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COMPARATIVE EFFECT OF TWO VARIETIES OF TOMATO (*SOLANUM LYCOPERSICUM* L.) TO SALINITY

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Abstract

Description of the subject: The majority of irrigation water in Algeria is of underground origin and laden with salt, whose harmful effects vary with the plant sensitivity and the nature of salt.
Objective: This work deals with the comparative study of two tomato genotypes sensitive, Marmande and Saint-Pierre, grown in aboveground and irrigated with water enriched in sodium salts in the form of NaCl or Na_2SO_4.
Methods: The seed was sown in a hydroponic system during 110 days after sowing (DAS); the plants were treated with 30.45 meq.L-1 sodium chloride or sodium sulfate. The plant's samples were collected from 20th, 65 and 110th DAS.
Results: Our results showed that the salt stress exert a depressive effect on growth more pronounced on the Marmande variety than the Saint-Pierre variety and that NaCl is more harmful than Na_2SO_4. However, an osmotic adjustment partially achieved by the accumulation of proline is recorded in the Marmande variety than Saint-Pierre in the presence of NaCl than Na_2SO_4.
Conclusion: Based on the results obtained, it is assumed that cv. Saint-Pierre is more tolerant to salinity than the cv. Marmande due to a higher ability of maintaining the root function for the uptake and supply water to shoot under salt stress conditions.
Keywords: Tomato, Salinity, Marmande; Saint-Pierre; NaCl; Na_2SO_4; proline.

EFFET COMPARATIF DE DEUX VARIÉTÉS DE TOMATE (*SOLANUM LYCOPERSICUM* L.) À LA SALINITÉ

Résumé

Description du sujet : La majorité des eaux d'irrigation en Algérie sont d'origine souterraine et chargée en sel, dont les effets néfastes sur les cultures variant avec la sensibilité de la plante et la nature de sel.
Objectifs : Nouvelle idée, nouvelle connaissance, nouvelle méthode, nouvelle approche, rapporter un fait nouveau, non encore publié, ou apporter un nouvel éclairage, peut-être une question.
Méthodes : Ce travail à porte sur l'étude comparative de deux variétés de tomate (Marmande et Saint-Pierre) cultivées en système hydroponique en condition semi contrôlées irriguées avec une eau chargée en sodium apporté sous forme de NaCl ou de Na_2SO_4 avec le même nombre d'équivalent gramme par litre (30,65meq/L) durant 20, 65 et 110 jours.
Résultats : Nos résultats ont montré que les deux sels exercent un effet dépressif sur la croissance plus marqué sur la variété Marmande que la variété Saint-Pierre et que NaCl est plus nocif que Na_2SO_4. Toutefois, un ajustement osmotique partiellement réalisé par l'accumulation de la proline est enregistré chez la variété Marmande que Saint-Pierre en présence de NaCl que de Na_2SO_4.
Conclusion : Sur la base des résultats obtenus, on suppose que cv. Saint-Pierre est plus tolérant à la salinité que le cv. Marmande en raison de sa plus grande capacité à maintenir la fonction racinaire absorbée et à fournir de l'eau pour tirer en conditions de stress salin.
Mots clés : Tomate, salinité, Marmande ; Saint-Pierre ; NaCl ; Na_2S0_4 ; proline.

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INTRODUCTION

According to the [1], approximately 800 million ha of land and 32 million ha of agricultural land are affected by salinity. 45 million ha (20%) of irrigated land is affected by this phenomena [2]. These areas are characterized by a marked decrease in rainfall, strong evaporation of water from the soil, and an increase in the extension of agricultural and pastoral activities with salinized water irrigation [3].

Plants are exposed to changes in their morphological and physiological behavior in the presence of salinity [4]; [5] and [6].

Therefore, a better understanding of the structural variations, ion distribution in crop plants induced by salinity should facilitate the identification of saline tolerance mechanisms [7]. The ability of plant cells to realize an osmotic adjustment and to accumulate through of the compatible solutes is the main factor of salt tolerance mechanisms. The accumulation of key osmolytes such as proline can provide a marker of the degree of tolerance to stress induced by osmoregulation. It is one of the major non-enzymatic antioxidants that plants need to counteract the inhibitory effects of salt stress [8].

Now it is well known that stress causes a number of effects on plants such as ion toxicity, hormonal imbalance [9 and 10]. This reaction results in metabolic, physiological and morphological changes [11]. One of the main physiological characters of environmental stress tolerance is osmotic adjustment, which plays a key role in resistance or tolerance of the plant to stress. This is achieved through an accumulation of osmoregulatory compounds leading to a reduction of the osmotic potential thus allowing the maintenance of the turgor potential [12].

One of the strategies for adaptation to salinity is to synthesize osmoprotective agents, mainly amino compounds and sugars, and to accumulate them in the cytoplasm and organelles [13]. The accumulation of these organic compounds has been demonstrated in several plant species subjected to salt stress.

Tomato (*Solanum Lycopersicum* L.) is one of the most important crops in terms of human consumption. It is cultivated in many regions and in different climates, including arid and semi-arid. Over the last decade, its production has grown to reach almost 160 million tons' fresh fruit in the world [14]. It is consumed as fresh or processed fruit due to its excellent nutritional properties. In addition, it is a rich source of vitamins, carbohydrates, proteins, mineral nutrients and other several important chemicals: carotenoids (lycopene, b-carotene, and lutein), tocopherols, and polyphenols [15]. Tomato is considered an ideal fruit model system because it can be easily grown under different conditions; it has a short lifecycle, and simple genetics due to the relatively small genome [16].

Furthermore, knowledge in tomato biology can be easily transferred to other economically important *Solanaceae* species [17]. Despite the economic relevance of this crop, the mechanisms underlying its response to abiotic stresses are not yet fully clarified and few information is currently available on the key role of stress-responsive genes [18 and 19]. Since salinity is a global scientific problem which affects crop productivity including vegetables. It is frequently answered in nature and it is one of the most negative environmental constraints for plants and tomato in particular [20 and 21].

The present study, conducted under semi-controlled conditions, aimed to examine the salinity tolerance by two saline solutions containing (NaCl and Na_2SO_4) in two tomato varieties most cultivated in Algeria (Marmande and Saint-Pierre). This effect is evaluated in terms of plant growth (plant height and dry matter production). Also to examine the physiological implications for photosynthetic activity and osmotic adjustment (expressed by chlorophyll and proline leaf content respectively). This experiment was designed to test the impact of sodium supplied as NaCl or Na_2SO_4 for 20, 65 and 110 days.

MATERIAL AND METHODS

2.1. Plant material and growth conditions

The present experiment was conducted during winter to spring 2011-2012 in the greenhouse “semi-controlled conditions”. Two tomato genotypes sensitive, Marmande and Saint-Pierre, were selected for this study. Seeds were surface-sterilized for 20 min in 20 % (v/v) sodium hypochlorite, rinsed and soaked in distilled water. This procedure is required to eliminate saponin from seeds and to avoid contamination by microorganisms during the germination process. The entire sterilization procedure, including soaking, took 1h and did not affect the germination process [22].

After that, seeds were placed on filter paper in round Petri dishes of 10cm diameter then they have been kept in the growth chamber at a temperature of 25°C in the dark with a relative humidity of 70%. Seeds were considered germinated when the radicle had extended at least 2 mm. After 10 days, the seedlings were transferred to plastic pots (23 cm diameter × 22 cm height) onto a semi-closed hydroponic device with river gravel substrate (3 mm diameter). The seedlings were irrigated every morning with a full nutrient solution during the period of first 15 days (pH 5,6; EC 2.11 mS cm-1, $NO3^-$ 10,20 meq/L; $S0_4^{-2}$ 1,50 meq/L; PO_4^{3-} 3,30 meq/L; Cl^- 0.60 meq/L; Na^+ 1,30 meq/L; K^+ 4.25 meq/L; Ca^{+2} 5.10 meq/L; Mg^{+2} 1.80 meq/L; NH_4^+ 1,80 meq/L) [23].

2.2. Salt stress

In this experiment, the irrigation solutions provided during the cultivation cycle were prepared by adding to the water the sodium supplied as NaCl, Na_2SO_4 with the same number of gram equivalent per liter as it is found in the underground water of Gassi Touil (Na^+ concentration is 30,45meq/L), and a standard nutrient solution used as a control (Table 1). These treatments were applied in three times according to the duration of stress: (1) short-term (20 days); (2) Medium term (65 days); (3) Long-term (110 days).

Table 1: The composition of water from the Gassi Touil source and various saline solutions tested (expressed in meq. L^{-1}).

	pH	EC	$NO3^-$	PO_4^{-3}	Cl^-	$S0_4^{-2}$	Na^+	Ca^{+2}	Mg^{+2}	K^+	NH_4^+
Water from the source	7,80	2.94	0.35	0.00	16.75	29.95	30.45	5.10	7.25	4.25	0.00
Tap water + Na_2SO_4	5,67	2,13	10,20	3,30	0,60	30,65	30,45	5,10	1,80	4,25	1,80
Tap water + NaCl	5,79	2.43	10,20	3,30	29,75	1,50	30,45	5,10	1,80	4,25	1,80

2.3. Dry matter determinations

A dry matter (DM) determination for plant growth is according to [24]. Three independent dry matter measurements analysis was performed on twinned leaves and stems. In addition to fresh weight (FW), dry weight (DW) was determined after desiccation at 105 °C for 48 h. Dry matter content (%) was estimated using the equation: DM % = [(FW - DW) / DW] * 100

2.4. Determination of physiological parameters

Estimation of Chlorophyll content

To study the Chlorophyll (Chl) contents in control and stress conditions. Pigment content was determined using the method of [25] in [26]. 0.1 g leaf tissue was put it in 10 ml of 95% acetone at 4°C in darkness for 48 h. The absorption was measured at 470, 645 and 663 nm using a UV-VIS spectrophotometer (Shimadzu UV-1700, Kyoto, Japan). Chlorophyll a (Chl a), chlorophyll b (Chl b) contents per unit area (mg/ml) were calculated by the formula [26].

Chl a = (9.78× A663 – 0.99 × A645), Chl b = (21.42 × A645 – 4.65 × A663) respectively.

Estimation of proline content

The proline was extracted and assayed according to methods described by [27], simplified and developed by [28] and modified by [29]. It was determined at the level of the root part and the aerial part (median leaf + stem). The principle is the quantification of the proline-ninhydrin reaction by spectrophotometric measurement at a wavelength of 528 nm. The proline couples with the ninhydrin forming a colored complex. The determination of the content of the proline is carried out according to the formula Proline (µg/g MF) = DO_{528} x 0.62 [29].

2.5. Experimental design and statistical analysis

The experiment was both set up as a completely randomized design with three replicates of each treatment. Data were subjected to One-Way ANOVA tests for each parameter at P < 0.05 using XLSTAT 2017, 4.467 software.

RESULTS

1.1. Effect of NaCl and Na_2SO_4 on growth

1.1.1. Effect on growth in length

Plant height in the control treatments did not differ from the two varieties. They showed a significant increase as a function of time (20, 65 and 110 days), with a slight decrease which was revealed for the Saint-Pierre variety after 110 days of culture (4.92 % of control at 65 days) (Fig.1). Whereas Na_2SO_4 treatments decreased plant height relative to controls at 20 days of treatment, and the decrease was greater for cv. Marmande than for cv. Saint-Pierre (28.79 % and 15.29 % respectively of control). After 65 days, plant height in the Na_2SO_4 treatment was higher in Marmande (13.81 % of control) than Saint-Pierre that presents a decreased plant height (2.39% of control). Conversely, plant height in NaCl treatment was decreased for Marmande variety (6.25 % of control) but she was increased in presence of Na2SO4 treatments from the Saint-Pierre (3.09 % of control). After 110 days, plant height in the NaCl treatment was increased in Saint-Pierre variety (3.09 % of control). On the other hand, this treatment was decreased the plant height of Marmande variety (14.77 % of control).

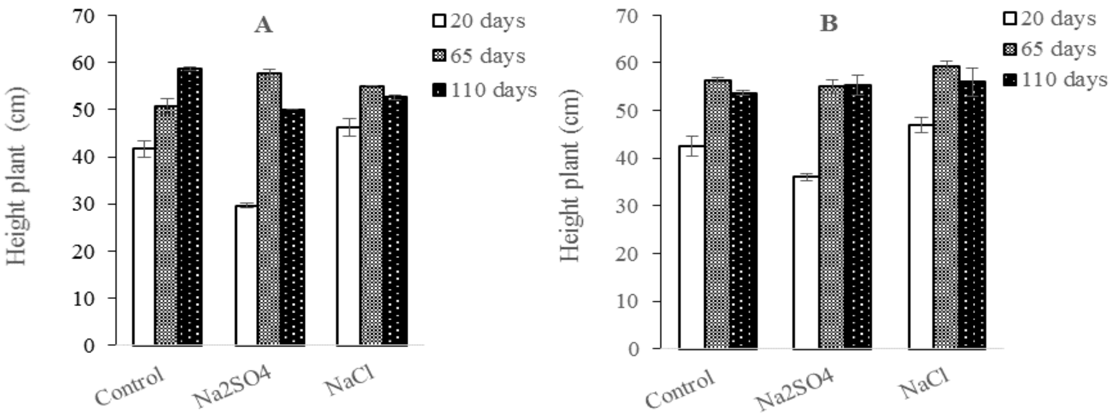

Figure 1. Plant height (cm) of the tow tomato cultivars [Marmande (A) and Saint-Pierre (B)] under 20, 65 and 110 irrigation's days of control, NaCl and Na_2SO_4 with Na^+ concentration is 30.45 meq/L). The data represent mean values ± SD (n = 3).

1.1.2. Effect on plant dry matter

Salinity can severely affect the dry matter content of different vegetative parts of the plant. The tomato cultivars' response to salinity has been quite diversified. It was found that the exposure time to salts affects the plant dry matter. Thus, a longer exposure to the salt content leads to solutes accumulation in the leaves stems and consequently increases the dry matter in the aerial tissues (Fig. 2). The dry matter according to the irrigation with the Na_2SO_4 solution of the two varieties of tomato was significantly decreased during 20 and 65 days. Nonetheless, at day 20 the decreased was more severely for the cv. Marmande than the cv. Saint-Pierre (61.62 % and 29.59 %) respectively of control. Thus, a longer exposure to the salt content (110 days) leads to solutes accumulation in the leaves and consequently increases the dry matter in the tissues. In the Na_2SO_4 treatment, the dry matter accumulated in the aerial tissues was increased in cv. Marmande (1.95% of control) and decreased in cv. Saint-Pierre (12.24% of control) respectively. It is to highlight that the irrigation with saline water containing NaCl induced a decrease of the dry matter, especially in the cv. Marmande during a long exposure (10.75% of control). On the other hand, this reduction is lower in cv. Saint-Pierre (1.14%).

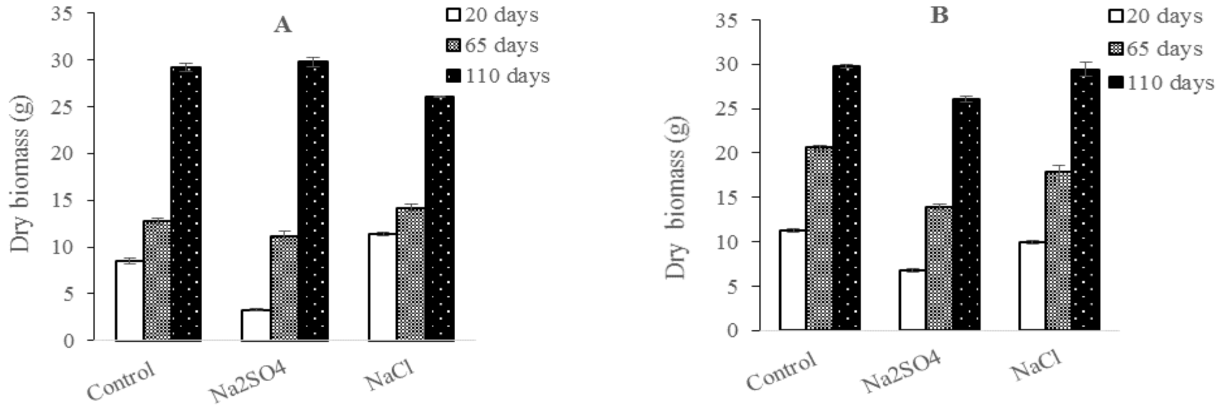

Figure 2. Dry matter of the aerial part (g) of the tow tomato cultivars [Marmande (A) and Saint-Pierre (B)] under 20, 65 and 110 irrigation's days of control, NaCl and Na_2SO_4 with Na^+ concentration is 30.45 meq/L). The data represent mean values ± SD (n = 3).

1.1.3. Effect on the chlorophyll content

Levels of pigments in control and salt-treated plants are illustrated by the histogram in Fig. 3.4. In the control plants, Chlorophyll (a) concentrations were presented a significant decrease between days 20 and 110 (68.18 and 77.54%) respectively. Conversely, chlorophyll (b) concentrations accumulated were (11.66 and 19.75%) for cv. Marmande and cv. Saint-Pierre respectively. However, both pigments (chlorophyll a and b) showed a significant decrease in Na_2SO_4-treated plants at days 110 (63.84 and 30.78 %), (64.70 and 21%) for the cv. Marmande and cv. Saint-Pierre respectively. The levels of pigments according to the irrigation with the NaCl solution of the two varieties of tomato was significantly decreased at day 110. Nonetheless, this decreased were more severely for the chlorophyll (a) (72.60 and 64.17%) that chlorophyll (b) (12.89 and 26.24%) respectively for the cv. Marmande and Saint-Pierre.

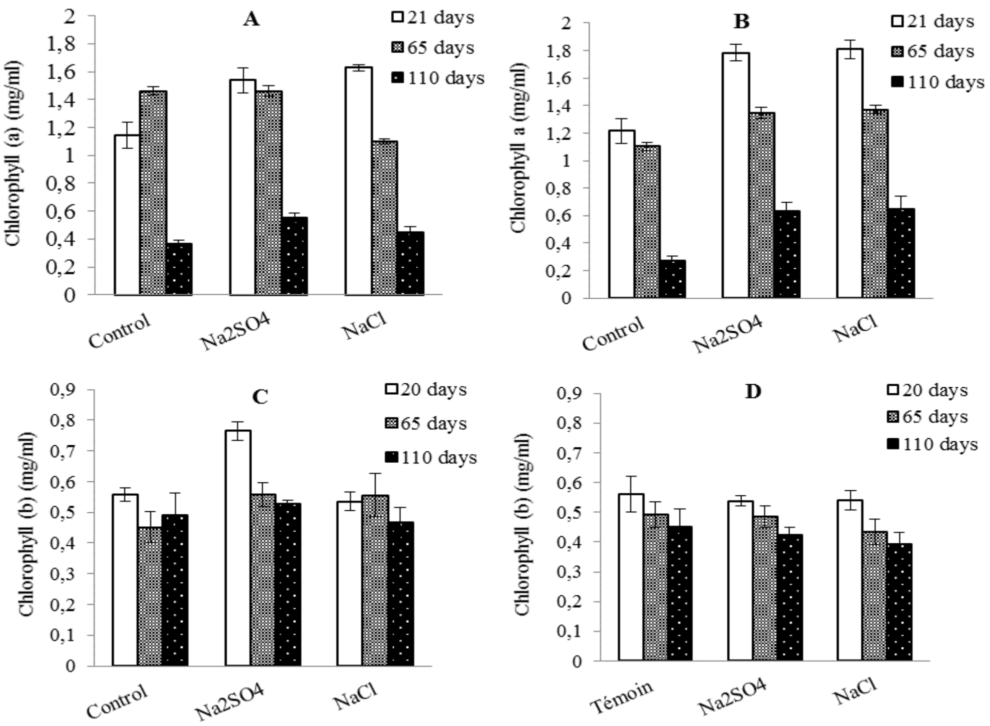

Figure 3. Leaf chlorophyll (a and b) content (mg/ml) of the tow tomato cultivars [Marmande (A and C) and Saint-Pierre (B and D) respectively] under 20, 65 and 110 irrigation's days of control, NaCl and Na_2SO_4 with Na^+ concentration is 30.45 meq/L). The data represent mean values ± SD (n=3).

1.2. Effect on water status

1.2.1. Effects of NaCl and Na_2SO_4 on proline content

The Proline content in *S. Lycopersicum* was estimated in the root and in the aerial part. The data are shown in Fig. 4 and 5. In control conditions, the proline concentration is very lower in the root (0.006 and 0.010 µg/g FM) and in the aerial part (0.019and 0.036 µg/g FM) for cv. Marmande and cv. Saint-Pierre respectively. As compared to the control, Na_2SO_4 treatment increase proline root contents by 200 and 250% for the cv. Marmande and by 10 and 240% for the cv. Saint-Pierre at day 20 and 65 respectively of control. NaCl treatment increases this content more rapidly. Effect of NaCl stress showed a similar pattern on proline contents in root when the plants were received the Na_2SO_4 but it was higher (1150 and 360% of control at day 20 of stress for the Marmande and Saint-Pierre respectively). Moreover, proline contents were found to be greater than that of control even in the aerial part under NaCl treatment. It was more increased for the cv. Marmande (226.31 and 152.63%) that in the cv. Saint-Pierre (44.44 and 216.66%) at 20 and 110 days respectively.

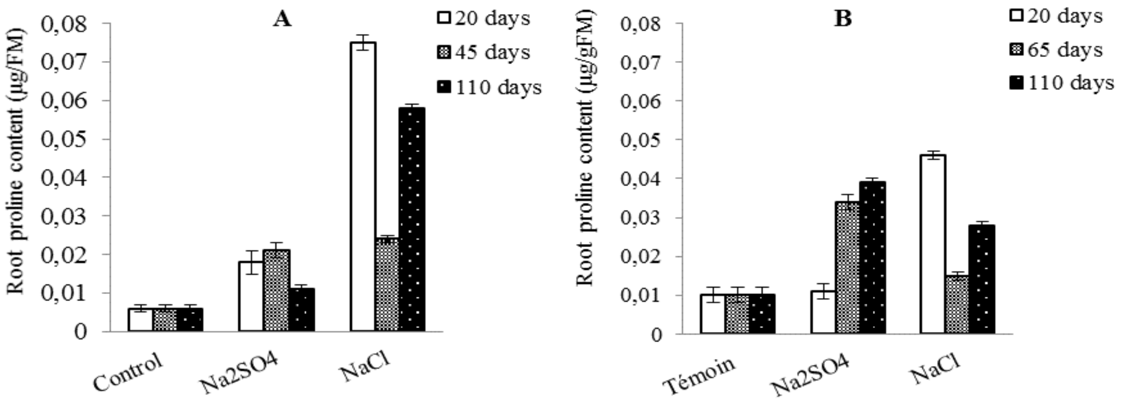

Figure 4. Root proline content (µg/g MF) of the tow tomato cultivars [Marmande (A) and Saint-Pierre (B)] under 20, 65 and 110 irrigation's days of control, NaCl and Na_2SO_4 with Na^+ concentration is 30,45 meq/L). The data represent mean values ± SD (n = 3).

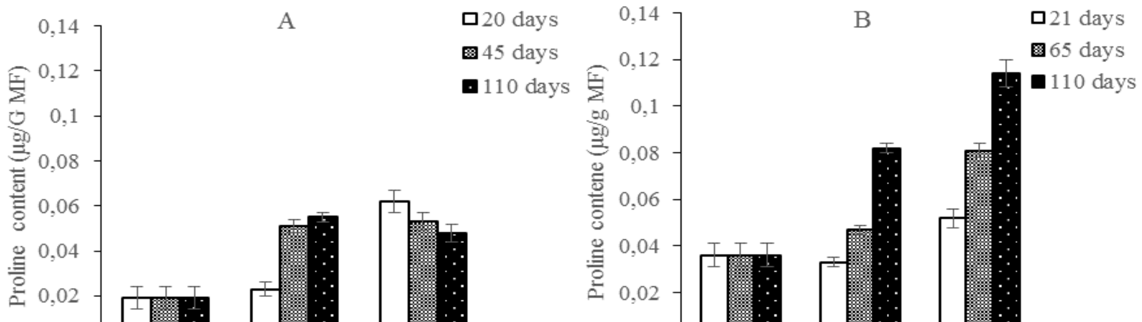

Figure 5. Proline content in the aerial part (µg/g MF) of the tow tomato cultivars [Marmande (A) and Saint-Pierre (B)] under 20, 65 and 110 irrigation's days of control, NaCl and Na_2SO_4 with Na^+ concentration is 30,45 meq/L). The data represent mean values ± SD (n = 3).

DISCUSSION

Salt stress had a negative influence on most of the parameters studied of S. Lycopersicum as has also been demonstrated in other studies [30], [31] and [32]. Our study suggests that this may be due to both osmotic and ion specific effects. According to [33] plants display a two-phase growth response to salinity. The first phase appears quickly and is due to osmotic stress caused by salt outside the plants.

The second phase takes the time to develop, and results from the toxic effect of salt inside the plant, as the ability of the cells to compartmentalize salt in the vacuole is exceeded. The present study indicates that S. Lycopersicum had entered the second growth response phase, as Na_2SO_4 inhibited growth relatively more than NaCl. The ion specific effect was only evident in the two highest salinity treatments, supporting the assumption that the greater growth reduction caused by Na_2SO_4 than NaCl was due to an inability of leaves to prevent salt from reaching toxic levels.

This translates into significant reductions in growth parameters such as plant height and dry matter and indicated that the cv. Marmande is more sensitive than the cv. St. Pierre toxic within the 110 days of treatment used here. These growth data were consistent with those reported in previous work [34]; inhibition of plant growth is one of the most sensitive parameters indicating that increased EC affecting the hydric potential in soil and plant. Moreover, the salt tolerance in plants is also related to their aptitude to maintain their chlorophyll level [35].

The chlorophyll response to salinity seems to depend on stress severity. Low salinities generally lead to an increase in chlorophyll levels whereas severe salinities often cause reduction [36]. In the present experiment, the cv. Saint-Pierre showed a good ability to tolerate elevated Na_2SO_4 concentrations that the cv. Marmande, while NaCl stress significantly reduced chlorophyll concentration. Under NaCl stress, decreases in photosynthetic pigments are related with high pH that increases the activity of Chl-degrading enzyme: chlorophyllases [37]. Other possibility for reduction in pigments may a reduction in biosynthesis of d-aminolevulinic acid (a precursor of chlorophyll) [38].

As regards the accumulation of proline in salt stress, the present study also showed that exposure of cv. Marmande to sodium stress increased proline content of root and in the aerial part. Proline provides tolerance against different abiotic stresses including NaCl stress by increasing endogenous level. It plays significant role in osmotic adjustment under salinity stress [40]; [41]; [42] and [39]. Such solutes at high concentrations are involved in maintaining turgor pressure and high concentrations of Na^+ and Cl^- in the vacuole. Besides acting as an osmolytes, Proline is known to contribute in stabilizing sub-cellular structures (membranes and proteins).

CONCLUSION

This work shows that salt stress exerts a depressive effect on both the morphological and physiological parameters studied in both of two tomato genotypes, Marmande and Saint-Pierre. However, the degree of affection depends on the duration, the type of salt tested and the variety studied.

Based on the results obtained, it is assumed that cv. Saint-Pierre is more tolerant to salinity than the cv. Marmande due to a higher ability of maintaining the root function for the uptake and supply water to shoot under salinity conditions. Thus, it can be retained that the measured physiological parameters are important indicators of the tolerance of this species to salinity. Further research is needed to explore the salt sensitivity of tomato to Na^+, Cl^- and SO_4^{-2} separately and ‘tissue tolerance’ needs to be assessed across many genotypes prior to generalizations being made on salt tolerance mechanisms in tomato.

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