



Ions and Organic Solutes as Implicated in the Ameliorative Effect of Exogenous Application of Calcium on Salt Stressed Tomato (*Lycopersicon esculentum* Mill.) Plants

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Authors' contributions

This work was carried out in collaboration among all authors. Author EEYH designed the study, wrote the protocol, and wrote the first draft of the manuscript. Author EEYH and author CBG managed the literature searches. Author EEYH, author ES and Author APN, contributed to the protocol writing and managed the analyses of the study. Author EEYH and author APN performed the statistical analysis. Author GA, author LBM and author CBG contributed to the protocol writing and data analysis. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study evaluated the role of sodium, potassium, proline and soluble sugars accumulation in the ameliorative effect of an exogenous application of calcium on the detrimental effect of salinity on tomato plants.

Study Design: The experiment was implemented as a Completely Randomized Design (RCD) with four treatments and three replications.

Place and Duration of Study: The experiment was realized in a green house of the Faculty of Agronomical Sciences, University of Abomey-Calavi, Benin Republic from June to July 2020.

Methodology: Three weeks old plants of the tomato cultivar "Padma" was submitted in pots to four treatments comprising the control (without NaCl); salt stress (120 mM NaCl) and a combination of 120 mM NaCl and exogenous application of 40 mM CaSO₄ or Ca (NO₃)₂ by irrigation every two days. Plants growth, sodium (Na) and potassium (K) as well as proline and soluble sugars contents of leaves and roots were determined after two weeks.

Results: Salt effect reduced significantly plant growth at $P = .05$, root K content at $P = .05$ and leaf K/Na ratio at $P = .01$ whereas it increased significantly leaf Na at $P = .05$ and root soluble sugars content at $P = .05$. The application of exogenous calcium (Ca) induced a significant amelioration of plant growth at $P = .01$ more marked with CaSO₄ than with Ca (NO₃)₂. This treatment induced a significant decrease at $P = .01$ in leaf and root Na content and a significant increase at $P = .05$ in root K content only for CaSO₄, a significant increase at $P = .01$ in leaf K/Na ratio and a significant increase at $P = .01$ in leaf and root proline content only for Ca(NO₃)₂.

Conclusion: The ameliorative effect of both forms of calcium was due mainly to sodium (Na) exclusion from leaves and potassium (K) accumulation associated to a maintain of high K/Na ratio mainly in leaves. The importance of proline accumulation as an indicator of this ameliorative effect was associated only to Ca(NO₃)₂.

Keywords: Ameliorative effect; calcium nitrate; calcium sulphate; proline; salt stress mitigation; sodium chloride; soluble sugars.

1. INTRODUCTION

Soil and water salinity is one of the major problems for plant production in many countries around the world. It is considered as the major abiotic factor that limits plant productivity and agricultural yield [1, 2]. Several studies have reported that salts present in soil and in irrigation water reduce plant growth [3-10] and consequently reduce crop production. Soil salinity affects plant growth, either through osmotic effects or ionic toxicity effects. Indeed, it has been established that the stronger accumulation of sodium especially in the leaves is a general consequence of salinity and that this accumulation generally causes a decrease in the absorption of calcium and potassium, two major ions for the cellular metabolism [11]. As reported by several authors, the synthesis of organic compounds, or osmo-protectants, such as proteins, soluble sugars, amino acids and more particularly proline is one of the strategies that the plant develops to attenuate the effect of salinity. The tolerance of plants to NaCl depends on their capacity to maintain sufficient absorption of essential nutrients, such as K [12].

Tomato (*Lycopersicon esculentum* Mill.), belonging to the family of *Solanaceae*, is one of the most important vegetable crops in the world. In the Republic of Benin, it is cultivated throughout the whole national territory [13]. Consumed mainly in its fresh form [14], tomato production shares a large portion of the market garden crops produced in the country. Indeed, with an annual production of 253,150 tons in 2018 [15], the tomato is the first vegetable fruit most consumed in Benin. However, its production met several biotic and abiotic factors, among which is salinity. As for other plants species, salt stress reduces tomato plants' growth [16,17]. Studies in the Republic of Benin have shown that several tomato cultivars produced in the country undergo significant reductions in growth under NaCl salt stress [8]. It is well known that improving a plant's tolerance to salinity can be achieved, in addition to genetic selection, through the use of suitable physiological tools [18]. Thus, to attenuate the negative effects of salinity on plants and to circumvent this stress, one of the approaches consists in enriching the nutrient contents of the substrate used for growing plants through

exogenous application of elements such as nitrogen, potassium, calcium, magnesium or phosphorus [19,20,21]. Particular emphasis has been put on calcium because of its ability to induce a protective effect on plants in hostile environmental conditions [18]. Calcium plays a vital role in salt stress tolerance as it induces the activities of anti-oxidant enzymes and reduces lipid peroxidation of cell membranes under abiotic stress [22,23]. Numerous studies have suggested that apoplastic Ca^{2+} directly suppresses symptoms produced by mineral toxicities. The enhancement effect of external Ca^{2+} in plants exposed to salinity may be associated with the maintenance of an optimal K^+/Na^+ ratio and with homeostasis in the cytosol in relation to an inhibition of the influx of Na^+ efflux of K^+ or the promotion of efflux of Na^+ and influx of K^+ through the plasma membrane [24, 25, 26].

In our previous study, using exogenous application of different forms of K^+ and Ca^{++} , we have demonstrated that only Ca^{++} was able to mitigate the effects of salinity on tomato plant growth [27]. The objective of this study is to evaluate the implication of Na^+ , K^+ , proline and soluble sugars accumulations in the ameliorative effect of Ca on the growth under NaCl salt stress of a cultivar of tomato grown in Benin Republic.

2. MATERIAL AND METHODS

2.1 Plant Material

In this experiment, we used tomato cultivar "Padma" identified as relatively sensitive to NaCl [8].

2.2 Experimental Design and Conditions

The experiment was done in green house of the Faculty of Agronomical Sciences, University of Abomey-Calavi, Benin Republic from June to July 2020. Day/night mean temperatures were $26^\circ\text{C}/23^\circ\text{C}$ with a mean humidity of 55%. The experiment was conducted as described by Kinsou et al. [8] with three plants per pot. Four treatments were used consisting in watering the plants every other day with 200 ml / pot of 0 mM (Control: C); 120 mM NaCl (Salt: S); 120 mM NaCl + 40 mM CaSO_4 (S + CaSO_4) and 120 mM NaCl + 40 mM $\text{Ca}(\text{NO}_3)_2$ (S + $\text{Ca}(\text{NO}_3)_2$). The experimental set-up as a completely randomized design with three replicates. We have chosen 40 mM CaSO_4 and $\text{Ca}(\text{NO}_3)_2$ because our previous study [27] has shown that among CaSO_4 ; CaCl_2

; KNO_3 and K_2SO_4 , no form of K was able to ameliorate plant growth under salt stress and that among CaSO_4 and CaCl_2 , CaSO_4 was more efficient in reducing salt detrimental effect on plant growth than CaCl_2 . We added $\text{Ca}(\text{NO}_3)_2$ to evaluate its capacity to attenuate salt effect on tomato plants growth as another form of calcium. The experiment was evaluated after two weeks of treatment.

2.3 Growth Determination

Relative plant height growth, relative fresh mass growth and relative dry mass growth of shoot and roots were determined as described by Kinsou et al. [8]. Plant height (cm), fresh and dry mass (FM and DM) of the shoots and roots were first determined before application of the treatments (X0). They were determined again after 2 weeks of treatment (X1). Relative growth of each parameter was determined according to the formula: $(X1-X0) / X0$. The fresh mass of the aerial and roots parts was determined by weighing. The samples from each part were then transferred to an oven at 80°C for 72 hours for the determination of the dry mass. Thus, relative plant height growth (RPHG), relative shoot fresh mass growth (RSFMG), relative shoot dry mass (RSDMG), relative root fresh mass growth (RRFMG) and relative root dry mass growth (RRDMG) were determined after two weeks of treatment.

2.4 Extraction and Estimation of Ion Concentrations

Ions determination was done as reported by Gouveitcha et al. [28]. Leaves and roots were individually dried in an oven at 80°C for 72 hours, ground in a mortar, and the powder was dried for 24 hours. To determine the concentrations of Na^+ and K^+ , 20 mg of the leaf and root powders were placed in 10 ml jars and digested in nitric acid (68%) at room temperature. The solutions were filtered through Whatman paper (85 mm, Grade 1). The filtrate was used for the determination of cations (Na^+ and K^+) using a flame spectrophotometer (Sherwood Model 360). Ion quantities were expressed in mg g^{-1} of dry mass (DM).

2.5 Extraction and Determination of Proline and Soluble Sugars

Proline extraction is performed according to Bates et al. [29]. Samples of 100 to 200 mg of leaf (youngest leaf fully opened), or root all in a 15 ml vial, to which 10 ml of sulfosalicylic acid

has been added. The extraction was done in a water bath: 70° C for 30 minutes and then filtered to collect the filtrate in 10 ml tubes. To 2 ml of the filtrate was added 2 ml of ninhydrin solution and 2 ml of acetic acid cc. The mixture was again brought to a water bath: 90° C. for 1 hour. The mixture is then cooled and the chromatophore formed is extracted with 3 or 4 ml of toluene. This mixture is then stirred vigorously for 10 seconds and left to stand, in order to separate the aqueous phase from the organic phase (toluene). The optical density of the organic phase containing the chromatophore is measured with a spectrophotometer at a wavelength of 520 nm using a UV-visible spectrophotometer (Jenway 7305) and results were expressed as μg proline g^{-1} FM (Fresh Mass).

Total soluble sugars were estimated by the anthrone reagent method using glucose as the standard according to [30] as used by [18]. Samples of leaves (the youngest, fully open) or roots of 500 mg, to each tube, 4 ml of 70% ethanol were added. The whole thing is crushed in a mortar placed on crushed ice. The shreds are transferred to in Eppendorf tubes, vortexed and centrifuged at 4° C, for 10 min, at 8000 rpm. They are then filtered with Whatman paper (85 mm, Grade 1) before adding 2 ml of 70% ethanol; the mixture is vortexed and centrifuged for 10 min at 8000 rpm at a temperature of 4° C. Standards are prepared with three repetitions. One (01) ml of anthrone is added to 200 μl of each standard solution, the whole is brought to a water bath at 100° C for 10 min. After rapid cooling, the optical density is read with a spectrophotometer at a wavelength of 625 nm using a UV-visible spectrophotometer (Jenway 7305). Soluble sugars concentration was

expressed as mg soluble sugars g^{-1} FM (Fresh Mass).

2.6 Statistical Analysis

The data collected were processed using descriptive statistics using an Excel spreadsheet and presented in the form of tables and graphs. Analysis of the effect of treatments was based on one way analysis of variance (ANOVA). Means were compared using the Student, Newman and Keuls test. Statistical analyses were performed using JMP Pro 12 software [31].

3. RESULTS

3.1 Effect of NaCl and Exogenous Calcium on Plant Growth

Salt stress induced a significant reduction at $P = .001$ in aerial part growth of tomato plants whatever the growth parameter taking into account. The growth reduction due to NaCl was about 55.46% for relative plant height growth (RPHG) (Fig. 1); 69.49% relative shoot fresh mass growth (RSFMG) (Fig. 2); 66.97% relative shoot dry mass growth (RSDMG) (Fig. 3). The exogenous application of calcium attenuated the reduction of plant growth due to NaCl. Growth amelioration was about 93.35% for CaSO_4 and 69.79% for $\text{Ca}(\text{NO}_3)_2$ in comparison with stressed plants, in the case of relative plant height growth (Fig. 1). Taking into account the relative shoot fresh mass growth, the ameliorative effect of exogenous calcium was about 117.76% and 107.21% respectively for CaSO_4 and $\text{Ca}(\text{NO}_3)_2$ (Fig. 2). For the relative shoot dry mass growth, the ameliorative effect of exogenous calcium was about 88.03% and 129.58%, respectively for CaSO_4 and $\text{Ca}(\text{NO}_3)_2$ (Fig. 3).

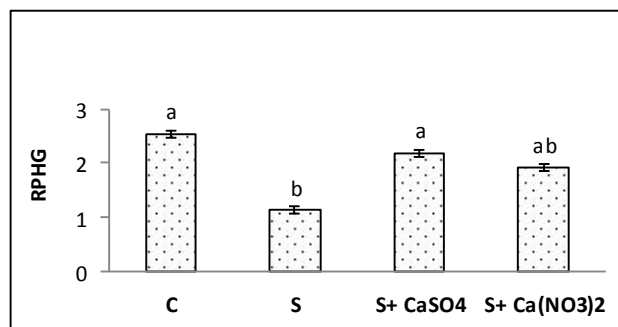


Fig. 1. Effect of exogenous application of two forms of calcium on relative plant height growth of tomato plants grown under salt stress after two weeks (n = 3; vertical bars are standard errors). Means with different letters are significantly different at $P = .001$

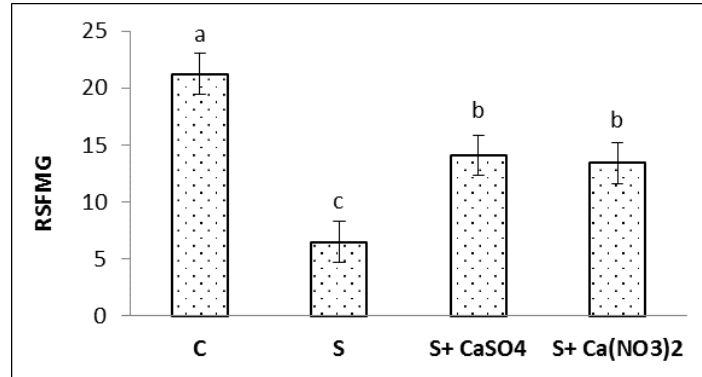


Fig. 2. Effect of exogenous application of two forms of calcium on relative shoot fresh mass growth of tomato plants grown under salt stress after two weeks (n = 3; vertical bars are standard errors). Means with different letters are significantly different at $P = .001$

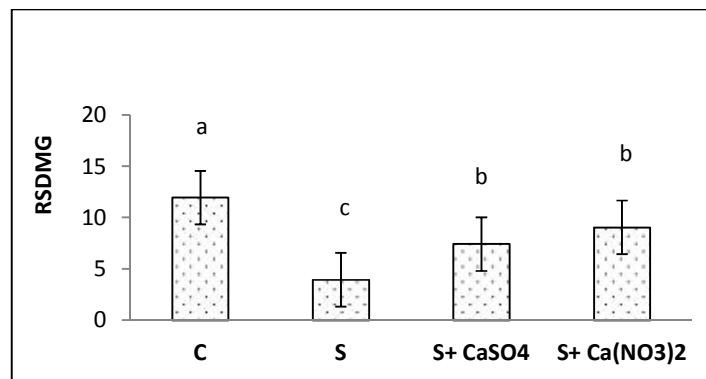


Fig. 3. Effect of exogenous application of two forms of calcium on relative shoot dry mass growth of tomato plants grown under salt stress after two weeks (n = 3; vertical bars are standard errors). Means with different letters are significantly different at $P = .001$

Salt stress induced a significant reduction at $P = .01$ in root growth of tomato plants whatever the growth parameter taking into account. The growth reduction due to NaCl was about 67.4% and 38.47% respectively); relative roots fresh mass growth (RRFMG) and relative roots dry mass growth (RRDMG). The ameliorative effect of exogenous calcium on relative roots fresh mass growth was about 76.06% and 166.68% respectively for CaSO_4 and $\text{Ca}(\text{NO}_3)_2$ while for the relative roots dry mass growth, the ameliorative effect was about 68.79% and 156.30% respectively for CaSO_4 and $\text{Ca}(\text{NO}_3)_2$. The ameliorative effect was significant at $P = .01$ for all the five growth parameters evaluated for CaSO_4 whereas in the case of $\text{Ca}(\text{NO}_3)_2$ the ameliorative effect was significant at $P = .01$ only for four growth parameters. Thus, CaSO_4 was more efficient in tomato salt stressed plant growth improvement than $\text{Ca}(\text{NO}_3)_2$. However, the highest improvement rate was obtained for

three of the five growth parameters evaluated in the case of $\text{Ca}(\text{NO}_3)_2$ (shoot dry mass and the two roots growth parameters).

3.2 Effect of NaCl and Exogenous Calcium on Plant Ions Content

The salinity caused a significant increase at $P = .05$ in Na content in both leaves and roots (Table 1). The increases were about 243.47% in comparison with the control for leaves and about 16.90% in roots. The exogenous application of calcium induced a significant reduction at $P = .01$ of Na content in leaves for both forms of calcium in comparison with NaCl. The reductions were about 45.56% and 41.77% in comparison with the stressed plants respectively for CaSO_4 and $\text{Ca}(\text{NO}_3)_2$. In roots, the reduction of Na content by exogenous application of calcium in comparison with NaCl was significant at $P = .01$ only for CaSO_4 (44.5%). It is important to notice

that the Na content in roots with exogenous application of both forms of Ca was lower than that of the control plants.

NaCl effect on leaves K content resulted in a non-significant decrease whereas a significant decrease at $P = .05$ was observed in roots (18.72% in comparison with the control non-stressed plants). The exogenous application of calcium induced a non-significant increase in leaves K content (6.41% and 10.89% respectively for CaSO_4 and $\text{Ca}(\text{NO}_3)_2$). In roots, the salt caused a significant decrease at $P = .05$ in K content with a reduction rate of 18.72%. The exogenous application of calcium induced an increase in roots K content but the increase was significant at $P = .05$ only for CaSO_4 (23.59%). Overall, the exogenous application of both calcium forms improved plant mineral nutrition; however, CaSO_4 had the best improving effects

on plants mineral nutrition: significant exclusion of Na from both leaves and roots; and significant accumulation of K in roots.

Salt stress induced a significant reduction at $P = .01$ in K/Na ratio only in leaves. The exogenous application of calcium induced a significant increase at $P = .01$ of the K/Na ratio in leaves for both calcium forms. The increases were about 95.55% and 88.93% compared to plants stressed respectively for CaSO_4 and $\text{Ca}(\text{NO}_3)_2$. In the roots, only CaSO_4 caused a significant increase at $P = .01$ in K/Na (93.97% in comparison with the stressed plants). Overall, the exogenous application of both calcium forms improved plant K/Na ratio; however, CaSO_4 had the best improving effects on both leaves and roots K/Na ratio. It is important to notice that the K/Na in roots with exogenous application of both forms of Ca was higher than that of the control plants.

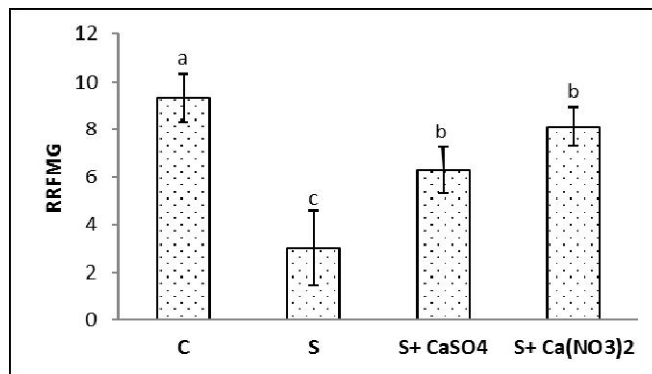


Fig. 4. Effect of exogenous application of two forms of calcium on relative root fresh mass growth of tomato plants grown under salt stress after two weeks (n = 3; vertical bars are standard errors). Means with different letters are significantly different at $P = .01$

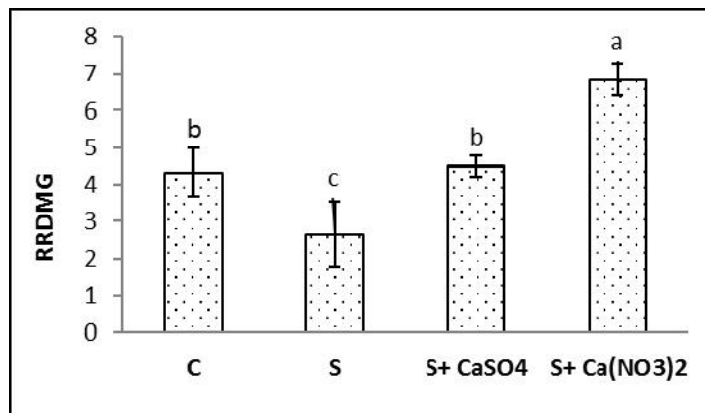


Fig. 5. Effect of exogenous application of two forms of calcium on relative root dry mass growth of tomato plants grown under salt stress after two weeks (n = 3; vertical bars are standard errors). Means with different letters are significantly different at $P = .01$

Table 1. Effect of exogenous application of two forms of calcium on Na et K ions content in leaves and roots of tomato plants grown under salt stress after two weeks (n = 3). Values are means \pm standard errors

	Leaves		Roots	
	Na (mg g ⁻¹ DM)	K (mg g ⁻¹ DM)	Na (mg g ⁻¹ DM)	K (mg g ⁻¹ DM)
C	0.023 \pm 0.00d	0.184 \pm 0.00a	0.071 \pm 0.00ab	0.219 \pm 0.00a
S	0.079 \pm 0.00a	0.156 \pm 0.00a	0.083 \pm 0.00a	0.178 \pm 0.00b
S + CaSO ₄	0.043 \pm 0.00c	0.166 \pm 0.01a	0.046 \pm 0.00b	0.191 \pm 0.00ab
S + Ca(NO ₃) ₂	0.046 \pm 0.00b	0.173 \pm 0.02a	0.066 \pm 0.01ab	0.220 \pm 0.01a

Means with different letters within column are significantly different at $P = .05$ or $P = .01$

Table 2. Effect of exogenous application of two forms of calcium on ion selectivity ratio (K/Na) of leaves and roots of tomato plants grown under salt stress after two weeks (n = 3). Values are means \pm standard errors

	Leaves	Roots
C	7.784 \pm 0.57a	3.077 \pm 0.04ab
S	1.980 \pm 0.11c	2.141 \pm 0.09b
S + CaSO ₄	3.872 \pm 0.31b	4.153 \pm 0.20a
S + Ca(NO ₃) ₂	3.741 \pm 0.55b	3.676 \pm 0.84ab

Means with different letters within column are significantly different at $P = .01$.

3.3 Effect of NaCl and Exogenous Calcium on Proline and Soluble Sugars Contents

Salt stress caused a non-significant increase in proline content in both leaves and roots (Fig. 6). The exogenous application of calcium induced a significant increase at $P = .05$ in proline content in both leaves (32.42%, compared to the stressed plants) and roots (22.33%, compared to the stressed plants) in comparison with NaCl only for Ca(NO₃)₂. However, proline content is higher in roots than in leaves whatever the growing conditions.

Salt stress caused a significant increase at $P = .05$ in soluble sugars content only in roots (80.76% compared to control (Fig. 7). The exogenous application of calcium induced a non-significant increase in soluble sugars content only for Ca(NO₃)₂. A non-significant decrease (25.53%) was observed in roots for CaSO₄. However, soluble sugars content is higher in roots than in leaves whatever the growing conditions.

4. DISCUSSION

4.1 Effect of NaCl and Exogenous Calcium on Plant Growth

Salinity caused a significant reduction in the various growth parameters of the aerial and root

parts taken into account of young plants of tomato cultivar "Padma". This growth reduction has been reported in several genotypes or varieties of tomato [32, 16, 8]. The reduction in plant growth under salt stress is generally attributed to a combination of the osmotic effect and the specific effect of Na⁺ and Cl⁻ ions [33,34]. The present results revealed that the exogenous application of both forms of calcium are efficient in attenuating salt detrimental effects in tomato plant growth. Other authors have reported that exogenous application of calcium improved the negative effects of salinity on plants growth in different species such as strawberries (*Fragaria ananassa*) [35]; guava (*Psidium guajava*) [19] and amaranth (*Amaranthus cruentus*) [36]. In our previous study [27], we have reported that exogenous calcium application was more efficient in attenuating the depressive effects of NaCl on tomato plants than potassium application. Similar results were also reported in two cultivars of tomato mainly for exogenous CaSO₄ application associated to salicylic acid [18]. However, opposite results were observed in sunflowers where supplemental calcium application was not able to improve the damage caused by NaCl [37].

Our results revealed also that between CaSO₄ and Ca(NO₃)₂, CaSO₄ was more efficient in tomato plant growth improvement under salt stress than Ca(NO₃)₂. According to [36], calcium sulfate (CaSO₄) has been one of the most

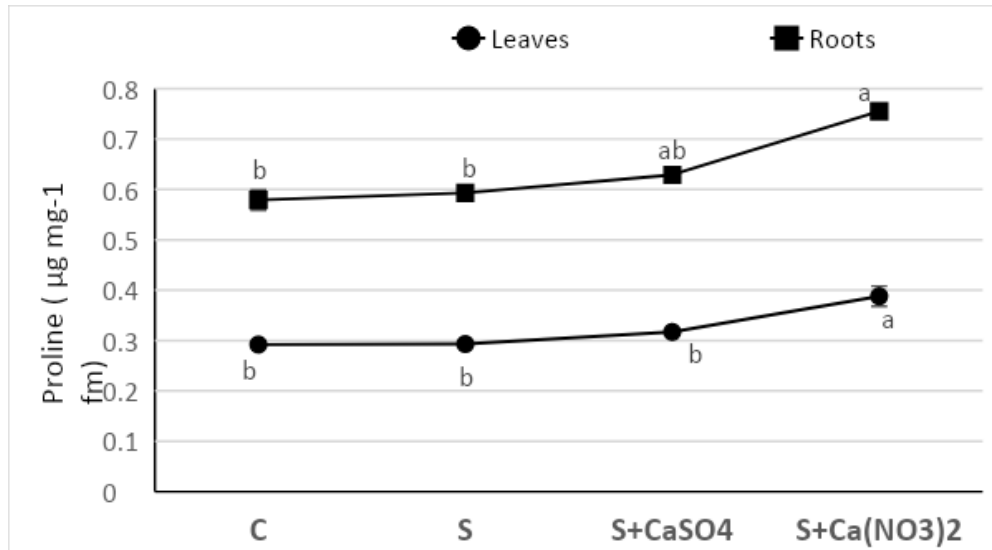


Fig. 6. Effect of exogenous application of two forms of calcium on proline content of tomato plants grown under salt stress after two weeks (n = 3). Values are means ± standard errors. Means with different letters are significantly different at P = .05

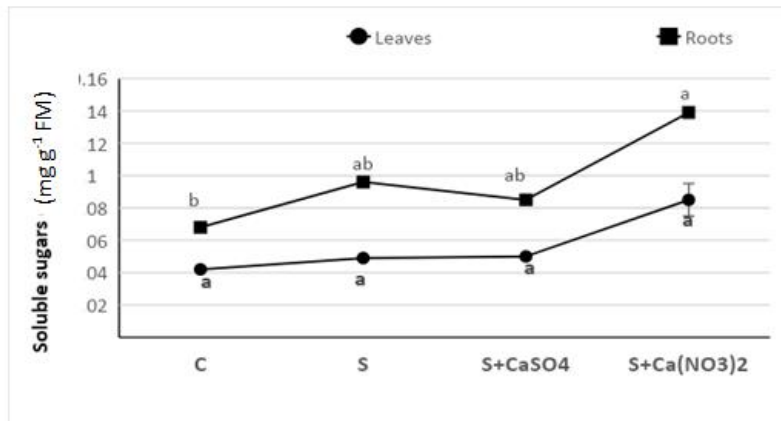


Fig. 7. Effect of exogenous application of two forms of calcium on soluble sugars content of tomato plants grown under salt stress after two weeks (n = 3). Values are means ± standard errors. Means with different letters are significantly different at P = .05

effective compounds in alleviating the negative effects of salinity on aerial part in amaranth among CaSO₄, CaCl₂, KNO₃ and K₂SO₄. More over several studies have demonstrated that exogenous application of different form of potassium (K) ameliorated salt tolerance in wheat [38] and in peanuts plants [39]. Thus, we can hypothesize that the response of salt stressed plants to an exogenous application of different compounds depends on the nature of the compounds used but also on the plant species. The efficacy of calcium in attenuating

the depressive effects of NaCl on plant growth is due to its ability to induce a protective effect on plants under hostile environmental conditions [18] and its role on the metabolic functions of the plant.

4.2 Implication of ions and organic solutes in the ameliorative effect of calcium on plant growth under salt stress

Salinity caused an increase in Na content and a

decrease in K content in the leaves as well as in the roots in tomato cultivar Padma plants evaluated in this study. This response of plant ion nutrition to salt stress in a common behavior as reported by several authors [40,41,42,43,28], suggesting that the NaCl detrimental effect on plant growth was due mainly to both Na toxicity and K deficiency. As revealed by our results, the exogenous application of both calcium forms improved plant mineral nutrition by reducing Na accumulation and enhancing K accumulation in both leaves and roots. Reducing Na accumulation and enhancing K accumulation mainly in leaves is one of the common plant strategies to tolerate NaCl salt stress as reported in several glycophyte species [44, 45, 46, 43, 28]. In okra (*Abelmoschus esculentus*), Habib *et al.* [42] reported that the foliar application of both pure glycine betaine and sugarbeet extract reduced the adverse effects of salt stress on plant growth, yield and leaf K content. The same tendency was reported in amaranth plants submitted to exogenous application of Ca and K in different forms under salt stress [36]. Moreover, it has been reported that the ameliorative effect of the exogenous application of different forms of K on plant growth of wheat and peanuts under salt stress was due to both Na exclusion and K accumulation mainly from leaves [38,39]. Thus, the salt tolerance acquired by tomato plants following the exogenous application of both forms of Ca was due, at least partially, to Na exclusion and K accumulation in both leaves and roots. However, CaSO₄ had the best improving effects on plants mineral nutrition: significant exclusion of Na from both leaves and roots; and significant accumulation of K in roots.

Salt stress induced a reduction in K/Na ratio both in leaves and roots, even if this reduction was significant only in leaves. This tendency is the general response of plants to salt stress as reported [18, 36]. However, reducing Na⁺ in the shoot, while maintaining K⁺ homeostasis, is a key component of salinity tolerance in many crops [44]. The exogenous application of both calcium forms improved plant K/Na ratio; however, CaSO₄ had the best improving effects on both leaves and roots K/Na ratio. It is known that the ameliorative effect of the exogenous application of Ca or K on the detrimental effects of salinity is associated with the maintenance of an optimal K/Na ratio and with homeostasis in the cytosol in relation with an inhibition of the influx of Na, the efflux of K or the promotion of the efflux of Na and the influx of K through the plasma

membrane [24, 25, 26, 38, 39]. Our results show that maintaining a high K/Na ratio mainly in leaves was one of tomato plant strategies to tolerate NaCl salt stress corroborating the report of [45] which reported that exogenous application of calcium attenuates the toxic effects of NaCl, by facilitating higher K selectivity to Na, the application of Ca also maintains membrane integrity and selectivity, thus reducing the toxicity of Na⁺ and Cl⁻ in plants.

It is known that the synthesis of soluble sugars and proline is one of the strategies that the plant develops to attenuate the effect of salinity [26]. Our results showed that the salinity increased the content of soluble sugars in the leaves and roots of tomato plants which is a general trend in plants submitted to salt stress as reported in several plant species such as sugarcane (*Saccharum* sp.) [46, 47]; amaranth [43] and euphrates poplar (*Populus euphratica*) [48]. The exogenous application of calcium did not induce any significant change in soluble sugars content indicating that these solutes did not mediate the ameliorative effect of calcium on the growth of tomato plants submitted to NaCl salt stress. Salt stress caused a non-significant increase in proline content in both leaves and roots but the exogenous application of calcium induced a significant increase in proline content in both leaves and roots only for Ca(NO₃)₂. Proline accumulation is frequently reported in plant exposed to salt or water stresses. Cytosolic accumulation of proline is indeed involved in osmotic adjustment [49, 50]. Proline is also supposed to act as protective agent to enzymes, intracellular structures and cellular homeostasis balance and signaling [51,52]. Thus, proline accumulation appeared as an important component mediating the ameliorative effect of Ca(NO₃)₂ on tomato plants growth under salt stress. As CaSO₄ was the most efficient compounds in attenuating the detrimental effect of NaCl on tomato plant growth, this osmolyte did not apparently mediate the ameliorative effect of CaSO₄. It seems that the ameliorative effect of each form of Ca on plant growth was not mediated by the same way. This idea corroborated the results reported in two peanut cultivars where the exogenous application of K under salt stress inhibited the accumulation of proline in one cultivar compared to the stressed plants [39].

5. CONCLUSION

The external application of CaSO₄ and Ca(NO₃)₂

is efficient to attenuate NaCl detrimental effects on plant growth in tomato plants. However, CaSO₄ is more efficient than Ca(NO₃)₂. The ameliorative effect of both Ca forms was due mainly to Na exclusion from leaves and K accumulation associated to a maintain of high K/Na ratio in leaves. The importance of proline accumulation as an indicator of this ameliorative effect was associated only to Ca(NO₃)₂ indicating that ameliorative strategy can be specific to a compound used. Soluble sugars did not play a key role in the ameliorative effect of exogenous application of Ca on growth of tomato plants cultivated under NaCl salt stress.

DISCLAIMER

The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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