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Effects of silicon on antioxidant enzymes, CO_2, proline and biological activity of *in vitro*-grown cape gooseberry under salinity stress

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Abstract

Cape gooseberry (*Physalis peruviana* L.) cannot tolerate high levels of salinity. Salt stress is one of the most damaging abiotic stresses that affects plant development. However, there are some evidences that silicon (Si) can act as a mitigating agent of environmental stresses. Towards understanding the stress using tissue culture, we investigated the effect of *in vitro* NaCl-induced salt stress in cape gooseberry and the potential mitigating effect provided by Si. Nodal segments were inoculated in MS medium with different combinations of NaCl concentrations (0.5 and 1.0% NaCl) and silicic acid (0.0, 0.5, and 1.0 g L^{-1}). The experimental design was completely randomized, in a factorial design with a control treatment [(2x3) + 1], totalizing 7 treatments. The control treatment consisted of an additional treatment without the addition of any of the factors. Isoenzyme activity, proline content, CO_2 level and biological activity were assessed after 30 d. We found an increase in all antioxidant enzymes (SOD, CAT and POD) activities when salt stress was imposed, indicating that the plant has an efficient system to protect itself against stress. Among these enzymes, Si played a mitigating role only in POD activity. In relation to other evaluated enzymes, NaCl enhanced the EST and MDH activities, followed by a subsequent decrease in MDH. Si showed different performances according to the concentration of NaCl. Electrophoretic analysis represented by bands illustrated these behaviors. Proline content increased as salinity increased, and Si effect was observed by increasing the levels of this amino acid. High CO_2 level was found at the concentration of 1.0% NaCl, and Si contributed to decrease this variable to normal levels. The reduction in the respiratory rates may contribute to the allocation of carbon to other chemical reactions such as the synthesis of new tissues. Salt stress also increased the biological activity of leaves and there was no effect of Si for this variable. We conclude that salt stress causes a great damage to *in vitro*-grown cape gooseberry plants and the addition of 1.0 g L^{-1} Si can ameliorate that damage for some characteristics. However, more studies are necessary since the physiological changes played by Si have been poorly understood for dicots.

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Keywords: abiotic stress, beneficial element, biospeckle laser, isoenzymes, respiration, soluble osmolytes.
Abbreviations: NaCl_sodium chloride, Si_silicon, MS_Murashige and Skoog, SOD_superoxide dismutase, CAT_catalase, POD_peroxidase, EST_esterase, MDH_malate dehydrogenase.

Introduction

Physalis peruviana L., popularly known as Cape gooseberry, is a species known for its nutritional properties and exuberant nature of its fruits. It is rich in vitamins A, B, and C and is high in minerals and bioactive compounds such as physalins (Puente et al., 2011). Cape gooseberry cultivation is still recent in Brazil, but it is expanding. The small commercial exploitation in Brazil is likely caused by the seasonal nature of its production, the lack of a Brazilian tendency to consume small fruits, and the scarcity of research on cape gooseberry cultivation that is available to farmers (Muniz et al., 2012). Conversely, the production and exploitation of cape gooseberry have already been established in other countries. Colombia, for instance, is the world's largest producer of cape gooseberry, with approximately 700 ha cultivated in 2011 (Miranda et al., 2014) in regions at 1800-2800 m above sea level. It is the second most exported fruit in Colombia that has 600,000 ha of agriculturally cultivated land affected by salinity (Posada et al., 2000; Miranda et al., 2014).

Cape gooseberry, like most horticultural plants, is considered to be a glycophyte, namely, a plant that does not tolerate high levels of salinity. Salt stress is considered a major environmental threat to agriculture because it affects many aspects of plant physiology (Hashemi et al., 2010). Most plants, especially glycophytes, are very sensitive to high concentrations of Na^+ that can lead to cell death caused by changes in intracellular homeostasis that result in dysfunctions in the membrane, a reduction in metabolic activity, and side effects that cause growth inhibition (Miranda et al., 2014). The morphological and physiological responses of plants to salinity vary according to genotype, development stage, and the intensity and duration of stress. Some plant mechanisms that aim to maintain growth in adverse environments are the production of compatible solutes such as proline, changes in respiratory rate and metabolism, and leaf anatomy modifications (Parida and Das, 2005). Currently, researchers often use *in vitro* systems to

study the effects of abiotic stress on plants. These types of experiments performed under artificial conditions are considered fully viable since they simulate the field environments where plants are exposed to adverse conditions (Clayes et al., 2014). Furthermore, these systems offer some advantages such as the control of stress level and onset, low variability in treatment and growth conditions, and the ability to cultivate a large number of plants in a small space (Lawlor, 2013).

Silicon (Si) is one of the elements considered beneficial to plants as its presence can improve some features, while its absence does not significantly affect the life cycle of most plants (Vasanthi et al., 2014). In addition to encouraging and promoting plant growth, Si also alleviates many abiotic stresses including salt stress (Shakoor, 2014). Studies have shown that Si can increase tolerance to salinity in some important crops and contributes on regulation of the biosynthesis of a number of soluble compounds (Zhu and Gong, 2014).

In this context, the main objective of this study was two-fold: (1) to investigate the effect of NaCl-induced salt stress on cape gooseberry grown *in vitro* and (2) to evaluate whether there is some beneficial effect of Si on stressed plants.

Results

Isoenzyme assessments

SOD activity increased after plants were exposed to NaCl treatments as shown by comparison with the control (Fig. 1) and by analysis of the electrophoretic pattern shown in Fig. 2A. Data also showed that the addition of 1.0 g L^{-1} Si to the culture medium contributed to declining SOD activity as did treatment with 1.0% NaCl plus 0.5 g L^{-1} Si.

Meanwhile, CAT activity did not depend on the relationship between factors, i.e. there was no interaction between NaCl and Si (Fig. 1). Analysis of NaCl showed that enhanced salinity caused increased CAT activity. The electrophoretic pattern of CAT, shown in Fig. 2B, indicates that when plants were subjected to salt stress (0.5% NaCl) there was a significant increase in the expression of this enzyme.

For POD, activity increased to 67% when salinity increased from 0.5 to 1.0% (Fig. 1). The evaluation of POD in the present study confirmed the hypothesis that reductions in CAT activity were likely caused by increased antioxidant activity of other enzymes. Indeed, we also verified clear increases in POD expression when CAT showed reduced expression (Fig. 2C). Salt stress significantly decreased POD activity; however, added Si improved the POD activity under salt stress.

For EST, enhanced activity was apparent under all stress conditions. At 1.0% NaCl, we observed a mitigating effect of Si when added to the medium at concentrations of 0.5 and 1.0 g L^{-1} (Fig. 1). High expression of EST was observed at the highest concentration of NaCl in the absence of Si as well as at 0.5% NaCl with 0.5 g L^{-1} Si (Fig. 3A).

For MDH, we observed increased activity under salt stress that was mitigated by the addition of 0.5 g L^{-1} Si to the 1.0% NaCl treatment (Fig. 1). According to Fig. 3B, MDH expression was increased more in treatments with higher concentrations of NaCl. Nevertheless, we observed that when Si was added, the expression of this enzyme decreased.

Proline content

For proline content, we observed significant effects caused by the interaction between NaCl and Si. According to the analysis of the NaCl level in each Si concentration, we verified that the content of this amino acid increased as salinity increased to 1.0% (Fig. 4). We also found that at 1.0% NaCl the proline content remained high even in the presence of Si (Fig. 4).

CO_2 level

In this study, the concentration of O_2 released by plants was quantified, but there was no statistical difference between treatments with an average concentration of 5.84% g^{-1} h^{-1} O_2 measured throughout (data not shown). Conversely, the CO_2 concentration increased as the salinity increased (Fig. 4). Compared to the control, the presence of Si in the medium resulted in a reduction in CO_2 content.

Biological activity

Biological activity, measured by leaf illumination using the biospeckle laser technique, was significant for the isolated factors only (Fig. 5) indicating a non-significant interaction between NaCl and Si. Increased activity was also observed with increasing salinity of the medium (Fig. 4). In comparison with the control treatment, we noted that both salinity levels contributed to this increased activity, with the activity increasing by 75% at the highest concentration (Fig. 4). In this study, the action of Si had no significant effect on interaction with NaCl. However, when we observed effects in isolation we found that Si was involved in reducing the biological activity. Fig. 6 shows illustrative outcomes for all treatments as maps of activity obtained by the SD using pseudo-colors ranging from blue to red, where blue means lower activity and red means higher activity.

Discussion

The salt stress conditions imposed on plants in this study led to the overproduction of reactive oxygen species (ROS) that cause injury to cell structures, often affecting them irreversibly (Barbosa et al., 2014). However, the plants have an efficient antioxidant system that regulates the activity of many enzymes such as SOD and CAT. SOD and CAT are considered to be antioxidant enzymes capable of eliminating the ROSs from the cell (Zhu and Gong, 2014). SOD is an enzyme with high O_2^- removal power; it catalyzes the dismutation reaction of superoxide anion resulting in O_2 and H_2O_2 (Scandalios, 1993). The removal of the produced H_2O_2 (highly toxic) by CAT is an essential action for the cell to avoid inhibition of other enzymes such as those controlling the Calvin cycle (Pan et al., 2006). Thus, the importance of these two enzymes in the control of the adverse reactions that occur in the cell is clear. In this study, SOD fulfilled its role as its activity was significantly enhanced when plants were under salt stress. However, added Si did not increase SOD activity as we expected. This can be explained by the fact that the effect of Si added to the growth media is time- and plant species-dependent (Zhu & Gong, 2014). In a study on barley, Liang et al. (2003) reported that the Si effect is time-dependent and became stronger as the experiments continued. Therefore, SOD evaluation following Si treatment should, for example, be performed at different days.

The high expression of CAT in this study can be explained by its role in defense against cellular H_2O_2 formation (Barbosa et al., 2014). CAT is another important enzyme involved in ROS scavenging. However, in this study, when the salt concentration increased to 1.0%, we found a decrease in CAT activity, independent of the Si concentrations. This can be explained by the fact that in the most severe stress

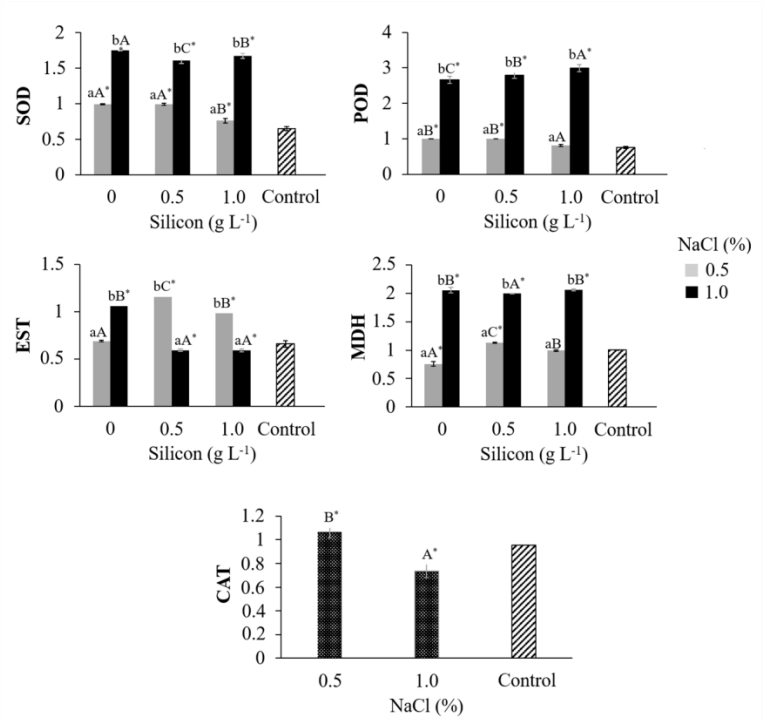

Fig 1. Effect of NaCl and silicon treatments on the activities (mmol min^{-1} mg^{-1}) of superoxide dismutase (SOD), peroxidase (POD), esterase (EST), malate dehydrogenase (MDH) and catalase (CAT) enzymes in *in vitro*-grown cape gooseberry plants. Means followed by the same lowercase letter inside each silicon concentration and uppercase letter inside each NaCl concentration do not differ by Tukey test ($p \leq 0.05$). * and ns; significant and non-significant contrasts, respectively, in comparison to the control treatment by Dunnett's test ($p \leq 0.05$).

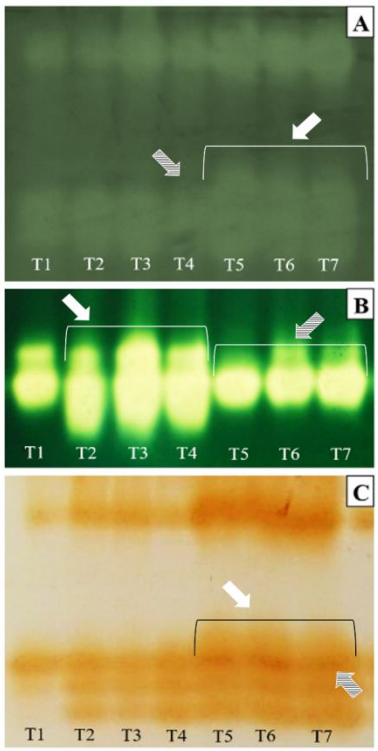

Fig 2. Isoenzyme patterns of cape gooseberry plants grown *in vitro* under salt stress. (A) Salt stress at 1.0% increased the expression of SOD (white arrow) and Si at 0.5 g L^{-1} decreased the SOD (hatched arrow); (B) Meanwhile, the first level of salt stress (0.5% NaCl) increased the CAT expression (white arrow) followed by a decrease at 1.0% NaCl (hatched arrow). For CAT, Si did not play a mitigating role; (C) The expression of POD increased when salt stress increased to 1.0% (white arrow) revealing that this enzyme was more effective against stress than CAT does. For POD, Si showed contributed to enhance the expression of this enzyme at concentration of 1.0 g L^{-1} (hatched arrow).T1 = control; T2 = 0.5% NaCl, T3 = 0.5% NaCl + 0.5 g L^{-1} silicic acid; T4 = 0.5% NaCl + 1.0 g L^{-1} silicic acid; T5 = 1.0% NaCl; T6 = 1.0% NaCl + 0.5 g L^{-1} silicic acid; T7 = 1.0% NaCl + 1.0 g L^{-1} silicic acid.

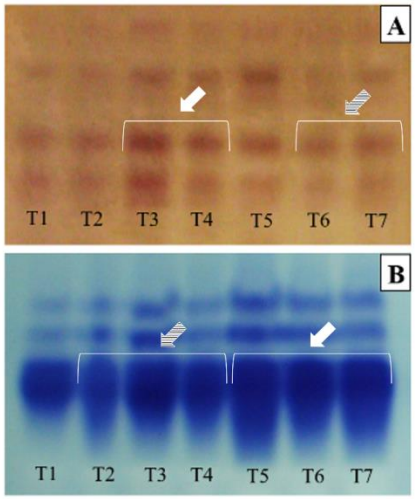

Fig 3. Isoenzyme patterns of cape gooseberry plants grown *in vitro* under salt stress. (A) The EST expression enhanced with increase in NaCl. Si contributed to increase the EST expression when salinity was about 0.5% NaCl (white arrow) and decrease when salinity was 1.0% (hatched arrow). (B) For MDH, changes in bands pattern were also observed. Si propitiated a decreased expression at 0.5% NaCl (white arrow) while contributed to increase the expression at 1.0% NaCl (hatched arrow). T1 = control; T2 = 0.5% NaCl, T3 = 0.5% NaCl + 0.5 g L^{-1} silicic acid; T4 = 0.5% NaCl + 1.0 g L^{-1} silicic acid; T5 = 1.0% NaCl; T6 = 1.0% NaCl + 0.5 g L^{-1} silicic acid; T7 = 1.0% NaCl + 1.0 g L^{-1} silicic acid.

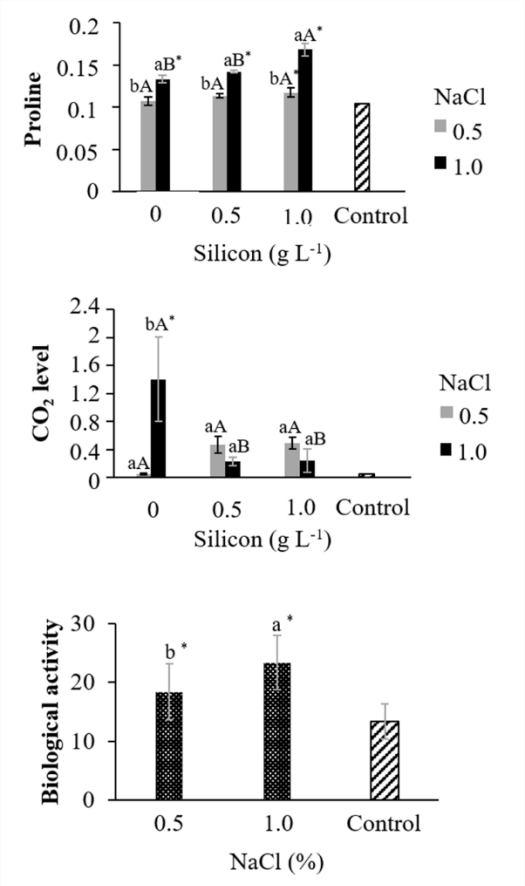

Fig 4. Salt stress increases the proline content, CO_2 level and biological activity of cape gooseberry plants cultivated *in vitro* under salt stress and silicon. Means followed by the same lowercase letter inside each silicon concentration and uppercase letter inside each NaCl concentration do not differ by Tukey test ($p \leq 0.05$). * and ^{ns}; significant and non-significant contrasts, respectively, in comparison to the control treatment by Dunnett's test ($p \leq 0.05$).

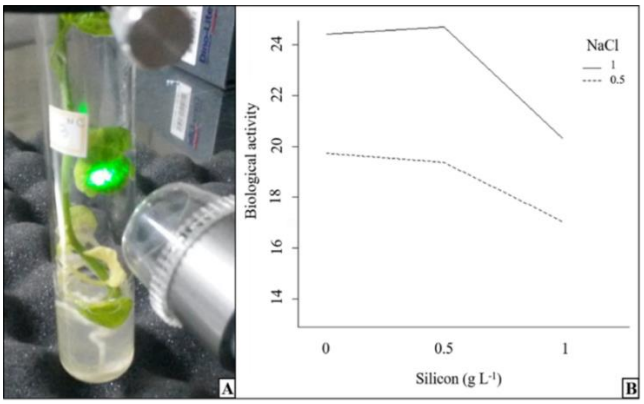

Fig 5. Cape gooseberry leave illuminated by biospeckle laser (A). The non-intercept of the lines means that the interaction between the factors (NaCl and Si) is not significant, i.e., there is not dependency between them. However, by the analysis of the graphic, the lines tend to touch each other after the concentration of 1.0 g L^{-1} silicon (B).

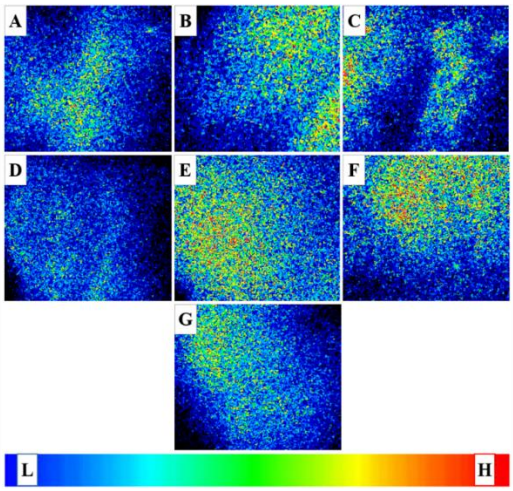

Fig. 6. Biological activity by SD analysis of cape gooseberry leaves as a function of different treatments concerning salt stress and silicon. These images show the increase in the biological activity of the leaves when the plants are exposed to increasing salinities (0.5 and 1.0%) compared to the control. **A**: control; **B**: 0.5% NaCl + 0.0 g L^{-1} Si; **C**: 0.5% NaCl + 0.5 g L^{-1} Si; **D**: 0.5% NaCl + 1.0 g L^{-1} Si; **E**: 1.0% NaCl + 0.0 g L^{-1} Si; **F**: 1.0% NaCl + 0.5 g L^{-1} Si; **G**: 1.0% NaCl + 1.0 g L^{-1} Si. The colored bar represents the range of biological activity: colors that tend to blue show low activity (L), and colors that tend to red indicate high activity (H).

conditions CAT production is decreased to reduce its activity and, therefore, cell energy consumption. In licorice (*Glycyrrhiza uralensis*) cultivated under salt stress, CAT activity was also reduced and the authors attributed this reduction to the expression of other antioxidant enzymes, such as the peroxidases, that have higher H_2O_2 decomposition capacity than CAT (Pan et al., 2006).

PODs, in general, are highly specific enzymes in H_2O_2 scavenging. In plants, PODs exist in several isoforms, are found in vacuoles and cell walls, and are involved in different cellular processes. Some are naturally expressed, whereas others are induced by environmental stress as has been found in studies showing that low POD activities indicate less severe stress symptoms and high activities indicate more severe symptoms (Barbosa et al., 2014). POD activity may serve as a biochemical biotic and abiotic stress marker and in the early identification of morphogenic processes during cell differentiation, growth, and multiplication of plants (Barbosa et al., 2014). Pan et al. (2006) found that induced stress in licorice increased the activity of POD while CAT activity reduced significantly, indicating that POD had greater power to break down the SOD-generated H_2O_2 than CAT. Similar behavior was observed in rice roots exposed to salinity (Khan et al., 2002). In the present study, Si was efficient in attenuating that effect at 1.0% NaCl concentration. These results corroborated those reported previously that Si increased leaf and root POD activities in salt-stressed plants (Liang et al., 2007; Zhu & Gong, 2014).

EST is associated with ester hydrolysis reactions and is directly linked to lipid metabolism and the degenerative processes of cell membranes (Santos et al., 2004). When Si was added to the medium, we verified that EST expression decreased in some bands. This can be attributed to the action of Si on salinity tolerance. It is known that in typical Si-accumulating plants, this element may reduce the Na^+ levels in the cell cytoplasm and increase K^+ by stimulating H^+-ATPase enzymes in the plasma membrane (Coskun et al., 2016). However, it remains unknown whether Si actually regulates, directly or indirectly, the transport activity or the expression of protein antiporter Na^+/H^+ under salt stress (Zhu and Gong, 2014). This protein plays an important role in maintaining low Na^+ concentrations by removing Na^+ from the cytosol (Yue et al., 2012). Notably, however, cape gooseberry is a Si-non-accumulating species, and, thus, these effects observed for many species cannot be found in this case. In tomato, which belongs to the same family as cape gooseberry and is also considered to be a Si-non-accumulating plant, the addition of Si did not affect the Na^+

and Cl^- concentrations in leaves, indicating that Si does not have a significant effect in this species because tomato cannot accumulate it. Notwithstanding, water storage in tomato improved with the addition of Si, and this higher water content contributed to the dilution of salts, thereby reducing their toxicity (Romero-Aranda et al., 2006).

MDH is associated with the biosynthesis of oxaloacetate (OAA) by interconversion of oxaloacetate to malate in the Krebs cycle and, therefore, plays an important role in cellular respiration and wide variety of biosynthetic reactions such as amino acids synthesis, gluconeogenesis, and redox potential maintenance (Taiz and Zeiger, 2013). The increased expression of MDH in this study may have been caused by increased enzyme activity in different cell compartments or by the induction of its activity as indicated by its increased band intensity. Such increased band intensity could be caused by increased respiration in plants in the presence of NaCl (especially in higher concentration) as degenerative processes were potentially initiated with these treatments, resulting in the activation of the enzymes involved in respiration. At 0.5% salinity, MDH activity decreased when Si was added, indicating that Si contributed to attenuate the salt damage.

Proline is considered to be one of the most important compatible solutes produced by cells. It accumulates in large amounts in response to environmental stresses. It also contributes to osmotic adjustment, membrane and protein stabilization, free radicals scavenging, and cellular redox potential buffering under stress conditions (Parida and Das, 2005). Gene expression induction in response to salt stress is also performed by proline (Chinnusamy et al., 2005). Proline accumulates at high levels under salt stress, leading many researchers to suspect that this amino acid also serves as an alternative energy source in abiotic stress responses. Furthermore, shoot plants seem to accumulate more proline while the roots in the developmental stage catabolize it at high rates, suggesting that this amino acid is an important source of energy for some tissues (Verslues and Sharp, 1999). The high proline content observed in the present study may have been caused by several factors such as the expression of genes, which encode key enzymes for proline synthesis, reduction of the conversion rate of proline oxidation, and reduction in the use of proline in the synthesis of protein and improvement of protein turnover (Sabbagh et al., 2014). According to Amini and Ehsanpour (2005), the accumulation of proline is considered a characteristic of tomato plant sensitivity to salinity and can be used to select tolerant plants. These authors observed that tomato cultivars grown *in vitro* under salt stress showed an increase in leaf proline content, with maximum peaks observed with 160 mM NaCl. Increased proline content was also found in species grown *in vitro* under salt stress such as *Populus euphratica* (Watanabe et al. 2000), tomato and cauliflower (Wahid et al., 2014), and *Bacopa monnieri* (Ahire et al., 2013). Miranda et al. (2014) observed that leaves of *P. peruviana* showed higher proline levels as the exposure time of the plants to NaCl increased.

The increased release of CO_2 to the environment is an indication of greater plant respiration. One of the secondary stresses resulting from salinity exposure is oxidative stress, which occurs especially in the leaves, resulting from uncontrolled metabolism involving key processes such as photosynthesis, respiration, photorespiration, and cellular metabolism. The key reactions of O_2 consumption and CO_2 production by the plants can be measured through gas exchange of tissues, whole plants, or even ecosystems (Leakey et al., 2009). However, the effect of salinity on respiratory rate is complex because many studies show that respiration increases with salinity, while others show that it reduces, and yet more show that there is no change at all. For the plant, the advantage of having a higher respiration rate is that more ATP is produced, providing vital energy for the growth of new tissue and defense mechanisms such as osmotic adjustment and sodium exclusion (Jacoby et al., 2011). However, the cost of a high respiration rate is that carbon is spent in respiration rather than being allocated for the synthesis of new tissues, thus limiting plant growth. There are indications that in the shoot, respiratory homeostasis, which is the plant's ability to maintain a constant respiratory rate in different environmental conditions, is related to salt tolerance, although further confirmation of this in most species is required (Jacoby et al., 2011). Detmann et al. (2013) have observed a strong correlation between Si concentration and the levels of compounds closely related to respiration (isocitrate and 2-oxiglutarate) and certain amino acids. The authors relate that when they are studied in conjunction, Si clearly plays an important role in regulating the flow rate of 2-oxiglutarate for the metabolism of amino acids, confirming that this metabolism is an intricately controlled network.

The higher biological activity observed in the leaves of plants grown with NaCl can be explained by increases in enzyme activity. Under stressful conditions, for example, the plant will, at first try to adapt to the adverse environment by rearranging its enzymatic apparatus. The higher respiration rate and production of osmolytes that were observed in this study are examples of how the metabolic activity of the plant is altered, reflecting the analysis of biological activity. The biological activity of carrot's respiration rate was observed using the biospeckle laser technique and showed that, in addition to other factors, respiration could be one of the causes of the changes observed in biological activity (Alves et al., 2013; Braga et al., 2005). When the plants are subjected to some form of environmental stress, biotic or abiotic, they respond by enhancing their biological activity, as their defense systems are triggered (Parida and Das, 2005). This response may continue or may decrease over time because of increases in the stress duration. Biospeckle laser is an optical technique that has been developed to analyze biological materials non-destructively (Zhao et al., 1997). It has already been applied in many studies on both animals and plants (Botega et al., 2009). Various studies have been performed using the biospeckle laser to study the biological activity of plant tissues in different situations. For example, studies carried out using this technique with bean seeds showed increased biological activity when they were inoculated with certain fungi, depicting an increased defense system against biotic stress (Rabelo et al., 2011). Apples that suffered mechanical injury showed a decline in activity at the location where the physical damage occurred (Pajuelo et al., 2003). According to Romero et al. (2009), the biological activity of tomato fruits increased as they matured, while another study showed that a decrease in chlorophyll content was accompanied by increased biological activity in apple fruits (Zdunek and Herppich,2012).

Materials and Methods

Plant material and experimental conditions

Nodal segments (± 1 cm) derived from cape gooseberry previously cultivated *in vitro* were inoculated in Murashige and Skoog medium - MS (Murashige and Skoog, 1962) with 30 g L^{-1} added sucrose, solidified with 1.8 g L^{-1} phytagel, and pH adjusted to 5.8. The culture medium was supplemented with different combinations of NaCl concentrations (0.5%

and 1.0%) and silicic acid (0, 0.5, and 1.0 g L^{-1}). The experimental design was completely randomized in a factorial scheme 2 × 3 (two concentrations of NaCl and three silicic acid concentrations) plus an additional treatment represented by the control (without the addition of any of the factors). Each treatment consisted of 40 test tubes with one plant each.

NaCl concentrations used in this experiment were chosen based on the results obtained from a preliminary study. Concentrations above 1.0% proved to be very toxic for cape gooseberry, resulting in almost no growth. The silicon was added to the medium in the form of very pure silicic acid ($SiO_2.xH_20$). After application of the treatments, the explants were maintained in a growth room at 25°C ± 2 °C with a photoperiod of 16 h of light:8 h of darkness, and a fluorescent light intensity of 35 µmol m^{-2} s^{-1}. The following evaluations were performed after 30 d.

Isoenzyme assessments

For isoenzyme electrophoresis, we collected plants and stored them at -86°C. The frozen material was ground in a pre-cooled mortar with liquid nitrogen and polyvinylpyrrolidone (PVP). The powder was suspended in 300 µL extraction buffer Tris-HCl 0.2M (pH 8.0) containing 0.1% β-mercaptoetanol. The material was homogenized by agitation in a vortex and kept in a refrigerator for 12 hr, followed by centrifugation at 14,000 rpm for 30 min at 4°C. Electrophoresis was performed on discontinuous, non-denaturing polyacrylamide gels (7.5% separating gel and 4.5% stacking gel). The gel/electrode buffer system was Tris-Glycine (pH 8.9). Aliquots of 60 µL of each sample's supernatant were applied to the gel and the electrophoretic run was conducted at 120 V for 5 hr. After the run, the gels were tested for the superoxide dismutase (SOD), catalase (CAT), peroxidase (POD), esterase (EST), and malate dehydrogenase (MDH) enzymes according to Tanksley and Orton (1983). The interpretation of the results was based on visual analysis of electrophoresis gels, taking into account the presence, absence, and intensity of the expression of each band. Furthermore, the gel was photographed and the average intensity of the bands was processed on three images of each gel for each enzyme using 1D Image Analysis Software (Vilber Lourmat).

Proline content

Proline content was determined using the method of Messer (1961), and the reading was performed in a spectrophotometer (Model UV-330G, Gehaka) at 509 nm. The pattern used was a solution of proline 10^{-4} M diluted with water in 1:1 ratio.

CO_2 level

The CO_2 level analysis was performed by the introduction of a needle that pierced the plastic that blocked the opening of the tube. This plastic prevented gas exchange with the environment but allowed the needle entry to remove air. This needle was attached to the PBI - Dansensor Checkpoint O_2/CO_2 (Micro C, Datospir) device that operates with an electrochemical reader absorbing approximately 15 mL of the sample atmosphere to instantaneously read the amount of O_2 and CO_2 percentages. These values were then divided by the weight of the plants in those tubes and by the 8 h related to the final reading time (default).

Biological activity

For the measurement of biological activity, cape gooseberry leaves inside test tubes (fully sealed with a lid and plastic-film) were illuminated by a coherent light, and the interference patterns formed were captured by a digital microscope (Dino-Lite Premier AM7013MZT). The microscope was arranged to form the back-scattering configuration (Ribeiro et al., 2014). The coherent light used was from a green diode laser (532 nm) with its beam split by a concave lens to cover the entire sample. The distance between the lens and the sample was 0.75 m. The dynamic interference patterns formed by the interaction of light with the material studied were collected by a portable digital microscope Dino-Lite brand, model AM 413zt. In each session, a set of 128 images in gray levels were stored at a rate of one every 0.08 s with a resolution of 1,280 X 1,024 pixels. The analysis and interpretation of the data were performed by image analysis with numerical approaches (Absolute Value of the Differences - AVD) (Braga et al., 2011) and graphical approaches (Standard Deviation - SD) that returned information related to the biological activity (BA) of the sample (Nothdurft and Yao, 2005). These biological activities are not related to a specific phenomenon, but a set of phenomena such as cell growth and reproduction and intracellular processes related to the movement of organelles, the cytoplasmic flow, or even to chemical reactions (Braga et al., 2009).

Statistical analysis

Statistical analyses were performed using R software (R Development Core Team, 2008). Data were subjected to analysis of variance and, in event of significant differences of factors or interaction between them, the means were compared by Tukey's test ($p \leq 0.05$). Comparisons to the additional treatment (control) were analyzed by Dunnett's test ($p \leq 0.05$).

Conclusion

Salt stress induced by NaCl is harmful to *in vitro*-grown cape gooseberry plants. The use of Si as a salinity-mitigating agent is observed for enzyme activities only at 1.0% NaCl, mainly for POD. Proline increases as salinity increased and Si contributed to enhance the level of this amino acid. High respiratory rate is verified under salt stress and Si plays a role in reduction of this variable. Salt stress increases the biological activity of leaves; however, for this characteristic, there is no response to Si application.

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