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[1]Department of Agronomy, Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogota, Colombia
[2]Division Urban Plant Ecophysiology, Faculty of Agriculture and Horticulture, Humboldt-Universität zu Berlin, Berlin, Germany
[3]Institute of Food Chemistry, Universität Hamburg, Hamburg, Germany

Salinity effects on proline accumulation and total antioxidant activity in leaves of the cape gooseberry (*Physalis peruviana* L.)

Diego Miranda[1*], Gerhard Fischer[1], Inga Mewis[2], Sascha Rohn[3], Christian Ulrichs[2]

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Summary

In Colombia, the cape gooseberry is grown in salt-affected areas. The effect of increasing sodium chloride (0, 60 and 120 mM NaCl) stress was investigated on the growth, proline content and total antioxidant activity (TAA) in leaves of cape gooseberry plants kept in 2 L pots and grown under greenhouse conditions. Plant leaves were analyzed 45, 55, 65 and 75 days after transplanting (DAT). The vegetative growth (measured as total plant and organ dry weight [DW], leaf number and leaf area, as well as plant height) was significantly lower at 120 mM NaCl, as compared to the control and 60 mM treated plants. At 60 mM NaCl, all determined leaf parameters and total plant DW were markedly reduced, as compared to non-salinized plants. The leaf proline content increased significantly during the evaluation period and tended to be higher with increasing NaCl concentrations, but without statistical differences. The TAA, measured as µM Fremy's salt per g^{-1} FW, increased constantly during the evaluation period and, from day 55, was significantly higher than in leaves of non-salinized plants. After 75 DAT of salt stress, the TAA and proline content did not differ between the 60 and 120 mM NaCl treatments. For all sampling dates, the 120 mM salt concentration significantly enhanced the free radical scavenging activity, as compared to the control and the 60 mM NaCl treatment. All treatments showed a nearly 12% increase in the radical scavenging activity during the experiment's duration. In conclusion, cape gooseberry plants protect themselves from salinity (120 mM NaCl) stress by increasing leaf antioxidant activity, as confirmed by the higher radical scavenging activity, but not significantly with proline synthesis.

Introduction

Salt stress poses a major environmental threat to agriculture, limiting the productivity of crop plants (Türkan and Demiral, 2009). This is due to the fact that salinity affects most aspects of plant physiology, growth and development (Borsani et al., 2003). Most plants, especially glycophytes, are very sensitive to high Na^+ concentrations (Kaya et al., 2007). In addition, salinity has a stronger effect on fruit crops than on field, forage or vegetable crops (Mengel et al., 2001). High concentrations of Na^+ can lead to cell death by disturbing the intracellular homeostasis, which leads to membrane dysfunction, attenuation of metabolic activity, and secondary effects that cause growth inhibition (Ashraf and McNeilly, 2004).

However, some plants are able to tolerate saline and drought stress by reducing the cellular osmotic potential with a net increase in solute accumulation in a process called osmotic adjustment (Munns, 2002). Among the metabolic responses to salt stress, the synthesis of compatible osmolytes, as well as non-toxic organic compounds, is responsible for the osmotic equilibrium between the cytoplasm and different cell compartments. Thus, many plants synthesize amino acids and amides (proline, alanine, glutamine, asparagine), quarternary ammonium bases (betaines), as well as various sugars, polyols (e.g., mannitol, sorbitol), and cyclites (pinitol) as a non-specific reaction to stress from excess salt, drought or frost (Larcher 2003). These organic compounds are thought to mediate osmotic adjustment, protecting the subcellular structures and preventing oxidative damage with their free radical scavenging capacity (Smirnoff, 1993). It is generally accepted that, under conditions of extreme salinity, proline accumulation serves as a defense against osmotic challenge by acting as a compatible solute and appears to be the preferred organic osmoticum in many plants (Manchanda and Garg, 2008). A characteristic pattern of stress metabolites is associated with different plant groups. For instance, soluble carbohydrates are found in Poaceae and proline in Brassicaceae (Larcher, 2003).

Ionic and osmotic effects (Tarakcioglu and Inal, 2002) and oxidative stress (Borsani et al., 2003) induced by salinity and sodicity can reduce plant growth and alter ionic ratios. Oxidative stress is characterized by the overproduction of active oxygen species, predominantly represented by superoxide radicals (O_2^-), singlet oxygen ($^1O_2$), hydrogen peroxide (H_2O_2) and hydroxyl radical ($OH^{\cdot}$), which cause tissue injury (Foyer et al., 1994). The degree of oxidative cellular damage in plants subjected to abiotic stress is controlled by the capacity of the antioxidative systems (Acar et al., 2001) to overcome salt-mediated oxidative stress. Plants detoxify reactive oxygen species (ROS) by up-regulating antioxidative enzymes, such as superoxide-dismutase, ascorbate peroxidase, catalase, glutathione reductase and glutathione peroxidase (Türkan and Demiral, 2009).

Pre-harvest factors may affect the content and stability of phytochemicals in plants and influence their antioxidant capacity (Wang, 2006). De Pascale et al. (2003) reported that it is possible to improve the contents of carotenoids and ascorbic acid and, thereby, the antioxidant activity in tomatoes by irrigating with saline water.

In Colombia, the cape gooseberry (*Physalis peruviana* L., Solanaceae) was grown on 743 ha in 2011 (Agronet, 2013) at sites ranging from 1800 to 2800 m above sea level. The cape gooseberry is not only an important source of vitamins (A and C) for Colombian highland farmers but also has become the second most important export fruit (Fischer et al., 2007). The cape gooseberry, like most horticultural crops, is glycophyte; generally believed to have little or no tolerance to salinity (Grattan and Grieve, 1999). If agricultural crops are to be used in potentially arable soils, a moderate degree of salt resistance may be useful to avoid yield reduction, especially in more arid regions (Larcher, 2003). In that vein, the mechanisms involved in the responses to salt stress must be understood in order to improve productivity under salinity stress conditions (Manchanda and Garg, 2008).

Nearly 600,000 ha of the agriculturally cultivated area in Colombia are affected by salinity (Casierra et al., 2000). Furthermore, cape gooseberry orchards on the Bogota Plateau are impaired by salinization. However, no attempts have been made to study proline accumulation and total antioxidant activity in cape gooseberry leaves

* Corresponding author

under these conditions. In order to verify the possibility of stress-induced proline synthesis and total antioxidant activity in cape gooseberry leaves, the effect of two increasing NaCl concentrations was studied under greenhouse conditions.

Materials and methods

Plant material and management

This experiment was carried out in the laboratories and glass greenhouse of the Urban Plant Ecophysiology Division at Humboldt University, Berlin, Germany, using the *P. peruviana* 'Colombia' ecotype. Seeds were germinated in blond peat substrate. The seedlings were transplanted in the greenhouse 20 days after sowing. Forty-five days after seeding, 340 seedlings were transplanted to black plastic pots (2 L) containing perlite as a substrate, where they grew over a period of 75 days. The pots were placed in double rows on elevated greenhouse tables, with a plant spacing of 0.5 m between rows and 0.35 m between plants within a row, resulting in a density of 5.7 plants per m^2. The climatic conditions in the greenhouse during the experiment were 24.9/20.4 °C mean air day/night temperature, 60.3% mean relative humidity and 484.1 µm m^{-2} s^{-1} mean photosynthetic active radiation, as indicated in Tab. 1. The day-length at the experiment's start (7 of April) was 13.3 light hours, which increased to 17.0 light hours when the greenhouse experiment ended (30 of June).

The nutrient solution for the adult cape gooseberry plants was prepared by dissolving 0.2 g Kristalon™ (19-6-20-3 + micronutrients) per liter of water. The electrical conductivity (EC) of the nutrient solution was 1.4 dS m^{-1}. The application was made with a Dosatron, which applied a 0.5% concentration with two applications per day, each 3 min in length.

Tab. 1: Ranges of mean day and night temperature, relative humidity and photosynthetic active radiation (PAR) (±SD) in the greenhouse at the four sampling periods of the experiment.

Days after transplanting (DAT)	Air temperature (°C)		Relative humidity (%)	PAR (µm m^{-2} s^{-1})
	Day	Night		
36-45	26.0 ± 4.5	20.9 ± 2.4	66 ± 3.6	535.8 ± 34.6
46-55	23.9 ± 4.8	19.5 ± 2.5	60 ± 5.7	502.7 ± 67.6
56-65	27.5 ± 5.2	22.3 ± 2.1	55 ± 2.0	500.4 ± 49.1
66-75	22.3 ± 4.4	19.1 ± 1.8	60 ± 3.9	397.6 ± 56.9

Sodium chloride treatments

Three treatments of 0, 60 and 120 mM NaCl were studied over 12 weeks. Each plot consisted of 68 plants, 340 plants in total. NaCl applications began 2 days after transplanting (DAT). The electrical conductivity (EC) of the irrigation water was measured twice a week using a conductivity meter (Hanna Instruments HI 9811, Ann Arbor, MI) and averaged at: 0.70 (non-saline, control), 6.36 (60 mM NaCl), and 12.53 (120 mM NaCl) dS m^{-1} over the course of the experiment. The pH was recorded using a portable pH meter (Testo 206, KKInstruments, Berlin) and averaged at: 7.9, 8.06, and 8.12, respectively. Samples of fully expanded leaves (six leaves per plant from three plants per repetition) were collected and shock frozen in liquid nitrogen before analysis.

Growth measurements

The plants were harvested at 75 DAT and separated into roots, stems and leaves. The plant material was dried at 70 ºC for 48 h to determine dry weight (DW). The leaf area was measured with a portable leaf area meter (Model LI-3000 A, LI-COR, Lincoln, NE).

Proline and total antioxidant activity determination

Samples of fully expanded leaves, obtained from the medium third of the plants and the medium third of the reproductive shoots, located after the first natural branch ramification, were collected at 45, 55, 65 and 75 DAT, between 9.30 and 10.30 am, and, then, shock frozen in liquid nitrogen. The proline content was determined for the 0, 60 and 120 mM NaCl treatments according to Bates et al. (1973). Purified proline (Sigma-Aldrich, Steinheim, Germany) was used as the standard. Approximately, 0.5 g of plant material was homogenized in 10 mL of 3% aqueous sulfosalicylic acid and the homogenate was filtered through a paper filter (Whatman # 2). Two mL of filtrate were reacted with 2 mL ninhydrin and 2 mL of glacial acetic acid in a test tube for 1 hour at 100 °C and the reaction was terminated in an ice bath. The reaction mixture was extracted with 4 mL toluene and mixed vigorously with a test tube stirrer for 15-20 sec. The chromophore containing toluene was aspirated from the aqueous phase, warmed to room temperature and the absorbance read at 520 nm, using toluene for a blank. The proline concentration was determined from a standard curve and calculated on a fresh weight basis as follows:

[(µg proline/mL × mL toluene) / 115.5 µg / µmoles]/[(g sample)/5] = µmoles proline/g of fresh weight material.

The total antioxidant activity (TAA) was determined using electron spin resonance spectroscopy (ESR) (Rohn and Kroh, 2005), in accordance with the following procedure: 1-2 leaves of the middle part of the plant (using three plants per repetition) that were frozen in liquid nitrogen were ground with a mortar and pestle. 500 mg of sample and 5 mL of methanol (70%) were weighed in centrifugation tubes and vigorously shaken for 2 min. Following centrifugation at 2000 rpm for 10 min (Eppendorf centrifuge HermLe Z320, Hamburg, Germany), the undiluted supernatant was used for ESR spectroscopy with Fremy's Salt (potassium nitrodisulphonate) as a stabilized radical. The measurements were taken according to Roesch et al. (2003). 100 µL of Fremy's salt (1 mM) + 100 µL of sample were measured for 20 min against a blind (100 µL buffer) and Fremy's radical ESR spectrum was obtained after 20 min, when the reaction was complete. Signal intensity was obtained by integration (phenolic compounds or further aromatic compounds) and antioxidant capacity (expressed as moles of Fremy's Salt reduced by one mole antioxidant) was calculated by comparison with a control reaction using methanol. The results are expressed as units of µM Fremy's Salt per g fresh weight (FW).

Free radical scavenging activity

The quantitative measurement of the radical scavenging properties of the sample extracts of cape gooseberry leaves was carried out in a universal bottle. The reaction mixture contained 50 µL of test samples (or 80% MeOH as a blank) and 5 mL of a 0.04% (w/v) solution of Fremy's Salt in methanol. Different known antioxidants, vitamin E (alpha tocopherol) and butylatedhydroxytoluene (BHT, Sigma) were used for comparison or as a positive control. The discoloration was measured at 517 nm after incubation for 20 min. The measurements were carried out at least in triplicate. Fremy's Salt radical's concentration was calculated using the following equation:

Fremy's Salt scavenging effect (%) = Ao – A1 / Ao x 100,

where Ao was the absorbance of the control and A1 was the absorbance in the presence of the sample extract of cape gooseberry leaves according to Oktay et al. (2003).

Statistical analysis

The experiment involved a completely randomized design with three replicates. The data were statistically analyzed by ANOVA using the SAS software (SAS 9.1); significant differences between treatments were calculated by Tukey's test ($p \leq 0.05$).

Results

Plant growth

Salinization of the irrigation water significantly affected all the measured vegetative growth parameters of the cape gooseberry (Fig. 1 and 2). At 60 mM NaCl, all the leaf parameters (DW, total area and number per plant) were significantly ($p \leq 0.05$) reduced. The considerably lowered leaf number ($p \leq 0.05$) with increasing salinity induced a significantly greater unit leaf area ($p \leq 0.05$), 40.8 cm^2 at 60 mM NaCl. At the intermediate salinity, only root and stem DW and plant height remained unchanged ($p > 0.05$) by the salinity. The higher growth reductions at 120 mM NaCl were found in root DW (46.8%), plant DW (34.3%) and leaf DW (33.9%), while plant height (99.0 cm) was only slightly, but significantly ($p \leq 0.05$), affected, i.e. 8.3% lower than the control plants.

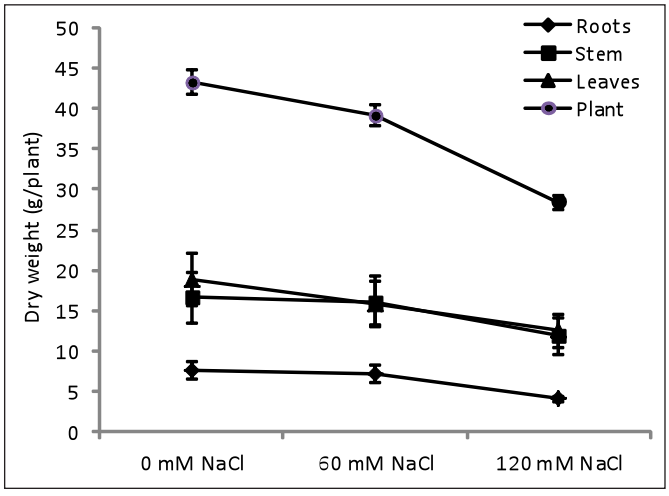

Fig. 1: Effect of NaCl on root, stem, leaf and plant dry weight of cape gooseberry at 75 DAT. Bars indicate ±SE.

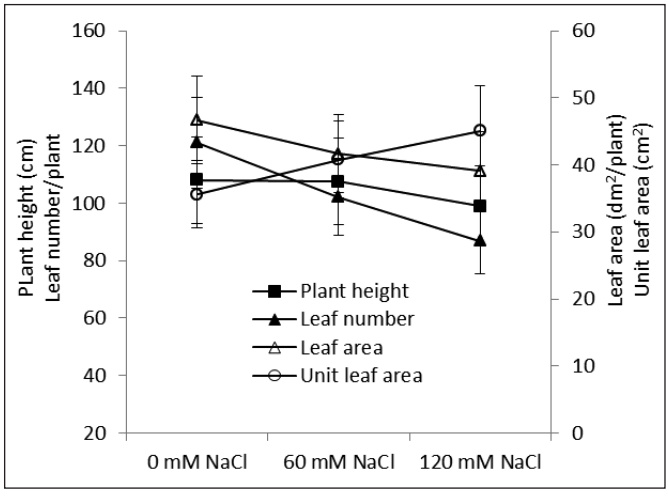

Fig. 2: Effect of NaCl on plant height, leaf number, leaf area and unit leaf area of cape gooseberry at 75 DAT. Bars indicate ±SE.

Proline content

The leaf proline content showed an overall increase during the time of salinity exposition, with no clear tendency from 45 to 65 DAT, but, at 75 DAT, the content was significantly higher ($p \leq 0.05$) than at 65 DAT (Tab. 2). The salt treated plants tended to have higher proline concentrations than the control plants, but without statistical differences ($p > 0.05$). After 75 DAT, both salinity levels exhibited the highest proline contents, which were nearly identical (about 0.8 μM g^{-1} FW).

Tab. 2: Effect of NaCl salinity on the proline content (μM g^{-1} FW) in leaf tissue of cape gooseberry plants between 45 and 75 DAT.

NaCl (mM)	Days after transplanting (DAT)			
	45	55	65	75
0	0.51 aA	0.18 aC	0.23 aB	0.46 aA
60	0.64 aB	0.36 aC	0.56 aB	0.80 aA
120	0.54 aC	0.47 aC	0.68 aB	0.82 aA

Means in columns followed by the same letters are not significantly different according to Tukey's test ($p \leq 0.05$). Lowercase letters are for comparing between NaCl concentrations and uppercase letters between sampling days.

Total antioxidant activity

In this methodology, the scavenging of the synthetic stable radical Fremy's salt was followed. The present study revealed that 3.11, 3.25 and 3.86 μM Fremy's salt were scavenged on average by 1 μM antioxidant present in the foliar tissue of plants subjected to stress with 0, 60 and 120 mM NaCl, respectively. The total antioxidant activity (TAA) increased constantly during the period of exposure to saline stress. The maximum TAA was 4.18 μM Fremy's salt per g FW for plants exposed to 120 mM NaCl at 75 DAT, as compared to the minimum of 2.72 μM in the control plants on the first sampling date (Tab. 3). The 120 mM NaCl treatment resulted in a significantly ($p \leq 0.05$) higher TAA than the lower salt concentrations at days 45, 55 and 65.

Tab. 3: Effect of NaCl salinity on the total antioxidant activity (μM Fremy's salt/g FW) in leaf tissue of cape gooseberry plants between 45 and 75 DAT.

NaCl (mM)	Days after transplanting (DAT)			
	45	55	65	75
0	2.72 bC	3.16 bB	3.15 bB	3.46 bA
60	2.74 bC	3.17 bB	3.29 bB	3.78 abA
120	3.28 aC	3.84 aB	4.18 aA	4.14 aA

Means in columns followed by the same letters are not significantly different according to Tukey's test ($p \leq 0.05$). Lowercase letters are for comparing between NaCl concentrations and uppercase letters between sampling days.

Free radical scavenging activity

In all treatments, the free radical scavenging activities of the leaf plant extract increased until 55 DAT (Tab. 4), but, after that date, no clear tendency was noticeable. It was obvious that, at all sampling dates, the 120 mM salt concentration significantly enhanced ($p \leq 0.05$) the free radical scavenging activity, as compared to the control and 60 mM treated plants. In general, all the treatments showed a nearly 12% increase in scavenging activity over the course of the experiment.

Tab. 4: Effect of NaCl salinity on the free radical scavenging activity (%) by antioxidants in the extract of foliar tissue of cape gooseberry plants between 45 and 75 DAT.

NaCl (mM)	Days after transplanting (DAT)				Mean
	45	55	65	75	
0	50.7 b	62.3 b	58.7 b	61.6 b	58.3 b
60	46.8 b	55.6 c	55.9 b	60.0 b	54.6 b
120	57.6 a	73.8 a	60.0 a	68.4 a	65.0 a

Means in the same column followed by the same letters are not significantly different according to Tukey's test ($p \leq 0.05$).

Discussion

Plant growth

A plant's metabolism is affected in several ways by salt stress and, therefore, growth is reduced (Manchanda and Garg, 2008). A lower biomass production induced by salinity is common in most plants (Larcher, 2003) and total plant and organ DW and leaf number have been reported to be the most commonly used criteria in order to characterize salinity stress and tolerance (Levitt, 1980). If the rooting medium has an excess of salts, the osmotic pressure will be increased, thereby decreasing the plant's ability to take up water and slowing growth (Manchanda and Garg, 2008).

A common result of a decrease in the osmotic potential of a plant's cells under salt stress is a malfunction in the enzymatic system of the plant, which reduces CO_2 fixation and N assimilation and, consequently, causes a shortage in plant structural material production (Aslam et al., 1984). Also, the impaired photosynthesis under severe salinity stress, as a result of smaller leaves and a higher stored carbohydrate usage as compared to plants in a non-salt stressed environment, reduces biomass production (Mengel et al., 2001). Furthermore, carbon dioxide uptake can be limited by the inadequate photosynthesis caused by stomatal closure, which further reduces the growth rate (Zhu, 2001).

Root growth was extremely affected, expressed in the root biomass reduction (Fig. 1) and low number of secondary roots (data not shown), at 120 mM NaCl, in agreement with Neumann (1995), who found that high salt concentrations inhibit the initiation of new roots and radical growth in the strawberry. Azooz et al. (2004) described the reduced hormone delivery from root to leaves as the principal effect of salinity on the inhibition of crop growth.

Growing leaves are particularly sensitive to water stress (induced by salinity) because foliar tissue has very high transpiration rates (Jones, 1992). Furthermore, leaf expansion responds rapidly to changes in the water potential gradient between the substrate solution and the roots (Cramer and Bowman, 1991), which is markedly influenced under salinity conditions. In our study, the salinity conditions reduced the number of leaves (Fig. 2) due to the lower plant growth rates (Miranda et al., 2010a; Munns and Termaat, 2008) and shorter leaf duration (Manchanda and Garg, 2008) resulting from the premature leaf fall of adult and necrotic leaves, as compared to non-salinized plants.

Leaf growth variations under saline stress have been found to be correlated with foliar Na^+ content in *Triticum* (Schachtmann and Munns, 1992) and the soybean (Doğan, 2011). Similarly, in the cape gooseberry, leaf Na^+ concentrations increased with greater NaCl salinity (Miranda et al., 2010b). Abscission of old leaves is common under salt stress (Munns and Termaat, 1986) because the principal site of Na^+ toxicity, in a great number of plant species, is the leaf blade, where Na^+ accumulates after being placed in the transpiration stream (Munns and Termaat, 2008). Our results indicated that stem elongation was less affected than total leaf area (Fig. 2) by salinity stress, which coincides with the findings of Chartzoulakis and Klapaki (2000) in pepper plants.

The impact of increasing salt concentrations on cape gooseberry growth shows that this plant begins to manifest a reduction in leaf DW starting at just 60 mM, where plant height, stem and root DM are not yet affected, which indicates a resistance to relatively high salt concentrations. Miranda et al. (2010a) classified the cape gooseberry as a moderate salt tolerant crop, wherein 30 mM NaCl stimulated growth indices such as crop growth rate, relative growth rate, unit leaf rate and leaf area, as compared to non-salinized plants.

Proline content

The accumulation of compatible solutes, such as proline, a preferred organic osmoticum in many plant species, is one of the key mechanisms used by higher plants to respond to salt-stress conditions (Larcher, 2003). Compatible solutes are used for osmotic adjustment and for maintaining the functional state of macromolecules, probably by scavenging ROS (Xiong and Zhu, 2002). Proline accumulates more in the leaves of plants that are more tolerant to salinity stress than in salt sensitive plants (Türkan and Demiral, 2009). Considering that the cape gooseberry is classified as a moderately salt tolerant plant (Miranda et al., 2010a), this behavior could not be attributed to the accumulation of proline in the present study because the proline content of the two NaCl treatments only tended to be higher than that of the control plants starting at 55 DAT, because there were no statistical differences.

The increase in proline content during the vegetative growth phase in the NaCl treatments suggests that the plants tried to stabilize their protection mechanism (Szabados and Savouré, 2009) with increased salt accumulation in the leaves. Also, in wheat plants, a salt stress provoked an increase in leaf proline content, but higher accumulation was recorded at the vegetative and boot stages than at the reproductive stage (Ashram et al., 2007). An increase in leaf proline content also depends on environmental stress factors, such as high light intensity, extreme temperatures and low relative humidity, as Claussen (2007) reported in tomatoes, but this behavior could not be confirmed in our experiment. Furthermore, at the lowest medium temperature (22.3 °C) and PAR, the highest proline concentration was measured (Tab. 1).

Total antioxidant activity

Pastori et al. (2000) stated that many stress situations can generate increased foliar antioxidant activity. Higher levels of antioxidants, whether constitutive or induced, have been reported to induce a greater resistance to different types of environmental stress conditions (Young and Jung, 1999). In the present study, the TAA increase with increasing NaCl concentrations agrees with the results of De Pascale et al. (2003), who observed that salinity increased lipophilic and hydrophilic antioxidant activities in tomato fruits. A correlation between antioxidant capacity and salinity tolerance was reported by Türkam and Demiral (2009) and other authors cited therein, for several plant species (cotton, rice, wheat, pea, sesame, among others) and fruit species, such as citrus fruits by Gueta-Dahan et al. (1997). According to Wang et al. (2009), who found that tolerant species generally withstand salt- or drought-induced oxidative stress with improved antioxidant enzyme activities, the cape gooseberry can be classified as a moderate salt tolerant species due to its increasing TAAs with increasing NaCl concentrations. The moderate salt tolerance of this species has been previously reported in terms of germination (Miranda et al., 2010c), growth indices

(MIRANDA et al., 2010a) and leaf Ca^{2+}, K^+ and Na^+ accumulation (MIRANDA et al., 2010b).

RAMACHANDRA et al. (2004) demonstrated that the antioxidant response level depends on stress duration and intensity, plant species, development, and metabolic state. Results for the soybean have shown that the activity of antioxidant enzymes under salinity depends on kind, age and type of plant organs, as well as on the salinity level (DOĞAN, 2011).

Adult *Physalis* plants produced more TAA than young plants (Tab. 4), corresponding to the report of BUCHANAN et al. (2002) that states that resistance or sensitivity to the stress depends on the developmental age of the plant. Consequently, reactive oxygen species (ROS) are continuously produced in plants.

Fruit crop maturity significantly influences antioxidant capacity (WANG, 2006). However, whereas the TAA increases with fruit maturity; in leaves of the red raspberry, blackberry and strawberry, the opposite was seen, i.e. the TAA decreased with leaf age (WANG and LIN, 2000).

Given the high content of antioxidants in cape gooseberry fruits (RAMADAN, 2011), its cultivation in moderately saline soils or with saline water irrigation may also increase antioxidant activity in fruits, as found in tomatoes by DE PASCALE et al. (2003), in the Cois HC01 hybrid, when watering with saline water of up to 4.4 dS m^{-1} EC, and in the Leovovil and Cervil tomato varieties by GAUTIER et al. (2010), using 7.6 dS m^{-1} water, and, in turn, also increase the content of total soluble solids (DE PASCALE et al. (2003) in fruit organs.

Similar to the TAA, the free radical scavenging activity also increased with increasing NaCl concentrations. Antioxidants use scavenging free radicals as a mechanism for inhibiting lipid peroxidation. The fact that ROS-induced lipid peroxidation of membranes reflects stress-induced damage at the cellular level is well accepted (JAIN et al., 2001).

The lack of difference found between the non-salinized plants and the plants treated with 60 mM NaCl for free radical scavenging activity confirmed that this salt concentration was probably not high enough to produce oxidative stress conditions in the plants. On the other hand, in agreement with DUH and YEN (1997), the appropriate concentration of crude extracts of non-stressed cape gooseberry leaves may act as a free radical scavenger and may react with free radicals to convert them into more stable products and terminate radical chain reactions. WU et al. (2006) found, in *P. peruviana* leaf extracts, a strong superoxide anion scavenging, antioxidant, and anti-inflammatory activity, underlining the role of this fruit species in folk medicine. Also, MIRANDA et al. (2010b) found no change in leaf Ca^{2+} concentrations in cape gooseberry plants with increasing salinity levels, which could indicate the effect of Ca^{2+} in alleviating salinity stress symptoms due to its important role in stability and integrity of biological membranes and tissues (MENGEL et al., 2001).

In addition, it could be possible that the irrigation and nutrition management in the present study did provide normal conditions, where the ROS production produced by the low saline stress was efficiently scavenged by the plants' antioxidant systems. WANG (2006) reported that management practices such as fertilization and organic farming, as well as climatic and soil conditions (light, temperature, CO_2) influence a crop's antioxidant content and activity. Thus, in the present study, from days 56 to 65, the mean temperature increase was high, from 19.6 to 31.2 ºC, and significantly correlated with the TAA increase ($r=0.68$; $p<0.05$). Also, WANG and ZHENG (2001) recorded a higher scavenging capacity of strawberry plants against active oxygen species when day/night temperatures were high (30/22 ºC and 25/22 ºC), as compared to low temperatures (18/12 ºC). ULRICHS et al. (2008) found a higher antioxidant activity, expressed as lycopene, β-carotene and total phenolic contents, in tomatoes growing in soils inoculated with vesicular arbuscular mycorrhizal fungi, as compared to conventionally grown tomatoes.

Free radical scavenging activity

Only the plants that received the highest sodium chloride concentration considerably increased their free radical scavenging activity, as compared to the other treatments. As discussed above, a number of adverse abiotic factors can disturb equilibrium between ROS production and scavenging activity; among these factors, PRASAD et al. (1994) and TSUGANE et al. (1999) mentioned high luminosity, temperature and salinity. According to WANG et al. (2009), the elevated scavenging capacity of cape gooseberries treated with 120 mM NaCl may reflect an increased ROS scavenging capacity, in order to provide protection from oxidative damage to lipids of the plasma membrane due to salt and drought stresses.

The percentage increase of free radical scavenging activity was almost the same between the non- and salinized plants, i.e. an increase of c. 12% in the leaves of all treatments. This indicates that the increased scavenging activity over the course of the experiment was determined only by the plant's age, independent of the salt concentrations applied. The degrees of scavenging capacity for different active oxygen species can vary with plant species or cultivar. In fruits of blackberries, blueberries, cranberries, raspberries and strawberries, the scavenging capacity for superoxide radicals ranged from 40.8% to 72.0%, for hydrogen peroxide from 50.7% to 73.9%, for hydroxyl radicals from 52.4% to 77.3%, and for singlet oxygen from 6.3% to 17.4% (WANG, 2006). These values are in accordance with the determined scavenging capacity of non-salinized cape gooseberry leaves (58.3%).

The results of the present study suggest, in agreement with DUH and YEN (1997), that the appropriate concentration of crude extracts of cape gooseberry leaves may act as a free radical scavenger and may react with free radicals to convert them to more stable products and terminate radical chain reactions.

Conclusions

The increasing NaCl concentrations, from 0 to 120 mM NaCl, affected leaf growth the most and, consequently, the biomass production of the plants.

A NaCl concentration of 60 mM did not affect the root and stem growth, which indicates a resistance to relatively high salt concentrations, which are too high for many other plants; so, this crop can be grown in many areas with ECs as high as 3 to 6 dS m^{-1} in the soil solution.

The enhanced total antioxidant activity with the higher sodium chloride concentrations confirms that *P. peruviana* uses antioxidant production in order to alleviate salt stress.

The plants exposed to the highest sodium chloride concentration considerably increased the free radical scavenging activity.

For further studies on the cape gooseberry under salt conditions, it is recommended that different types of antioxidant enzymes and low-molecular antioxidants be determined in order to recognize the stress defense mechanism for use in plant selection and breeding purposes.

Beyond this preliminary experiment on proline and TAA activity in cape gooseberry leaves in relation to salt stress, their contents in fruits should also be determined because other species have shown increased antioxidant activities in fruits grown under these stress conditions.

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Address of the authors:

Prof. Dr. Diego Miranda, Department of Agronomy, Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogota, Colombia.

E-mail: dmirandal@unal.edu.co

Prof. Dr. Gerhard Fischer, Department of Agronomy, Faculty of Agricultural Sciences, Universidad Nacional de Colombia, Bogota, Colombia

Sascha Rohn, Institute of Food Chemistry, Universität Hamburg, Hamburg, Germany

Prof. Dr. Dr. Christian Ulrichs and Dr. Inga Mewis, Division Urban Plant Ecophysiology, Faculty of Agriculture and Horticulture, Humboldt-Universität zu Berlin, Berlin, Germany