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Salt Stress: Effects on Nitrogen Metabolism in Tomato Plants Differing in Salt Tolerance

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ABSTRACT

The cultivated tomato (*Solanum lycopersicum* L.) is a widely-grown crop plant and the focus of a large agricultural industry. Tomato is cultivated in almost every corner of the world, but a major portion of the world tomato production is concentrated in a rather limited number of warm and not humid areas. Although such areas generally provide optimal environmental conditions for tomato production, a high level of salinity frequently encountered in the soil or in the irrigation water poses serious constraints to tomato production. The aim of this work was to analyze the relationship between salinity and nitrogen (N) metabolism, in order to evaluate the effects of using sea water for tomato irrigation and to optimize N use efficiency and thus yield amount and quality. Three tomato genotypes differing in their relative level of salt tolerance were exposed to salinity stress: cv. 'Edkawi' (EDK), salt tolerant; cv. 'Gimar' (GIM), relatively salt sensitive and its near isogenic line for the *nor* gene (NOR) defective in ethylene synthesis. Tomato plants were grown hydroponically for 4 weeks in control (EC = 3 mS cm^{-1}) or in saline conditions (EC = 10 mS cm^{-1}). Plant growth, ethylene emission, Cl^-, NO_3^- and free amino acids (a.a.) contents and the activities of nitrate reductase (NR; EC 1.6.6.1), glutamine synthetase (GS; EC 6.3.1.2) and NAD-dependent glutamate dehydrogenase (GDH; EC 1.4.1.2) were measured in roots to compare control and salt-treated plants. Results confirmed the NO_3^-/Cl^- antagonism and suggested nitrate content as a marker of plant salt tolerance. The relationship between root NR activity and NO_3^- content suggests a complex response of tomato plants to imposed salt stress.

Keywords: abscisic acid, ethylene, glutamate dehydrogenase, glutamine synthetase, nitrate reductase

INTRODUCTION

Several nitrogen (N) fertilizers are applied in fields, since N is a key element of a large number of cell components such as amino acids, proteins, nucleic acids, porphirins, cytochromes (Ullrich 1992). Therefore the absorption of N by plants plays a significant role both in crop yield and quality (Marschner 1995). Unlike other nutrient elements, it can be utilised by plants in two ionic forms: NH_4^+ cation and NO_3^- anion (Marschner 1995), but nitrate is often the major source of N available to higher plants. When absorbed by the plant, the first step in nitrate assimilation is its reduction to nitrite by nitrate reductase (NR; EC 1.6.6.1). Nitrate reduction is considered the rate limiting and regulatory step in the N assimilation pathway. NH_4-N is produced by the following action of nitrite reductase and is then assimilated into an organic form as glutamate and glutamine. The enzymes responsible for the biosynthesis of these amino acids are glutamine synthetase (GS; EC 6.3.1.2), glutamate synthase (GOGAT; EC 1.4.7.1) and glutamate dehydrogenase (GDH; EC 1.4.1.2).

The cultivated tomato (*Solanum lycopersicum* L.) is a widely-grown crop plant and the focus of a large agricultural industry (Bebeli and Mazzucato 2008). Although a tropical plant, tomato is grown in almost every corner of the world, but a major portion of the world tomato production is concentrated in a rather limited number of warm and not humid areas, in particular regions around the Mediterranean Sea. Although such areas generally provide optimal climates for tomato production, a high level of salinity frequently encountered in the soil or in the irrigation water poses serious constraints to tomato production. Natural soil pedogenetic processes in warm and dry regions could often result in saline soils formation with low agricultural potential. Furthermore, tomato cultivation requires irrigation in these areas and an inadequate irrigation management could lead to salinisation of water resources and soils, defined as secondary salinisation (Cuartero and Fernández-Muñoz 1999).

The macroscopic effects induced by salinity are recognized in a reduction of vegetative growth and yield (Ramage 1979), with varying expression according to the species and genotype. In tomato, Cuartero *et al.* (1995) reported the influence of salinity on fruit size, sugar content, polygalacturonase activity and CO_2 production.

Salinity can reduce N accumulation in plants (Cram 1973) and such an effect has also been found in tomato (Feigin *et al.* 1987; Pessarakli and Tucker 1988). Nitrate uptake is particularly affected by salinity (Lips *et al.* 1990), since chloride competes with nitrate for uptake and translocation within the plants by nitrate transporter proteins (Campbell 1999). As a consequence, salinity markedly affects nitrate assimilation since NR is an inducible enzyme and nitrate is needed to induce it (Kaiser and Huber 2001).

On the other hand, some evidences suggest that the metabolism of N compounds plays a key role in the ability of plants to tolerate salinity (Rains 1979). Most salinity and N-interaction studies in open field demonstrated that additions of N improved growth and yield of tomato when the degree of salinity was not severe (Papadopoulos and Rendig 1983). However, trials in lab and greenhouse have proved that salinity can reduce N accumulation in plants (Cram 1973) and such an effect has also been found in tomato (Feigin *et al.* 1987; Pessarakli and Tucker 1988). In addition, the NO_3^- influx rate may be related to the salt tolerance: salt-tolerant tomato cultivars showed higher NO_3^- influx rates than the more sensitive ones (Kafkafi *et al.* 1992; Perez-Alfocea *et al.* 1993).

Recently a key role of ethylene, a plant signal molecule, is emerged with regard to its multiple effects during plant

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development (Smalle and van der Straaten 1997), in root formation (Clark *et al.* 1999), in fruit ripening (Alexander and Grierson 2002) and in response to a number of biotic and abiotic stresses. Few data are however available for its involvement in plants exposed to salinity. By studying in Arabidopsis, tomato and tobacco mutants, it was shown that the ethylene signal interacts with a number of exogenous and endogenous factors controlling the downstream gene expression (Wang *et al.* 2002). Also abscisic acid (ABA) has been involved in plant adaptation to salinity, since it has been shown to mediate various responses to osmotic stress, as proline accumulation, stomatal closure and shoot growth inhibition (Ruggiero *et al.* 2004; Verslues and Zhu 2005).

The purpose of this work was to investigate in tomato plants the effects of salinity on different phases of N metabolism (uptake, reduction and assimilation) at the root level.

Tomato is a model plant for geneticists and molecular biologists; consequently to its high intra- and inter-specific genetic variability displayed and the great number of studies available, this vegetable appears particularly suitable for investigations directed to better comprehend the metabolic mechanisms and their genetic regulation responsible of its adaptation to grow in experimental saline conditions. In particular, tomato was used in this study because it has proven to be sensitive to salt stress (Ayers e Westcot 1989) and because of the availability of genotypes differing in their tolerance to salt conditions as cv. 'Edkawi' (Jones 1987; Habashi 1992; Picarella *et al.* 1995). Furthermore, the availability of the cv. 'Gimar' *nor*, a near isogenic line (NIL) of the cv. 'GIM', differing in homozygous genes affecting ethylene metabolism could allow us to achieve some indications about the role played by ethylene in response to salinity during the vegetative phase.

We studied the effect of salinity in the roots of the above described plants by analysis of N content and chemical form, rate of nitrate uptake and nitrate reductase (NR) activity. Investigation was completed by assessing changes in the activity of GS and GDH, in order to gain further information on the effects of salinity on assimilatory phase of N metabolism. Ethylene and ABA levels were also measured.

MATERIALS AND METHODS

Plant material and growing conditions

The different tomato (*Solanum lycopersicum* L.) genotypes studied in this work came from the Soressi germplasm collection at Tuscia University (Viterbo, Italy). A "salad" tomato type (cv. 'Gimar'), its near isogenic line (NIL), obtained by backcrossing the mutant genes *nor* (non ripening) to the cv. 'Gimar', and the salt tolerant cv. 'Edkawi' were analyzed. These genotypes are from now on reported as GIM ('Gimar'), NOR (cv. 'Gimar' *nor*) and EDK (cv. 'Edkawi'). Tomato seedlings were grown in plastic pots (6 plants per pot) containing 2.2 l of nutrient solution (NS) (Pinton *et al.* 1999), supplemented with 10 mM NaCl, which served as control (EC = 3 mS cm^{-1}), or with 70 mM NaCl, which served as treatment solution (EC = 10 mS cm^{-1}). Seedlings were kept into a climatic chamber under 200 microE m^{-2} s^{-1} PPF and 14/10 day/night regime (28/20°C day/night temperature cycling, 80% relative humidity). After 4 weeks from sowing, plant roots were used for chemical analysis and enzyme assays.

Measurement of ethylene production

Ethylene production was determined according to the method described by Antonelli *et al.* (2008). Plants were harvested and separated into roots and shoots. Whole root system was carefully placed in a test tube (50 ml). The tubes were sealed with rubber caps and incubated in the dark at 24°C for 2 h. Gas samples (1 ml) were withdrawn from the tube through a syringe and analyzed for ethylene by gas chromatography (Fraetovap 4200, Carlo Erba) equipped with a 80100 mesh alumina column. After analysis of ethylene, the roots were weighed and the fresh weight was used to calculate the rate of ethylene production.

Measurement of ABA concentration

ABA concentrations were determined as described in Vernieri *et al.* (1989). Briefly, roots samples (ranging from 0.1 to 1 g of fresh weight) were extracted with distilled water added in a ratio of 20:1 (v/w) for 16 h at 4°C, in the dark. Quantitative analysis was performed on crude aqueous extracts using a solid-phase radioimmunoassay (RIA) based on a monoclonal antibody (DBPA1) raised against free (S)-ABA. Procedure to validate the efficiency of the extraction method and the RIA results using DBPA1 monoclonal antibody on crude extracts of tomato tissues were described in detail by Vernieri *et al.* (1989).

Measurement of net NO_3^- uptake

Roots of intact plants were washed with 1 mM $CaSO_4$ solution for 24 h and then transferred in 30 ml aerated solution containing 0.2 mM KNO_3 and 0.5 mM $CaSO_4$. Samples (0.2 ml) for NO_3^- determination were removed every 15 min, for 90 min, the period during which uptake was observed to have a linear trend, and the net uptake was measured as NO_3^- depletion from the solution per unit of time according to Cataldo *et al.* (1975).

Enzyme extraction and assays

Frozen roots (*ca.* 1 g of fresh weight) were ground to a fine powder in a pre-chilled mortar under liquid N_2. Cold extraction buffer containing 50 mM HEPES-KOH (pH 7.4), 5 mM $MgCl_2$, 1 mM EDTA, 10% (v/v) glycerol, 0.1% (v/v) Triton X-100, 5 mM DTT, 1 mM PMSF and 1% (w/v) PVP was added in a ratio of 1:7 (w/v). The brei was filtered through four layers of cheesecloth and the homogenate was centrifuged at 1000 × *g* for 5 min at 4°C. The resulting supernatant was desalted at 4°C on a Sephadex G-25 column (PD-10, Pharmacia, Uppsala, Sweden) pre-equilibrated with extraction buffer minus Triton X-100. The desalted extract was then centrifuged at 30,000 × *g* for 5 min at 4°C. the supernatant was divided into 0.3 ml aliquots which were frozen in liquid N_2 and stored at -80°C until analysis.

The procedures described in Astolfi *et al.* (2001) were followed to determine nitrate reductase (NR; EC 1.6.6.1), glutamine synthetase (GS; EC 6.3.1.2), and NAD-dependent glutamate dehydrogenase (GDH; EC 1.4.1.2) activity.

Extraction and determination of free amino acid

Free amino acids content was estimated according to Winters *et al.* (2002), with minor modifications. Free amino acids were extracted from samples of root tissue with distilled water added in a ratio 0.1: 1 (w/v) and shaking in a boiling water bath for 25 min. Samples were then allowed to cool and centrifuged at 10,000 × *g* for 10 min. The supernatant was analysed using the ninhydrin assay. The ninhydrin reagent was prepared by making up a 3% (w/v) solution in DMSO. A working solution was arranged by adding 1 ml 0.085 M ascorbic acid to 49 ml pH 5.2-5.3 sodium acetate buffer prepared by mixing 0.2 M acetic acid with 0.2 M sodium acetate. A 0.2 ml sample was mixed with 0.1 ml of working solution and 0.1 ml of ninhydrin solution and rapidly transferred into a block heater at 100°C for 15 min. The reaction mixture was then rapidly cooled and diluted with 1 ml ethanol. The absorbance was measured at 570 nm.

Extraction and determination of nitrate

Nitrate content was measured colorimetrically according to Singh (1988), with minor modifications. Briefly 0.2 g of root tissue were crushed thoroughly in 10 ml 2% acetic acid and the filtered through filter paper. The assay was performed by adding 0.2 g of a powder containing 37 g citric acid, 5 g manganese sulphate monohydrate, 2 g sulphanilamide, 1 g NED and 1 g zinc to 5 ml of the aqueous extract. Samples were shaken and centrifuged at 1500 × *g* for 5 min. Absorbance of the supernatant was measured at 540 nm.

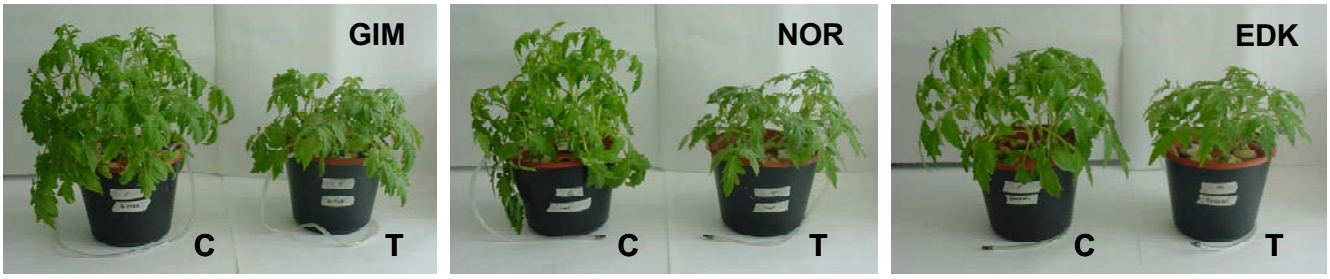

Fig. 1 Tomato (*Solanum lycopersicum* L.) plants were grown in plastic pots (6 plants per pot). In each picture, the plant on the left was supplied with 10 mM NaCl, which served as control (C) whilst the plant on the right was supplied with 70 mM NaCl, which served as treatment solution (T).

Extraction and determination of chloride ion

Roots samples were dried at 110°C for 24 h and then ground. Chloride ions were extracted from plant tissues in distilled water added in a ratio 1: 50 (w/v) and shaking in a water bath at 30°C for 1 h. The chloride ions content was determined spectrophotometrically from the filtrate using the mercuri(II)thiocyanate method described by Florrence and Farrar (1971).

Other measurement and statistics

Protein content was determined according to Bradford (1976), using BSA as standard.

The concentration of chlorophyll per unit area was estimated in attached leaves by a SPAD portable apparatus (Minolta Co., Osaka, Japan) using in particular the first fully expanded leaf from the top of the plant.

Each reported value in tables and graphs represents the mean ± SD of measurements carried out in triplicate and obtained from three independent experiments. Statistical analyses of data were carried out by ANOVA tests with the GraphPad InStat Program (version 3.06). Significant differences were established by *posthoc* comparisons (HSD test of Tukey) at $P < 0.01$ or $P < 0.05$. Moreover, the Student's *t*-test (GraphPad InStat Program, version 3.06) was used to determine the significance of differences between control and salt-treated plant.

RESULTS

Fig. 1 shows tomato plants grown at 10 mM and 70 mM NaCl, which served as control (C) and treated (T) plant, respectively. Visual observations (**Fig. 1**) suggested that plants supplied with 70 mM NaCl were poorly developed. After 4 weeks of growth in the control nutrient solution GIM plants accumulated more dry mass with respect to NOR and EDK plants, both at shoot level (**Fig. 2A**). The EC increase of nutrient solution caused a reduction of dry biomass but root growth appeared to be less affected by salt than shoot growth. In particular, the effect of the stress was particularly pronounced for the sensitive cv. GIM (-40%) with respect to its near isogenic line NOR (-20%) and the tolerant cv. EDK (-30%) (**Fig. 2A**). On the other hand, root dry weight was not affected by salt stress in GIM and EDK plants but significantly increased in NOR (+ 50%) (**Fig. 2B**). As a direct consequence, the shoot-to-root ratio decreased relatively to the salinity of the nutrient solution (box in **Fig. 2B**).

The chlorophyll content in tomato leaves was determined by a SPAD portable apparatus that provide a sensitive and accurate index of leaf chlorophyll content and relative data are showed in **Fig. 3**. Except for salt tolerant EDK plants, salt exposure was associated to a decline of leaf chlorophyll content (-15%).

The basal level of ethylene produced by roots of plants under normal conditions of growth was similar in GIM and EDK control plants, while, as expected, NOR roots showed a very low level of this hormone (**Fig. 4A**). However, the salt treatment induced a strong decrease of ethylene production in all genotypes. In particular, ethylene production dropped by 50% in EDK and 40% in GIM and NOR. As shown in **Fig. 4B**, GIM roots showed the lowest ABA content when plants were grown under control conditions. Salt treatment resulted in an increased ABA production both in GIM and EDK plants, but the extent of the increase was greater in salt sensitive GIM plants (+ 210%) than in salt tolerant EDK (+ 76%). On the other hand, ABA levels in NOR roots was slightly decreased by salt treatment.

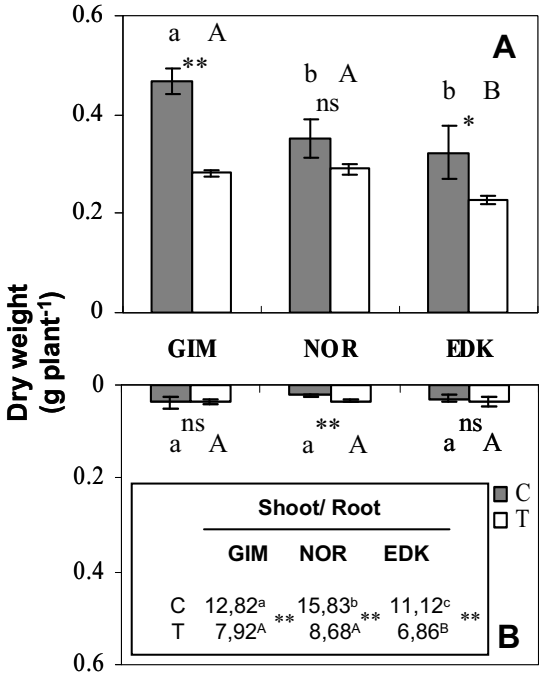

Fig. 2 Shoot (A) and root (B) dry weight of tomato seedlings after 4 weeks of growth in control (C) or in salt-treated solution (T). *In box*: shoot to root ratio. Data are means ± SD of three independent replications. Stars indicate significant differences between control (C) and salt-treated (T) plants (significance levels: * P<0.05, ** P<0.01, *** P<0.001). Different small letters indicate significant differences (at least P<0.05) among the three control plants. Different capital letters indicate significant differences (at least P<0.05) among the three salt-treated plants.

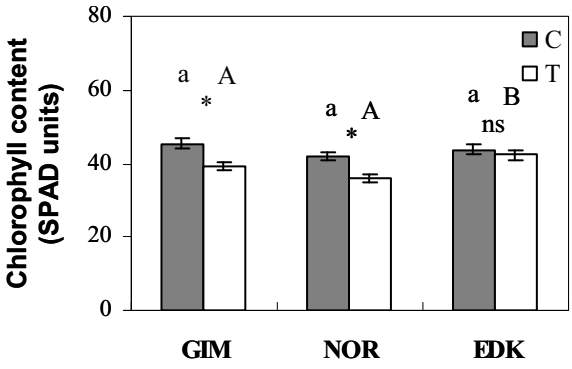

Fig. 3 Chlorophyll content of tomato seedlings after 4 weeks of growth in control (C) or in salt-treated solution (T). Statistics as in **Fig. 1**.

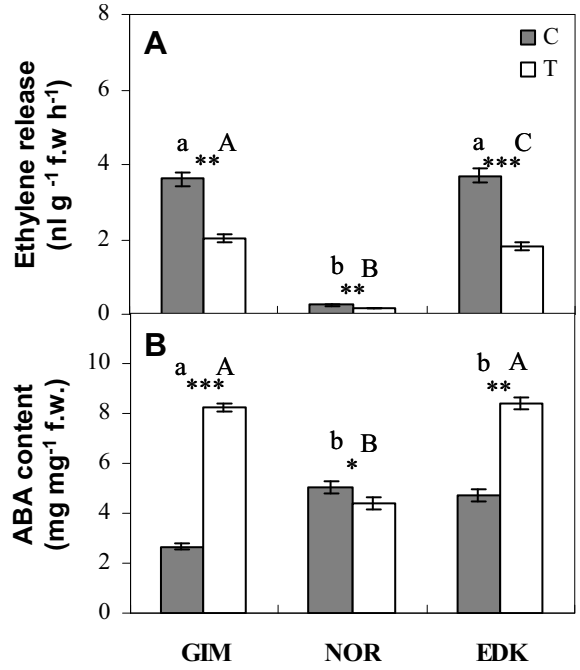

Fig. 4 Ethylene (A) and ABA content (B) in roots of tomato plants grown in control (C) or in salt-treated (T) solution. Statistics as in **Fig. 1**.

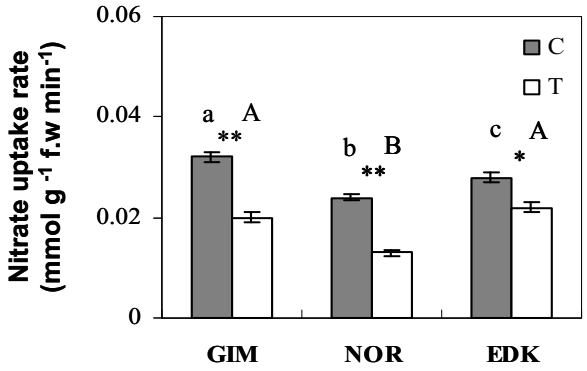

Fig. 5 Nitrate uptake rate in roots of tomato plants grown in control (C) or in salt-treated (T) solution. Statistics as in **Fig. 1**.

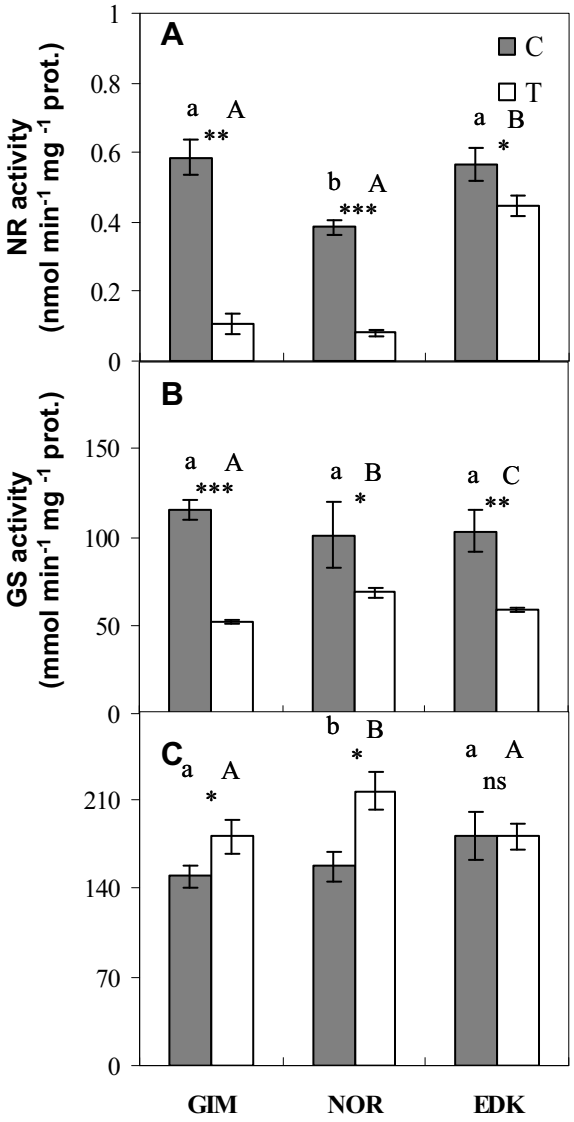

Fig. 6 NR (A), GS (B) and NAD-dependent GDH (C) activity in roots of tomato plants grown in control (C) or in salt-treated (T) solution. Statistics as in **Fig. 1**.

It is well known that chloride potentially competes with nitrate for uptake and translocation within the plants by nitrate transporter proteins (*Lips et al.* 1990; Campbell 1999). An *in vivo* experiment was carried out to investigate salt induced changes in plant capability to take up nitrate (**Fig. 5**). The imposition of salt stress significantly decreased nitrate uptake rates in all genotypes. However, the reduction was more pronounced in GIM and NOR (-40%) than in the salt tolerant EDK plants (-20%).

Root NR activity followed a similar pattern in all genotypes, where a significant decrease in enzyme activity was observed under stress condition (**Fig. 6A**). In particular, NR activity was 80% (in GIM and NOR) and 20% (EDK) lower when measured in roots from the salt treated condition. Also root GS activity was decreased by growth under salt stress condition in all tomato genotypes (**Fig. 6B**). In particular, the effect of the treatment was greater in salt sensitive GIM plants (-55%) than in NOR (-30%) or EDK plants (-40%). NR and GS activities exhibited a strong response to salt stress, whereas GDH activity did not show a similar pattern. As shown in **Fig. 6C**, under salt stress GDH activity was kept constant in EDK roots or even increased in the other two genotypes (+20 and +40% in GIM and NOR, respectively).

The pattern of changes in total free amino acids, NO_3^- and Cl^- contents in roots of control and salt stressed plants is shown in **Fig. 7**. Amino acids are well known as compatible solutes in plants under salinity (Nanjo *et al.* 1999) and several authors have been reported an increase of these compounds in plants subject to salt stress (Dubey 1997; Mansour 2000; Carillo *et al.* 2005). Very low amounts of free amino acids were found in roots of EDK control plants (containing 70% less than GIM and NOR plants). Imposition of salt stress slightly decreased total free amino acids levels at least in the two sensitive NILs, whereas a sharp increase (+30%) in amounts of free amino acids was found in EDK roots (**Fig. 7A**). NO_3^- content was significantly lower in roots of salt-treated compared to control plants. Reductions of nitrate content were greater for roots of GIM and NOR plants (-80%), than in salt-treated EDK roots (-20%). In particular, EDK salt-treated roots contained 3- and 5-fold higher nitrate content per g dry weight than GIM and NOR roots, respectively (**Fig. 7B**). We next investigated the relationship between NO_3^- and Cl^- concentration in control and salt-treated tomato roots by measuring chloride content (**Fig. 7C**). As expected, Cl^- ions increased under saline conditions in all studied genotypes except in EDK one. In particular, the increase of Cl^- reached values of 60 and 40% in GIM and NOR plants, respectively. Furthermore, data showed that the accumulation of Cl^- by EDK plants was 20 and 30% lower than GIM and NOR, respectively, by considering the absolute amount of Cl^- content in salt-treated roots.

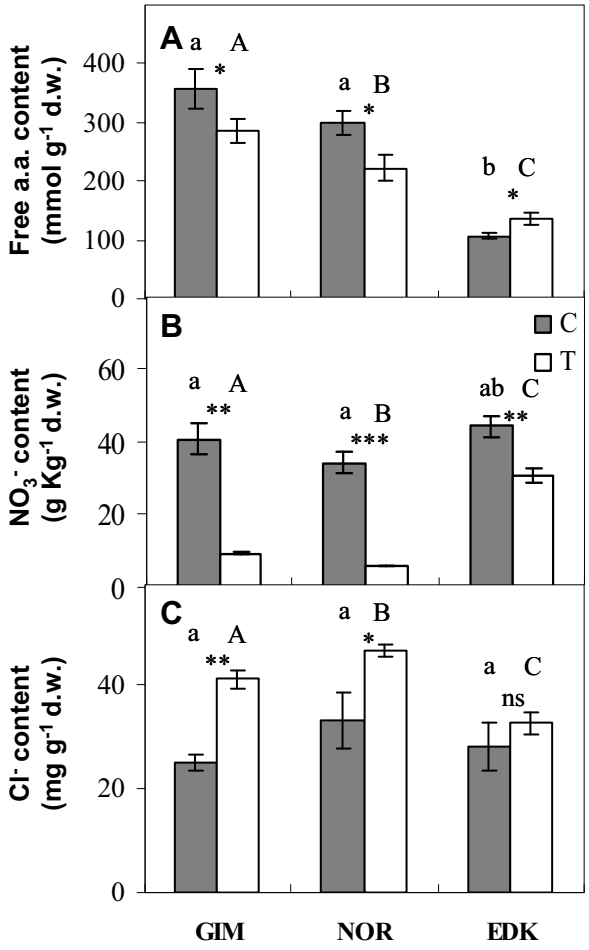

Fig. 7 Free amino acids (a.a.) (A), nitrate (B) and chloride ions (C) content in roots of tomato plants grown in control (C) or in salt-treated (T) solution. Statistics as in **Fig. 1**.

DISCUSSION

Tomato was used in this study because it has proven to be sensitive to salt stress (Ayers and Westcot 1989) and because of the availability of genotypes differing in their tolerance to salt conditions as cv. 'EDK' (Jones 1987; Habashi 1992; Picarella *et al.* 1995) or defective in ethylene synthesis as NOR (Grierson and Kader 1986).

Growth in saline soils generally leads to pronounced reduction of vegetative growth and yield of well-fertilized plants (Ramage 1979), with varying expression according to the species and genotype. In particular, plants suffering from severe salt stress show a characteristic shift in plant development leading to higher root-to-shoot dry weight ratio in plants grown under salt stress than in control plants (Cuartero and Fernández-Muñoz 1999). In this study, tomato leaves showed a significant response to salinity, as shown by reduced dry mass accumulation and decreased shoot-to-root ratio. A decline in the leaf chlorophyll content is generally produced by salt exposure (Parida and Das 2005), probably correlated to the indirect effect of NaCl on the content of essential nutrients. Our results showed that chlorophyll content declined slightly in both GIM and NOR leaves, while it was unaffected by salinity in EDK plants.

Salinity could interact with the N metabolism of plants in a number of ways. First, chloride potentially competes with nitrate for uptake and translocation within the plants by nitrate transporter proteins (Lips *et al.* 1990; Campbell 1999). Second, decreased nitrate uptake inhibit and decrease NR activity (Martinez and Cerdà 1989), since NR is an inducible enzyme and nitrate is needed to induce it.

In the present study, increasing the EC from 3 to 10 mS cm^{-1} decreased nitrate uptake rate in tomato roots 40% on a FW basis in both GIM and NOR plants. In contrast, large variations of nitrate uptake rate were not observed in EDK plants. There was a 20% decrease of this parameter in roots of salt-treated compared to control plants in this study.

NO_3^- content was significantly lower in roots of salt-treated compared to control plants. Reductions of nitrate content were magnified for roots of GIM and NOR plants (-80%), while it was reduced to a lesser extent in salt-treated EDK roots (-20%). Hence, not only the capacity of roots to take up NO_3^- but also the root potential for accumulating nitrate apparently was greater in salt-treated EDK plants compared to the other two genotypes. In particular, EDK salt-treated roots contained 3-fold and 5-fold higher nitrate content per g dry weight than GIM and NOR roots, respectively. Moreover, data showed that salt-treated EDK plants were able to accumulate Cl^- to a lower extent than the others genotypes, suggesting that not only in shoots (Perez-Alfocea *et al.* 1993) but also in roots was showed a negative correlation between NO_3^- and Cl^- concentration in salt-tolerant tomato plants.

In order to verify if rates of NO_3^- uptake and accumulation were balanced with rates of NO_3^- utilization, we investigated changes in activity of N-assimilating enzymes.

As reported, NR activity severely decreased (-80%) in roots of salt-treated compared to control of both GIM and NOR plants. Also the roots of EDK plants exhibited reduced NR activity, but salinity effect usually did not exceed 20%. The last finding indicated that NR activity followed a pattern similar to that of nitrate uptake rate and accumulation.

The evidence that NR activity is greater in leaves than in roots (Pereira and Splittstoesser 1986) helps to explain high NO_3^- accumulation at root level, since utilization and so decreasing of nitrate pools is expected to be larger in leaves than in roots. As a result, upon salt treatment total free amino acids levels decreased in tomato roots at least in the two sensitive genotypes. The sharp increase in free amino acids content found in EDK roots (+ 30%) indicates that their accumulation is most likely involved in salt stress tolerance. Amino acids are well known as compatible solutes in plants under salinity (Nanjo *et al.* 1999) and several authors have been reported an increase of these compounds in plants subject to salt stress (Dubey 1997; Mansour 2000; Carillo *et al.* 2005). Also in our previous work, we found a significant increase in leaf free amino acids concentration in salt treated tomato plants (Astolfi *et al.* 2005). It is interesting to note that very low levels of amino N were found in roots of EDK control plants (contained 70% less than GIM and NOR plants). Changes of nitrogenous metabolites in roots were likely the result of N assimilation. As reported, GS and GDH activity levels were similar among the three genotypes, but responded differently to salt stress. Effects of salt treatment were to reduce root GS activity, with maximum repressing effect in GIM roots (55%) and was smaller in EDK and NOR (-40 e -30%, respectively). The greater reduction of NR activity than that of GS might suggest that nitrate reduction would be more limiting to growth in tomato under salt stress than ammonium assimilation, confirming the general belief that NR activity is the rate-limiting step in N assimilation pathway (Campbell 1999). On the other hand, under salt stress GDH activity was kept constant in EDK roots or even increased in the other two genotypes (+20 and +40% in GIM and NOR, respectively).

The enzyme glutamate dehydrogenase represents an alternative route to the usual GS:GOGAT pathway of ammonia assimilation in plants and mediates the reductive amination of a-ketoglutarate to yield glutamic acid (Dubey and Pessarakali 2005). GDH is common in plant tissues but the physiological role of the enzyme remains undefined (Lam *et al.* 1995; Oaks 1995; Pahlich 1996). Although Robinson *et al.* (1991) and later Fox *et al.* (1995) have argued against its assimilatory role, it has been reported an increased GDH activity in plants under abiotic stress thus suggesting that GDH may have a role in the process of assimilation of NH_4^+ (Melo-Oliviera *et al.* 1996; Masclaux

et al. 2001). Our findings that increased GDH activity occurs at moderate salinity level in both sensitive genotypes but not in tolerant one suggests that under stressful condition of salinity GDH possibly plays an important role in assimilation and re-assimilation of ammonia. These findings are in agreement with the increased GDH activity observed in our previous studies in plants under stressful conditions of irradiance (Astolfi *et al.* 2001) and heavy metal toxicity (Astolfi *et al.* 2004). Furthermore, it has been suggested that plant GDH offers a means for improved diagnosis of the nutrient status of crops and functions as a sensor in the monitoring of environmentally induced stress (Osuji *et al.* 1998). In this context, we may speculate that EDK plants are the least suffering from salt stress.

It has been suggested that ethylene and ABA may modulate the physiological effects induced by salinity in different plant species (Gómez-Cadenas *et al.* 1998). In particular, it has been reported that exposure to salt stress results in increased ethylene production in rice (Lutts *et al.* 1996), lettuce (Zapata *et al.* 2003), citrus (Gómez-Cadenas *et al.* 1998) and also tomato (Botella *et al.* 1993), this increase being higher in salt-tolerant than in salt-sensitive cultivars, showing that the capacity to increase ethylene production under saline conditions could provide a higher tolerance to salinity. In this study, tomato plants showed a different behaviour in relation to the effect of salinity on ethylene production, since in them ethylene production was lower under saline than under control conditions. Such findings would indicate that no relation can be established between increases in ethylene production and salt tolerance of these genotypes during vegetative phase. This hypothesis is also consistent with our reported data showing that the lack of ethylene production in NOR plant results in no significant difference in plant capability to respond to salt stress between it and its near isogenic line (GIM). ABA has been involved in plant response to salt stress due to its control on many stress adaptation responses, including proline accumulation, stomatal closure and shoot growth inhibition (Ruggiero *et al.* 2004; Verslues and Zhu 2005). Except for NOR plants, we found a much higher ABA concentration in the salt-treated roots relatively to control ones, in according to other reports (Gómez-Cadenas *et al.* 1998). This result is consistent with ABA mediated regulation of root versus shoot growth which led to the sharp decrease of the shoot-to-root ratio observed in all salt-treated plants. The decrease of the shoot-to-root ratio coupled with the lacking increase in ABA production in NOR salt-treated plants remains to be explained. In this context, the involvement of other mechanisms independent of ABA in plant growth regulation could not be excluded. However, it should be considered that regardless of the absolute amount of ABA, the ratio between ABA and ethylene contents in roots was 3-fold higher under salt stress in GIM and EDK plants but it was nearly identical in roots of control and salt-treated NOR plants.

We can conclude that salinity affected N metabolism in all studied steps (uptake, reduction and assimilation), although the effect was different depending on the considered parameter. The greater reduction of NR activity than that of GS might suggest that nitrate reduction would be more limiting to growth in tomato under salt stress than ammonium assimilation. Lack of changes in GDH activity in EDK roots under stress could at least in part be related to their tolerance to salinity. Furthermore, this result confirms the potential usefulness to measuring GDH activity for monitoring of environmentally induced stress (Osuji *et al.* 1998).

The fact that EDK roots showed a reduced Cl^- accumulation and increased NO_3^- uptake and accumulation as compared with GIM and NOR could at least in part explain their better response to salinity and highlight the importance of Cl^-/NO_3^- ratio in modulating salt stress adaptation in plants. In addition, the large accumulation of amino compounds only found in the roots of salt stressed EDK plants could be considered a protective response against salinity.

Ethylene and ABA levels were affected by salinity, but in different ways in the three genotypes studied, therefore a clear relation between these and their different tolerance level to salinity stress could not be defined.

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