



Simultaneous application of salicylic acid and calcium improves salt tolerance in two contrasting tomato (*Solanum lycopersicum*) cultivars



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ABSTRACT

Soil salinity is one of the most important environmental factors responsible for serious agricultural problems. Tomato salt tolerance may be improved by genetic selection and by the use of adapted physiological tools. The aim of this study was to investigate the impact of exogenous application of salicylic acid (SA 0.01 mM) and calcium sulphate (CaSO₄ 5 mM), singly or in combination, on plant growth, photosynthetic pigments, nutritional behaviour and some metabolic parameters (total chlorophyll, carotenoids, soluble sugars, proline and lipid peroxidation) of two tomato cultivars (cv. Super Marmande and cv. Red River) exposed to salt stress (100 mM NaCl). Application of 100 mM NaCl reduced plant growth, total chlorophyll and carotenoid contents. Salt stress also induced an accumulation of Na⁺, a decrease in K⁺ and Ca²⁺ concentration and root sugar level, an increase in malondialdehyde (MDA) and proline concentration. Deleterious impact of salinity was related to modification in ion content rather than modification in the plant water status. Exogenous application of SA or Ca alone improved plant behaviour in the presence of NaCl. Nevertheless, the best results in terms of growth, photosynthetic pigment concentrations and mineral nutrition (limitation of Na⁺ accumulation and maintenance of K⁺ and Ca²⁺ content) were obtained in response to the combined SA + Ca treatment. Although the involved physiological parameters varied depending on the considered cultivar, our results suggest that Ca²⁺ and SA may interact to reduce the stress experienced by the plant in the presence of NaCl.

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1. Introduction

Tomato (*Solanum lycopersicum*) is one of the most consumed vegetables in the world and it plays an important role in the human diet. Tomato has long served as a model system for plant genetics, development, physiology and pathology, leading to substantial information regarding the biology of this economically important organism (Fau Robert et al., 2007). However tomatoes are frequently exposed to multiple environmental stresses. In particular, salinity is one of the most important environmental constraints affecting plant growth, development, and crop productivity (Dasgan et al., 2002; Juan et al., 2005; Gunes et al., 2007; Khan et al., 2010). Salinity alters many physiological and biochemical processes such as mineral nutrition, respiration rate, organic solutes/osmolyte synthesis, seed germination, enzyme activities and photosynthesis (Juan et al., 2005; Siddiqui et al., 2010). Salt tolerance mechanisms vary from plant species to plant species, from cultivar to cultivar, and rely on complex interacting

physiological and biochemical processes (Ashraf and McNeilly, 2004; Manaa et al., 2011).

Tomato salt tolerance may be improved not only by genetic selection but also through the use of adapted physiological tools. Salicylic acid (SA) and calcium are recognized as signal molecules, and have been intensively investigated for their roles in plant adaptation to changing environments. Especially, SA was found to improve plant tolerance to salt stress (Gunes et al., 2007; Misra and Saxena, 2009; Gautam and Singh, 2009; Wasti et al., 2012), heavy metals (Pál et al., 2002), water stress (Singh and Usha, 2003) and heat stress (He et al., 2005). Exogenous application of SA enhanced the photosynthetic rate and also maintained the membrane stability, thereby improving the growth of salinity stressed barley plants (El Tayeb, 2005). The damaging effects of salinity were also alleviated by exogenous application of SA in *Arabidopsis* (Borsani et al., 2001) and in tomato plants (Wasti et al., 2012). Kaydan et al. (2007) observed that pre-sowing soaking treatment of seeds with SA, positively, affected the osmotic potential, shoot and root dry mass, K⁺/Na⁺ ratio and contents of photosynthetic pigments (chlorophyll a, chlorophyll b and carotenoids) in wheat seedlings, under both saline and non-saline conditions.

Another approach to minimize the effects of salinity on plants consists of substrate nutrient enrichment (as N, P, K, Mg, and Ca) in order to reduce Na⁺ and Cl⁻ injuries in plants (Kaya et al., 2002; Song and

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Roe, 2008). Considerable interest has been focused on Ca^{2+} due to its ability to induce a protective effect on plants under adverse environmental conditions. Calcium plays a vital role in salt stress tolerance since it induces antioxidant enzyme activities and reduces lipid peroxidation of cell membranes under abiotic stress (Jiang and Huang, 2001; Khan et al., 2010). It has also been shown to stabilize cell membrane surfaces, prevent solute leakage from the cytoplasm, maintain normal photosynthesis and regulate the plant hormone metabolism (Hirschi, 2004; Song and Roe, 2008). Numerous data suggest that Na^+ competes with Ca^{2+} for binding sites under salinity conditions and that apoplastic Ca^{2+} directly alleviates symptoms produced by mineral toxicities. The ameliorative effect of external Ca^{2+} on plants facing salinity may be associated with the maintenance of an optimal K^+/Na^+ ratio and homeostasis in the cytosol in relation to an inhibition of Na^+ influx and K^+ efflux or promotion of Na^+ efflux and K^+ influx across the plasma membrane (Elphick et al., 2001; Demidchik and Tester, 2002; Shabala et al., 2006).

Most studies dealing with SA or Ca^{2+} involvement in salt tolerance only consider one of these compounds while their putative interactions received only little attention. To the best of our knowledge, no data are available in respect for *S. lycopersicum*. For this reason, we assess the influence of exogenous SA and calcium applied alone and in combination on tomato behaviour exposed to salinity during the vegetative phase of development on two contrasting cultivars.

2. Materials and methods

2.1. Plant material and growth conditions

Two contrasted sized genotypes of tomato (*S. lycopersicum*, production Vilmorin®, France) were used: a cherry tomato, cerasiforme (cv. Red River) and a medium-sized tomato (cv. Super Marmande). Seeds were surface-sterilized by soaking in a 5% (v/v) sodium hypochlorite solution for 15 min followed by three washes with sterile distilled water. Seeds were then germinated in Petri dishes with moistened filter paper and were incubated under fluorescent light ($90 \mu\text{mol m}^{-2} \text{s}^{-1}$ with a 16 h photoperiod at 25 °C). Ten days after sowing, seedlings were transferred to growth chamber (25 °C/70% relative humidity during the day and 20 °C/90% relative humidity during the night; photoperiod: 16 h daily with a light irradiance of $150 \mu\text{mol m}^{-2} \text{s}^{-1}$). They were grown in hydroponic nutrient solution continuously aerated containing: KNO_3 3 mM, $\text{Ca}(\text{NO}_3)_2$ 1 mM, KH_2PO_4 2 mM, MgSO_4 0.5 mM, Fe-EDTA 32.9 μM and micronutrients: H_3BO_4 30 μM , MnSO_4 5 μM , CuSO_4 1 μM , ZnSO_4 1 μM and $(\text{NH}_4)_6\text{Mo}_7\text{O}$ 1 μM for 10 days. At this time, plants were at the third leaf stage and different treatments were initiated (Table 1).

NaCl was added in increments of 25 mM d^{-1} up to a final concentration of 100 mM. This concentration was found in a salinised agriculture field and designed in the manuscript on the basis of previous published experiments (Dasgan et al., 2002; Manaa et al., 2011). 5 mM of CaSO_4 and 0.01 mM of SA were selected. These concentrations enhanced

growth of tomato seedling under salt stress as previously reported (Tuna et al., 2007; Wasti et al., 2012).

The pH of the nutrient solution was adjusted daily to 5.5 with a minimum amount of 0.1 mM KOH. After 15 days of treatments, the plants (35 days old) were harvested and divided into roots, stems, and leaves for physiological and biochemical parameter determinations.

2.2. Dry weight determinations and ions analysis

For plant growth and ion analysis, 20 independent dry matter measurements and ion analysis were performed on separated leaves, stems and roots. In addition to fresh weight (FW), dry weight (DW) was determined after desiccation at 80 °C for 48 h. Water content ($\text{mL} \cdot \text{g}^{-1}$ DW) was estimated using the equation: $\text{WC} = (\text{FW} - \text{DW}) / \text{DW}$.

The sensitivity index (SI) that means the difference between dry matter production of salt-treated plants and the control, expressed in percent of the latter, was calculated according to the following expression:

$$\text{SI}_{\text{treatment}} = (100 \times (\text{DW}_{\text{treatment}} - \text{DW}_{\text{control}}) / \text{DW}_{\text{control}})$$
 according to Saadallah et al. (2001).

For the measurement of cations, plant material was dried at 80 °C and digested with nitric acid (1% (v/v) HNO_3) according to the method of Wolf (1982). K^+ , Ca^{2+} , and Na^+ were analysed by flame emission using a spectrophotometer (Eppendorf Geratebau Netherler).

2.3. Determination of physiological and biochemical parameters

2.3.1. Chlorophyll and carotenoid analysis

The pigments were extracted from leaf disks in 80% acetone. The chlorophyll and carotenoid concentrations were determined by spectrophotometry according to the procedure described by Lichtenthaler (1987) and using the following equations:

$$C_a = 12.25A_{663} - 2.79A_{645}$$

$$C_b = 21.50A_{645} - 5.10A_{663}$$

$$C_{a+b} = 7.15A_{663} + 18.71A_{645}$$

$$C_{x+c} = [1000A_{470} - 1.82C_a - 85.02C_b] / 198$$

where C_a = chlorophyll a; C_b = chlorophyll b; C_{a+b} = total chlorophyll; C_{x+c} = carotenoids, A_λ = absorbance at λ (nm).

2.3.2. Proline and total soluble sugars

Proline concentration was determined using the method of Bates et al. (1973). Proline was extracted from organ samples (c.a. 200 mg FW) with 10 mL of 3% sulphosalicylic acid at 70 °C for 30 min. After addition of acid ninhydrin and glacial acetic acid to the extracts, the mixture was heated at 90 °C for 1 h in water bath. The reaction was then stopped by using an ice bath. The mixture was extracted with toluene and the absorbance of the toluene fraction was spectrophotometrically determined at 520 nm. Proline concentration was determined using calibration curve as $\mu\text{mol proline g}^{-1}$ FW.

Total soluble sugars were estimated by the anthrone reagent method using glucose as the standard according to Yemm and Willis (1954).

2.3.3. Lipid peroxidation

The level of lipid peroxidation was measured as 2-thiobarbituric acid-reactive substances (mainly malondialdehyde (MDA)) according to Madhava Rao and Sresty (2000). Frozen samples (0.25 g from 10 pooled plants) were homogenized with a pre-chilled mortar and pestle with 5 mL of ice-cold 5% (w/v) trichloroacetic acid (TCA) and

Table 1

Different treatments applied to the tomato seedling. (+) Indicates presence and (–) absence of considered solution.

	Nutrient solution	Salicylic acid (0.01 mM)	CaSO_4 (5 mM)	NaCl (100 mM)
(C) control	+	–	–	–
SA	+	+	–	–
Ca	+	–	+	–
SA + Ca	+	+	+	–
S	+	–	–	+
S + SA	+	+	–	+
S + Ca	+	–	+	+
S + SA + Ca	+	+	+	+

C: Control; SA: Salicylic acid; Ca: CaSO_4 and S: Salt (NaCl).

centrifuged at 12,000 $\times g$ for 15 min and at 4 °C. Assay mixture containing a 2 mL aliquot of supernatant and 2 mL of 0.67% (w/v) thiobarbituric acid (TBA) was heated to 100 °C for 30 min and then rapidly cooled to 4 °C in an ice-bath. After centrifugation (10,000 $\times g$ for 1 min at 4 °C), the supernatant absorbance was read (532 nm) and values corresponding to non-specific absorption (600 nm) were subtracted. MDA concentration was calculated using its molar extinction coefficient (155 $\text{mM}^{-1}\cdot\text{cm}^{-1}$).

2.4. Data analysis

The statistical analyses were performed with the “Statistica” software (version 6.0). Mean values and standard error (SE) were obtained from at least 20 replicates for physiological parameters (DW, ions content) and from 10 replicates for biochemical parameters (pigment content, proline, total soluble sugars, MDA) and analysed using Duncan’s multiple range test. A *P* value of <0.05 was considered to be statistically significant. A two-way ANOVA was performed to detect cultivar, treatment, and interaction effects, a *P* value less than 0.05 was considered statistically significant.

3. Results and discussion

3.1. SA and Ca induce ameliorative effects on the growth potential of stressed plants

Salinity is one of the major abiotic stresses in plant agriculture worldwide, and an excess amount of salt in the soil adversely affects plant growth and development by altering various plant metabolic processes, i.e. nitrogen and sulphur assimilation, antioxidant system and photosynthesis (Gunes et al., 2007; Manaa et al., 2011; Siddiqui et al., 2012). In this study, we found that the presence of NaCl 100 mM in

Table 2

Variability of biomass production sensitivity index (%) in tomato plants grown under 100 mM NaCl with application of SA and/or Ca. Data are means of 20 replicates \pm SE at *P* \leq 0.05. Letters correspond to Duncan’s multiple range test at 95%.

	Leaves	Stems	Roots	Whole plant
<i>Super Marmande</i>				
S	-39.2 \pm 8.4 a	-60.3 \pm 8.4 a	-35.8 \pm 4.4 a	-47.5 \pm 6.3 a
S + SA	-46.0 \pm 5.4 a	-49.6 \pm 7.6 b	-39.9 \pm 3.3 a	-47.9 \pm 8.0 a
S + Ca	-18.6 \pm 6.8 b	-36.8 \pm 3.7 c	-24.1 \pm 3.9 b	-27.7 \pm 2.7 b
S + SA + Ca	-17.5 \pm 5.3 b	-35.8 \pm 5.4 c	-3.0 \pm 2.9 c	-24.1 \pm 6.6 b
<i>Red River</i>				
S	-32.3 \pm 8.8 a	-60.1 \pm 6.8 a	-45.3 \pm 12.4 a	-41.9 \pm 6.3 a
S + SA	-11.2 \pm 3.9 c	-38.3 \pm 9.9 c	2.1 \pm 3.0 b	-12.1 \pm 7.4 b
S + Ca	-20.7 \pm 1.1 b	-44.8 \pm 5.4 b	3.8 \pm 3.5 b	-19.0 \pm 5.5 b
S + SA + Ca	-20.3 \pm 5.5 b	-41.4 \pm 6.9 bc	-5.9 \pm 2.1 c	-19.7 \pm 6.2 b

S: Salt (NaCl 100 mM); SA: Salicylic acid (0.01 mM) and Ca: CaSO₄ (5 mM).

the growth medium significantly reduced plant growth for each genotype (Fig. 1, Table 2). Some visible symptoms like: leaf necrosis, curling and chlorosis were observed on old leaves, only under salt treatment (see Supplementary Fig. S1), whatever the genotype. Plant dry weight was reduced by salt treatment (S), in Super Marmande and Red River respectively, by 46% and 42% as compared to control (Fig. 1A). The magnitude of salt response however varied according to the considered plant organ. (Fig. 1, Table 2) for both genotypes.

On the other hand, salinity induces a water stress component in relation to a decrease of external water potential. Our data suggest that, both cultivars were able to cope with this component of salt stress since no decrease in leaf WC was recorded after 15 days of exposure to 100 mM NaCl (Fig. 2). Furthermore, salt treatment induced an increase in leaf WC in Red River which might be related to an attempt to dilute toxic ions through a succulence strategy.

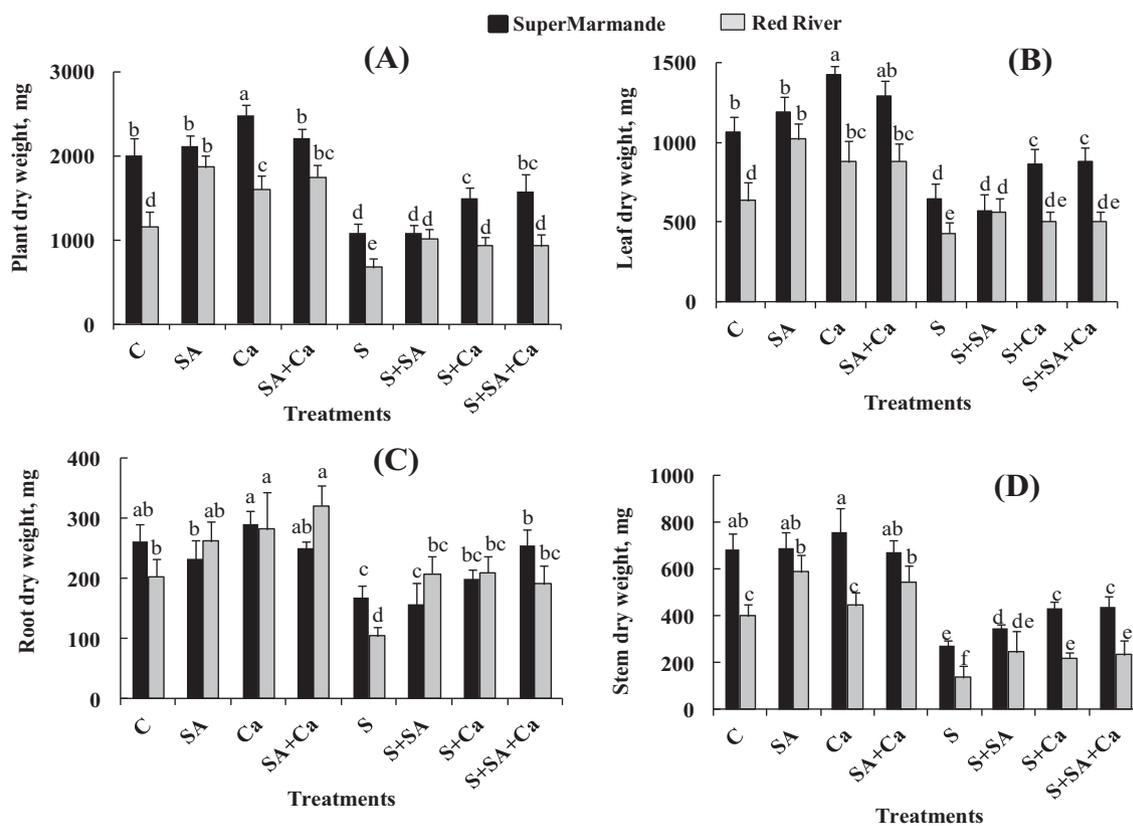


Fig. 1. Effect of SA and/or Ca application on dry weight of (A) whole plant, (B) leaves, (C) roots and (D) stems of the two tomato genotypes (cv. Super Marmande and Red River) submitted to 100 mM NaCl for 15 days. Data are means of 20 replicates \pm SE. Means with similar letters are not different at *P* \leq 0.05 according to Duncan’s multiple range test at 95%. C: Control; S: Salt (NaCl 100 mM); SA: Salicylic acid (0.01 mM) and Ca: CaSO₄ (5 mM).

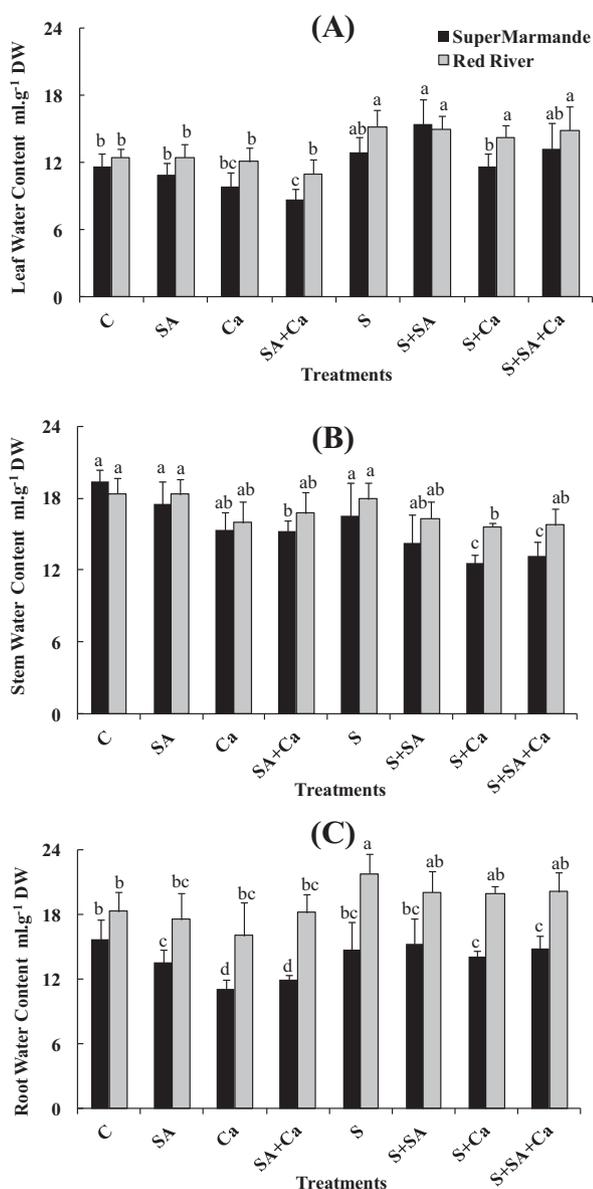


Fig. 2. Effect of SA and/or Ca application on water content, in leaves (A) and root (B) of the two tomato genotypes (cv. Super Marmande and Red River) submitted to 100 mM NaCl for 15 days. Data are means of 20 replicates \pm SE. Means with similar letters are not different at $P \leq 0.05$ according to Duncan's multiple range test at 95%. C: Control; S: Salt (NaCl 100 mM); SA: Salicylic acid (0.01 mM) and Ca: CaSO₄ (5 mM).

Exogenous use of various chemicals to alleviate the toxic effects of saline stress may have implications both from theoretical and practical perspectives (Wahid et al., 2007; Wasti et al., 2012; Hu et al., 2012; Idrees et al., 2012; Tanou et al., 2012). In the present study, the salt-exposed plants treated with both SA and Ca exhibited enhanced plant growth. In fact, the treatments (S + SA), (S + Ca) and (S + SA + Ca) significantly improved plant DW in Red River, respectively by 51, 40 and 39% as compared to saline condition (S) (Fig. 1A). However, in Super Marmande the combined SA and Ca application was more efficient in alleviating the adverse effects of salt stress on plant DW than SA application alone. Fig. 1 also indicated that the ameliorative effect exerted by the exogenous application of Ca and/or SA on the NaCl-induced growth inhibition was more prominent in roots than in leaves especially for cultivar Red River. It may thus be hypothesized that SA and Ca act as signalling compounds in an additive or even synergistic way that induces ameliorative effects on the growth potential of stressed plants. Indeed, simultaneous application of Ca and SA gave

better results than exogenous application of Ca or SA alone for some of the parameters conditioning plant growth.

Our data indicated that the ameliorative effect exerted by the exogenous application of Ca and/or SA on the NaCl-caused growth inhibition was more prominent especially in cultivar Red River, which is a cerasiforme type of tomato and Super Marmande a classical large-fruited tomatoes (*S. lycopersicum*). The *S. lycopersicum* cerasiforme group evolved through hybridization between *S. lycopersicum* and a wild relative species *Solanum pimpinellifolium* (Ranc et al., 2008). This distant phylogenetic origin may explain the differences observed between Red River and Super Marmande on their responses under salt treatments. Martínez et al. (2012) recently demonstrated that cerasiforme-type of tomato displays specific adaptation to salt stress, even during the reproductive phase of development.

Exogenous application of SA may influence a range of processes in plants, including seed germination (Korkmaz, 2005), ion uptake and transport (Gunes et al., 2005; Szepesi et al., 2009), membrane permeability (Barkosky and Einhellig, 1993), as well as photosynthetic and growth rates (Khan et al., 2003). In addition to enhanced plant growth, SA has been shown to play a role in mitigating the deleterious effects of some environmental stresses including salinity, heat and cadmium stress (Gunes et al., 2007; Guo et al., 2007; Hayat et al., 2010). In the same way, sufficient calcium concentration is required at all stages of plant growth and development, playing a fundamental role in regulating polar growth of cells and tissues and participating in plant adaptation to various stress factors (Song and Roe, 2008). Exogenous Ca²⁺ can enhance plant drought resistance, inhibit the synthesis of reactive oxygen species, protect the structure of cellular plasma membranes, and maintain normal photosynthesis (Tuna et al., 2007; Song and Roe, 2008). The present work showed that under both non-saline and saline conditions, the exogenous application of Ca²⁺ alone, through the rooting medium, had an ameliorative effect on total plant dry weight production of the two tested tomato genotypes.

Application of SA and Ca alone to salt-stressed plant did not significantly improved chlorophyll concentrations, whatever the genotype. However, their combined application (SA + Ca) was clearly more efficient in mitigating the adverse effects of salinity on total chlorophyll and carotenoids in Red River (Table 3). This effect of SA application on photosynthetic pigments was already reported (Arfan et al., 2007; Wasti et al., 2012) but the present study demonstrates that such an effect may be increased by Ca addition.

3.2. The enhancement of salt tolerance could be associated with an optimal selectivity for potassium and calcium nutrition

The presence of salt in root medium causes disturbances in the essential mineral nutrition of tomato plant (Juan et al., 2005; Manaa et al., 2011). In fact, salt treatment (S) induced an accumulation of Na⁺ and decreased the K⁺ and Ca²⁺ concentrations in leaves, stems and roots of both genotypes (Table 4). The magnitude of the response depended on the genotype and the considered plant organ. For example, root K⁺ and Ca²⁺ contents significantly decreased under salt treatment (S). The reduction was about 60% and 84%, respectively for K⁺ and Ca²⁺ in Super Marmande and about 18% and 76% in Red River.

Exogenous application of SA and Ca²⁺ singly or in combination to salt-stressed plant induced a significant decrease in leaf Na⁺ concentration. Once again, only combined SA and Ca application significantly increased root K⁺ and Ca²⁺ under saline conditions. This increase was higher in Super Marmande (61%) than in Red River (17%) as compared to plants treated only by 100 mM NaCl (Table 4). SA ameliorates the aptitude of the whole plant to ensure a sufficient supply of K⁺ by maintaining a high selectivity for this essential nutriment, in spite of an excess of Na⁺ in the medium. This result is in agreement with previous studies (Gunes et al., 2007; Szepesi et al., 2009), which demonstrated that salicylic acid strongly inhibited Na⁺ and Cl⁻ accumulations, but also stimulated N, Mg, Fe, Mn, and Cu concentrations of stressed

Table 3
Effect of SA and/or Ca application on total Chl, Chl a and b, Chl a/b ratio and carotenoid contents of two tomato genotypes (Super Marmande and Red River) exposed to 100 mM NaCl for 15 days. Data are means of 10 replicates \pm SE. Means with similar letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

	C	S	S + SA	S + Ca	S + SA + Ca
<i>Super Marmande</i>					
Total Chl (mg g ⁻¹ FW)	2.10 \pm 0.19 a	1.16 \pm 0.07 b	1.28 \pm 0.10 b	1.16 \pm 0.10 b	0.97 \pm 0.23 b
Chl a (mg g ⁻¹ FW)	1.44 \pm 0.09 a	0.80 \pm 0.09 b	0.88 \pm 0.10 b	0.80 \pm 0.13 b	0.66 \pm 0.15 c
Chl b (mg g ⁻¹ FW)	0.66 \pm 0.03 a	0.36 \pm 0.05 b	0.40 \pm 0.12 bc	0.36 \pm 0.07 b	0.31 \pm 0.05 c
Chl a/b ratio	2.14 \pm 0.32 a	2.14 \pm 0.26 a	2.23 \pm 0.37 a	2.11 \pm 0.35 a	2.12 \pm 0.41 a
Carot (mg g ⁻¹ FW)	0.33 \pm 0.03 a	0.18 \pm 0.02 b	0.20 \pm 0.04 c	0.18 \pm 0.03 b	0.18 \pm 0.03 b
<i>Red River</i>					
Total Chl (mg g ⁻¹ FW)	1.74 \pm 0.21 a	1.05 \pm 0.07 c	0.87 \pm 0.08 d	1.18 \pm 0.12 c	1.43 \pm 0.19 b
Chl a (mg g ⁻¹ FW)	1.22 \pm 0.12 a	0.69 \pm 0.09 c	0.50 \pm 0.08 d	0.80 \pm 0.10 b	0.98 \pm 0.07 b
Chl b (mg g ⁻¹ FW)	0.53 \pm 0.06 a	0.36 \pm 0.05 c	0.36 \pm 0.05 c	0.38 \pm 0.05 c	0.45 \pm 0.04 b
Chl a/b ratio	2.31 \pm 0.25 a	1.94 \pm 0.26 ab	1.51 \pm 0.66 b	2.10 \pm 0.28 a	2.20 \pm 0.26 a
Carot (mg g ⁻¹ FW)	0.26 \pm 0.01 a	0.16 \pm 0.02 c	0.12 \pm 0.02 d	0.18 \pm 0.01 c	0.23 \pm 0.01 b

C: Control; S: Salt (NaCl 100 mM); SA: Salicylic acid (0.01 mM) and Ca: CaSO₄ (5 mM).

maize plants. According to our data, a combination of SA and Ca could play a synergetic role in regulating ion absorption. It is noteworthy that the presence of SA in the medium increased Ca accumulation in the leaves (but not in stem) of Super Marmande stressed plants thus suggesting that SA may positively influence both Ca absorption and translocation processes. Indeed, our data revealed only a rather low increase in Ca²⁺ content under (S + Ca) treatment, despite the fact that there was an increment of Ca concentration by a factor of 6 as compared to salt treatment (S) (Table 4). Hence, SA may positively influence the efficiency of Ca transporters or selectivity of Ca channels under salt stress conditions at the root level. It is well known that Ca²⁺ acts as a second messenger in many biological systems, and influences the plant's ability to adjust to high salt environments. According to our results, we hypothesize that Ca²⁺ may be involved in SA transduction pathways and that this reciprocal influence of SA on Ca²⁺ absorption, and Ca²⁺ on SA transduction could explain the synergistic effects recorded in our experiments in response to the combined treatment.

3.3. Ca and SA may interact to reduce the oxidative damage experienced by salt stress

Lipid peroxidation is an indicator of membrane damages leading to electrolyte leakage under salt stress conditions (Katsuhara et al., 2005). Malondialdehyde quantification, a product of lipid peroxidation, is a common indicator of oxidative stress encountered by plant tissues.

Salt stress induced lipid peroxidation in both cultivars. Indeed, NaCl supplied in the growth medium promoted MDA generation by 157% and 150% as compared to the control, respectively, in leaves and roots of Red River genotype (Fig. 3). This effect was even more pronounced in Super Marmande, with an increase of about 257% and 260%, respectively, in leaves and roots.

Indeed, NaCl-treated plants supplemented with SA or Ca alone showed significant decrease of MDA content in tomato leaf and root, as compared to plants treated only by NaCl, whatever the genotype (Fig. 3A, B). However, no synergy was found between SA and Ca for tolerance to oxidative stress. It may thus be hypothesized that application of SA and Ca alone has a role in induction of antioxidant enzyme activity leading to decrease in ROS and lipid peroxidation on long term basis (Ganesan and Thomas, 2001; Gunes et al., 2007) and that SA and Ca do not interact in the management of the stressed plant's oxidative status.

3.4. Combination of SA and Ca could play a synergetic role in regulating organic solute/osmolyte accumulation under salt stress

Survival under stressful conditions depends on the plant's ability to perceive the stimulus, generate and transmit signals, and instigate biochemical changes that adjust the metabolism accordingly. Accumulation of organic solutes/osmolytes in plants contributes to osmotic adjustment that is important for physiological machinery by which

Table 4
Effect of SA and/or Ca application on Na⁺, K⁺ and Ca²⁺ contents in tomato plants (cv. Super Marmande (SM) and Red River (RED)) exposed to 100 mM NaCl for 15 days. Data are means of 20 replicates \pm SE. Means with similar letters are not significantly different at $P \leq 0.05$ according to Duncan's multiple range test.

	Leaves		Stems		Roots	
	SM	RED	SM	RED	SM	RED
<i>Na⁺ (mmol/g DW)</i>						
S	2.19 \pm 0.10 a	3.00 \pm 0.12 a	1.54 \pm 0.09 ab	2.13 \pm 0.11 a	1.22 \pm 0.09 a	1.59 \pm 0.10 a
S + SA	2.23 \pm 0.10 a	2.46 \pm 0.10 b	1.70 \pm 0.11 a	2.28 \pm 0.12 a	1.14 \pm 0.10 a	1.62 \pm 0.10 a
S + Ca	1.96 \pm 0.11 a	2.13 \pm 0.09 b	1.17 \pm 0.09 c	2.00 \pm 0.12 a	0.72 \pm 0.02 b	1.77 \pm 0.10 a
S + SA + Ca	2.10 \pm 0.12 a	1.87 \pm 0.10 c	1.44 \pm 0.11 b	2.11 \pm 0.14 a	1.23 \pm 0.08 a	1.67 \pm 0.10 a
<i>K⁺ (mmol/g DW)</i>						
C	1.58 \pm 0.07 a	1.12 \pm 0.10 a	2.63 \pm 0.10 a	2.11 \pm 0.09 a	1.35 \pm 0.05 a	1.24 \pm 0.10 a
S	0.52 \pm 0.05 bc	0.47 \pm 0.05 b	1.24 \pm 0.10 b	1.12 \pm 0.09 b	0.55 \pm 0.08 c	1.01 \pm 0.10 b
S + SA	0.54 \pm 0.04 bc	0.43 \pm 0.02 b	1.27 \pm 0.09 b	1.04 \pm 0.06 b	0.31 \pm 0.06 d	0.73 \pm 0.06 d
S + Ca	0.61 \pm 0.03 b	0.44 \pm 0.04 b	1.27 \pm 0.08 b	1.09 \pm 0.07 b	0.50 \pm 0.04 c	0.94 \pm 0.06 c
S + SA + Ca	0.51 \pm 0.04 c	0.42 \pm 0.03 b	1.04 \pm 0.09 b	1.14 \pm 0.06 b	0.88 \pm 0.05 b	1.18 \pm 0.07 b
<i>Ca²⁺ (mmol/g DW)</i>						
C	1.01 \pm 0.11 a	1.46 \pm 0.11 a	0.66 \pm 0.09 a	0.74 \pm 0.09 a	1.37 \pm 0.10 a	1.12 \pm 0.09 a
S	0.38 \pm 0.05 d	0.82 \pm 0.07 c	0.24 \pm 0.03 bc	0.47 \pm 0.08 b	0.21 \pm 0.05 d	0.26 \pm 0.06 c
S + SA	0.46 \pm 0.05 c	0.89 \pm 0.08 bc	0.21 \pm 0.03 cd	0.49 \pm 0.04 b	0.21 \pm 0.04 d	0.21 \pm 0.03 c
S + Ca	0.60 \pm 0.05 b	0.98 \pm 0.05 b	0.28 \pm 0.03 b	0.54 \pm 0.02 bc	0.28 \pm 0.01 c	0.35 \pm 0.02 b
S + SA + Ca	0.54 \pm 0.04 b	1.34 \pm 0.12 a	0.19 \pm 0.02 d	0.38 \pm 0.07 c	0.71 \pm 0.10 b	0.97 \pm 0.09 a

C: Control; S: Salt (NaCl 100 mM); SA: Salicylic acid (0.01 mM) and Ca: CaSO₄ (5 mM).

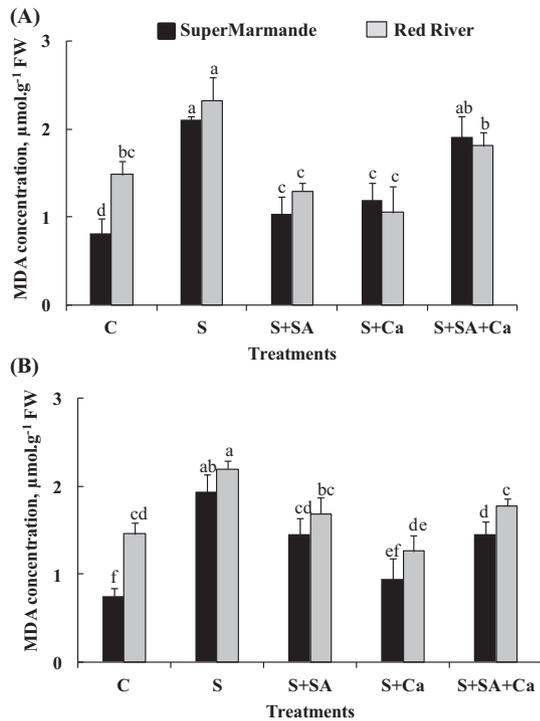


Fig. 3. Effect of SA and/or Ca application on malondialdehyde concentration, in leaves (A) and root (B) of the two tomato genotypes (cv. Super Marmande and Red River) submitted to 100 mM NaCl for 15 days. Data are means of 10 replicates \pm SE. Means with similar letters are not different at $P \leq 0.05$ according to Duncan's multiple range test at 95%. C: Control; S: Salt (NaCl 100 mM); SA: Salicylic acid (0.01 mM) and Ca: CaSO_4 (5 mM).

plants tolerate biotic and abiotic stresses (Khan et al., 2010). Among the studied organic osmolytes, proline showed the lowest accumulation in leaf and root subjected to (S + SA + Ca) treatment, when compared to NaCl treatment alone (S), especially in Super Marmande (Fig. 4A, B).

Previous studies reported a substantial increase in proline levels in response to NaCl treatment and SA may be, at least partly involved in this response (Misra and Saxena, 2009; Al-Wahaibi et al., 2012). The synthesis of proline in the presence of salinity and SA is regulated by activation of pyrroline-5-carboxylate reductase (P-5-CR) and γ -glutamyl kinase and inhibition of proline oxidase and proline dehydrogenase (Misra and Saxena, 2009). Whatever the underlying mechanisms, proline should be regarded as a typical "stress response" and a decrease in the leaf proline concentration of stressed plants exposed to the combined treatment may suggest a lower stress intensity experienced by these plants. The fact that decreases in proline (commonly considered as an important component of osmotic adjustment) did not induce any decrease in water content supports this view.

Sugars are also considered to play a major role in osmoregulation under abiotic stress conditions (Fallon and Phillips, 1989). Total soluble carbohydrates are important solutes that accumulate in cytosol under salt stress and may thus contribute to plant survival (Rejšková et al., 2007). In our study, tomato plants submitted to NaCl treatment showed a decrease in their root soluble sugar content, at all genotypes. For example, NaCl decreased root sugar concentration by 56% and 50% as compared to control, respectively, for Super Marmande and Red River (Fig. 4D). Application of SA and/or Ca alleviated this adverse effect of salinity on root sugar level. This effect was more prominent in Super Marmande and more efficient with combined treatment (S + SA + Ca) application which increased sugar content by 211%, as compared to salt treatment (S).

In contrast to proline, no synergistic impact of SA and Ca was found for soluble sugar concentration: in (S + SA + Ca) treated plants, soluble sugar concentration was almost similar to control, which could be considered as the ultimate consequence of photosynthesis maintenance

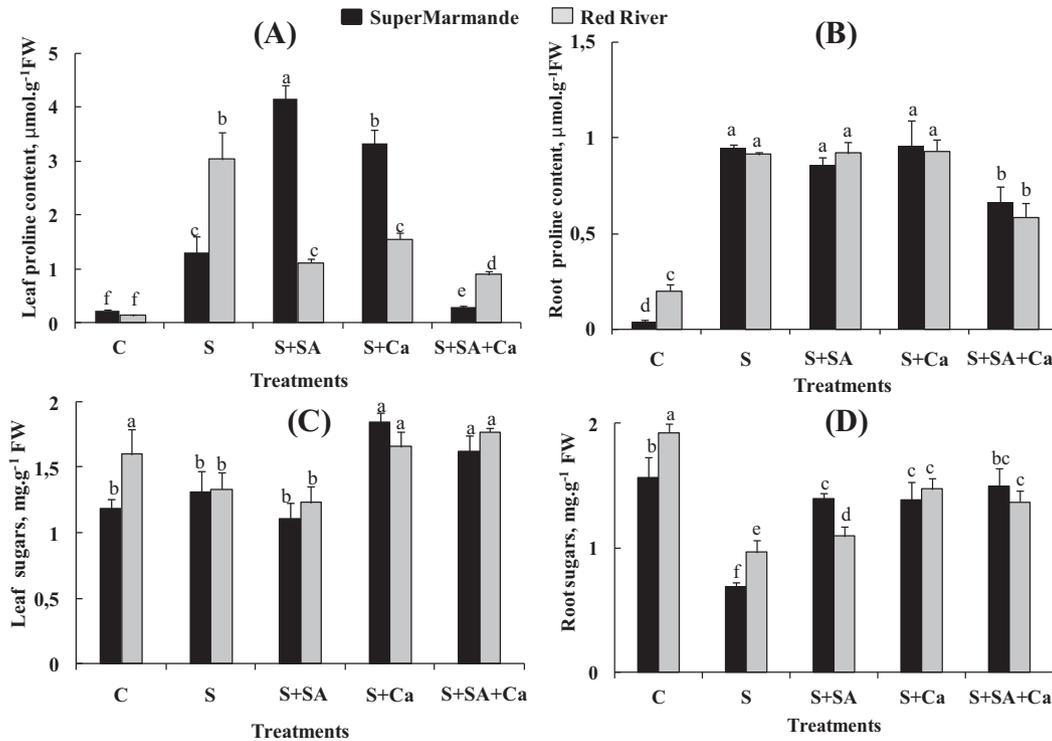


Fig. 4. Effect of SA and/or Ca application on proline (A, B) and sugars concentrations (C, D), of the two tomato genotypes (cv. Super Marmande and Red River) submitted to 100 mM NaCl for 15 days. Data are means of 10 replicates \pm SE. Means with similar letters are not different at $P \leq 0.05$ according to Duncan's multiple range test at 95%. C: Control; S: Salt (NaCl 100 mM); SA: Salicylic acid (0.01 mM) and Ca: CaSO_4 (5 mM).

and, once again, supports the hypothesis of an improved ability of those plants to cope with deleterious NaCl concentrations.

The results from the present study illustrate that application of SA and/or Ca improved the salt tolerance of tomato through protection of photosynthetic pigments and organic solute/osmolyte accumulation, which may be responsible for enhanced plant growth. The enhancement of salt tolerance in tomato could be associated with an optimal selectivity for potassium and calcium nutrition. The findings also indicated that combination of SA and Ca could play a synergetic role in regulating several properties conditioning plant salt resistance at the vegetative stage in a cultivar-dependent manner. Further works are required to quantify the impact of combined SA + Ca treatments on yield-related parameters of salt-treated tomato.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.sajb.2014.07.015>.

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