الأنزيمات المرتبطة بتمثيل الأمونيا -GS, GOGAT, NADH-GDH and NAD GDH ومحتواها يوجد به تثبيط ملحوظ وذلك مقارنة بأوراق النباتات التي لم يتم نقعها فى تركيز 10 ميكرو مول من صوديوم نيتروبروسيد أو التى توجد تحت الإجهاد الملحى.