ISSN 0100-2945 http://dx.doi.org/10.1590/0100-29452017155

CHANGES IN OSMOTIC AND IONIC INDICATORS IN *Ananas comosus* (L.) cv. MD GOLD PRE-TREATED WITH PHYTOHORMONES AND SUBMITTED TO SALINE MEDIUM[1]

YURI LIMA MELO[2], CIBELLEY VANÚCIA SANTANA DANTAS[3], YUGO LIMA-MELO[4],
JOSEMIR MOURA MAIA[5], CRISTIANE ELIZABETH COSTA DE MACÊDO[6]

ABSTRACT- The aim of this study was to evaluate the effect of NaCl on the hydroponic culture of cv. MD Gold pineapple pretreated with the phytohormones naphthaleneacetic acid (NAA) and 6-benzylaminopurine (BAP) using indicators of water stress and osmotic adjustment. Pineapple seedlings from saline treatments in the absence (-NB) and presence (+NB) of the phytohormones during the *in vitro* culture were grown in Hoagland & Arnon (1950) nutrient solution in the absence and presence of different NaCl concentrations (50; 100 and 150 mM) for 10 days in a greenhouse. Plants obtained from *in vitro* culture pretreated with phytoregulators (+NB) showed distinct physiological responses compared to non-treated plants (-NB) in relation to dry mass (DM) in roots, electrolyte leakage (EL) and Na^+ and K^+ concentrations in leaves and roots, and also regarding soluble sugars (TSS), free amino acids (TFAA) and proline (PRO) concentrations in leaves. Additionally, salt treatments induced similar responses in -NB and +NB plants, however differing in relation to intensity and the studied organs. The presence of NaCl in the solution reduced leaf DM in the -NB treatment, the leaf relative water content in -NB and +NB, and root RWC only in the -NB treatment. High levels of NaCl increased leaf EL in the +NB treatment. Potassium levels decreased with the increase of NaCl concentrations in nutrient solution for leaves and roots submitted to -NB treatment and for roots submitted to +NB treatment. Leaf potassium levels increased in +NB treatment regardless of salt treatment. No ionic toxic effects were identified except for roots subjected to 150 mM NaCl solution both for -NB and +NB conditions. TSS concentrations decreased with increasing NaCl concentration in leaves for both -NB and +NB treatments. Furthermore, TFAA and PRO showed increased levels in leaves subjected to saline stress, being more expressive in -NB treatment. In conclusion, pretreatment with growth regulators in pineapple plants minimized the effects of NaCl, despite higher concentrations of this salt affecting most of the evaluated parameters. Additionally, we conclude that the osmotic effect of salt stress was determinative for the physiological changes and that roots have different outcomes regarding the increment of growth regulators and NaCl concentrations.

Index Terms: *Ananas comosus* (L.), auxin, cytokinin, osmoregulators, salt stress.

ALTERAÇÕES DE INDICADORES OSMÓTICOS E IÔNICOS EM *Ananas comosus* (L.) cv. 'MD GOLD' PRÉ-TRATADOS COM FITOHORMÔNIOS E SUBMETIDOS AO MEIO SALINO

RESUMO- O objetivo deste trabalho foi avaliar o efeito do NaCl durante o cultivo hidropônico de abacaxizeiro 'MD Gold' pré-tratado com os fitohormônios ácido naftalenoacético (ANA) e 6-benzilaminopurina (BAP) utilizando indicadores de estresse hídrico e de ajustamento osmótico. Plântulas de abacaxizeiro, provenientes de tratamentos salinos na ausência (-AB) e presença (+AB) dos fitohormônios, durante o cultivo *in vitro*, foram cultivadas em solução nutritiva na ausência e em presença de diferentes concentrações de NaCl (50; 100 e 150 mM), durante 10 dias em casa de vegetação. Plantas provenientes do cultivo *in vitro* pré-tratadas com os fitohormônios (+AB), apresentaram respostas fisiológicas distintas daquelas não tratadas com os mesmos (-AB), com relação à massa seca (MS) em raízes, vazamento de eletrólitos (VE) e concentrações de Na^+ e K^+ em folhas e raízes e, também, com relação às concentrações de açúcares solúveis (AST), aminoácidos livres (AALT) e prolina (PRO) em folhas. Adicionalmente, o tratamento salino induziu respostas similares entre as plantas -AB e +AB, mas diferentes em relação a intensidade e aos órgãos estudados. A presença de NaCl na solução reduziu a MS de folhas no tratamento -AB, conteúdo relativo de água de folhas em -AB e +AB e em raízes apenas no tratamento -AB. Altos níveis de NaCl aumentaram o VE em folhas no tratamento +AB. Os níveis de K^+ diminuíram com o aumento da concentração de NaCl na solução nutritiva em folhas e raízes do tratamento -AB e em raízes no tratamento +AB e aumentaram, independente do tratamento salino, em folhas do tratamento +AB. Não foi possível identificar o efeito tóxico dos íons, com exceção das raízes submetidas a 150 mM de NaCl em +AB e -AB. As concentrações de AST diminuíram com o aumento da concentração de NaCl em folhas, nas plantas -AB e +AB. Já os AALT e PRO tiveram seus níveis aumentados em folhas submetidas ao estresse salino, sendo mais expressivo no tratamento -AB. Concluímos que o pré-tratamento com os fitohormônios em plantas de abacaxizeiro minimizaram os efeitos do NaCl , apesar de altas concentrações deste sal afetarem a maioria dos caracteres avaliados. Adicionalmente, conclui-se que o efeito osmótico do estresse salino foi determinante para as alterações fisiológicas observadas e que folhas e raízes apresentam respostas diferentes com relação ao incremento de fitohormônios e também às concentrações de NaCl.

Termos para indexação: *Ananas comosus* (L.), auxina, citocinina, estresse salino, osmorreguladores.

---

[1] (Paper 215-15). Received on September 03, 2015. Accepted February, 17, 2016. Financial support: EMPARN-EMBRAPA (OEPAS) and CAPES.

[2] PhD in Agronomy. PNPD/PPGCA/UEPB, Campina Grande-PB. E-mail: yurimelo86@gmail.com

[3] MSc in Agronomy. DCV/UFERSA, Mossoró-RN. E-mail: cibelley_rn@hotmail.com

[4] MSc in Biochemistry. DBBM/UFC, Fortaleza-CE. E-mail: yugo_lima@yahoo.com.br

[5] PhD in Biochemistry. Professor at the State University of Paraíba (DAE/CCHA/UEPB),Catolé do Rocha-PB. E-mail:jmouram@gmail.com

[6] PhD in Science. Professor at the Federal University of Rio Grande do Norte (UFRN,CB/DBG), Natal-RN. E-mail: cristianemacedo@ufrnet.br

INTRODUCTION

Salinity is the most limiting factor in crop productivity due to the presence of soluble salts and/or exchangeable sodium in the soil. Excessive concentrations of saline ions in the root environment can lead to damage caused by water deficit, or even toxicity by specific ions, especially sodium (Na^+) and chloride (Cl^-) (BENDERRADJI et al., 2011; FLOWERS et al., 2014), interfering in the vegetal development and leading to functional disorders, metabolic impairments such as unregulated phytohormone synthesis, among others (SILVA et al., 2009a; ZHANG, 2010).

The effects of water deficit and salinity on plants are minimized by metabolic mechanisms such as osmotic adjustment (ADEM et al., 2014). Accumulation of osmoregulators such as soluble sugars, amino acids (e.g. proline), inorganic ions (e.g. potassium), proteins and others (JOSEPH et al., 2015) prevent water loss and maintain plant turgor; thus balancing osmotic potentials between the cytoplasm and the vacuole, which facilitates water retention in the cytoplasm and allows Na^+ sequestration to the vacuole or apoplast (MUNNS; TESTER, 2008; SHABALA; MUNNS, 2012).

Despite being widely studied, osmoregulator accumulation and their physiological role in plants submitted to saline stress is paradoxically little understood and quite a controversial phenomenon due to different results found in the literature. For example, an increase in proline, proteins and soluble carbohydrate content was observed in ornamental pineapple (*Ananas porteanus* Hort Veitch ex C. Koch) belonging to the Bromeliaceae family, the same family as the species used in this study, which seems to have contributed to membrane integrity maintenance in plants submitted to NaCl treatment (MENDES et al., 2011). In other species such as physic nut (*Jatropha curcas* L.) (SILVA et al., 2009b), great millet (*Sorghum bicolor*) (OLIVEIRA et al., 2006) and the banana tree (*Musa* spp.) (CARVALHO et al., 2006), an accumulation of saline ions and/or compatible solutes has contributed to reducing the osmotic effects of salinity.

Thus, in order for saline stress indicators to be safely determined and the salinity factor to be studied in isolation, plant tissue culture and hydroponic techniques have been widely applied in identifying and selecting cultivars that are more resistant to salinity (MORAES et al. 2010; NASSAR et al. 2015). Moreover, such knowledge can be used in breeding programs aimed at increasing crop productivity and occupation of constantly abandoned salinized areas (MACÊDO et al., 2005).

In Brazil, salinized areas are mainly located in the northeastern semi-arid region (FAO, 2014; SÁNCHEZ et al., 2015). However, the Northeast Region has the highest percentage (40%) of national pineapple production, totaling 687,130 thousand fruits in 2014 (IBGE, 2014); thus highlighting this species as the most important among the Bromeliaceae family in tropical agriculture. Among the several cultivars of the species, MD Gold is a double-hybrid, sugar-rich, low-acid pine-free/thornless pineapple, being characteristics responsible for its worldwide expanding production and consumption (BARREIRO NETO et al., 2007; BARTHOLOMEW, 2009).

In a previous study, Melo et al. (2011) indicated that the *in vitro* culture of the MD Gold cultivar in the presence of salt is more efficient when associated with the application of an auxin (naphthaleneacetic acid - NAA) and a cytokinin (6-benzylaminopurine - BAP) to the culture medium, ensuring growth maintenance, increasing the number of leaves, producing new buds and accelerating the rooting process. However, the physiological adaptation phenomenon by way of mitigating the effects of saline stress through the action of phytohormones in the metabolism of pineapples when plants from the *in vitro* culture system are transported to other *ex vitro* culture systems (e.g. hydroponics) is not fully understood. Recent studies have attempted to improve plant tolerance to damage caused by salinity through exogenous induction of plant hormones (HAMDIA; SHADDAD, 2010).

In order to better understand phytohormone actions in *Ananas comosus* (L.) cv. MD Gold under salt stress and to identify the effects of such stress during hydroponic cultivation in relation to osmoregulation and ionic toxicity, in this study we investigated whether *Ananas comosu*s (L.) grown with NAA and BAP phytohormone supplementation are more tolerant to salinity through osmoregulation. To test this hypothesis, cv. MD Gold pineapple plants from *in vitro* cultures in the presence or absence of NAA and BAP were submitted to different NaCl concentrations and evaluated using methods related to indicators of water and ionic stresses, as well as osmotic adjustment. From the results of this study we intend to contribute to improving the cultivation of this fruit crop, increasing its importance in tropical agricultures.

MATERIAL AND METHODS

Ananas comosus cv. MD Gold pineapple plants measuring between 12 and 15 cm in height obtained from salt treatments, in the absence (-NB) and presence (+NB) of naphthaleneacetic acid (NAA; 1 mg L^{-1}) and 6-benzylaminopurine (BAP; 0.5 mg L^{-1}) phytohormones during *in vitro* cultures (MELO et al., 2011) were fixed on styrofoam plates and placed inside individual plastic containers to float in 800 mL Hoagland and Arnon nutrient solution (1950), diluted to 1:4 (v/v) in water, pH 6.0. The seedlings were placed in a greenhouse for acclimatization and to establish the roots during 30 days; the nutrient solution was changed weekly. Aeration pumps were used to maintain oxygenation in the nutrient solution during the experiment.

After the acclimatization period, different concentrations of NaCl (0; 50; 100 and 150 mM) were added to the Hoagland and Arnon (1950) nutrient solution, totaling four saline treatments. The seedlings were kept in a greenhouse under an average temperature of 35 ± 5°C and relative air humidity of 40 ± 10% for 10 days.

After the 10-day period of exposure to saline solution, the seedlings were collected and separated into shoots and roots. Plant material was analyzed for dry matter (DM), water deficit indicators as determined by relative water content (RWC) and moisture percentage (MP), in addition to the occurrence of electrolyte leakage (EL). Also, Na^+ and K^+ concentrations as indicators of ionic stress, and total soluble sugars (TSS), total free amino acids (TFAA) and proline (PRO) concentrations were evaluated as osmotic adjustment indicators.

To determine RWC, parts of the shoot and root systems of each seedling were weighed to obtain fresh mass (FM) and then soaked in deionized water at room temperature of 25 ± 5°C for six hours to obtain turgid mass (TM). Dry matter (DM) was obtained by TM dehydration for 72 h at 75°C. RWC calculations were based on the mathematical expression by Irigoyen et al. (1992) of: $RWC = (FM - DM/TM - DM) \times 100$. The moisture percentage was determined according to Slavick (1974) using the relationship: $MP = [(FM - DM)/FM] \times 100$.

EL was estimated according to Blum and Ebercon (1981) using the equation: $EL = (L_{1/}L_2) \times 100$, where: L_1 corresponds to the first electrical conductivity (EC) reading of the solution performed after 6 hours, and L_2 represents the second EC reading carried out after heating in a water bath at 100ºC.

Na^+ and K^+ extractions were carried out according to Sarruge and Haag (1974) with some modifications. K^+/Na^+ ratio was determined according to Maathius and Amtmann (1999).

The TSS concentration measurement was performed by the "phenol-sulfuric" method described by Dubois et al. (1956). TSS concentrations were determined based on the standard curve adjusted for increasing concentrations of D-glucose stock solution, with results expressed in µmol g^{-1} of dry matter and readings being conducted using a spectrophotometer at 490 nm absorbance.

TFAA concentration was determined by the method described by Peoples et al. (1989). TFAA concentrations were determined based on the standard curve adjusted for increasing concentrations of a standardized L-glutamine mixture, with results expressed in µmol g^{-1} of dry matter and readings being carried out using a spectrophotometer at 570 nm absorbance.

PRO concentration was determined according to a methodology described by Bates (1973). Concentrations were determined based on the standard curve adjusted for increasing L-proline concentrations, with results expressed in µmol g^{-1} of dry matter and readings being performed using a spectrophotometer at 520 nm absorbance.

The experimental design was completely randomized with a 4x2 factorial scheme; four salt treatments (control – 0; 50; 100 and 150 mM NaCl) and two treatments from *in vitro* cultures (-NB and +NB), corresponding to eight treatments of five replicates each, thus totaling 40 experimental units.

The results were submitted to analysis of variance (ANOVA), and they were submitted to Tukey's mean test when significant in the F-test ($p \leq 0.05$).

RESULTS AND DISCUSSION

In this study, plants from *in vitro* culture pre-treated with naphthaleneacetic acid and 6-benzylaminopurine phytohormones (+NB) showed different physiological responses from non-treated (-NB) plants. Moreover, saline treatment induced similar responses between -NB and +NB plants, although the intensity of the response was different in relation to both groups (+NB and -NB) and to the studied organs.

Increasing NaCl concentrations in the nutrient solution led to a proportional decrease of up to 62% (in 150 mM NaCl) of DM content in leaves from the -NB group (Table 1). In contrast, salt did not influence DM content of leaves in the +NB group. The same effect was observed for the plant roots treated and not treated with phytohormones. The addition of NaCl to the nutrient solution led to a reduction of DM in -NB plant roots. In leaves, 50 mM concentration caused a decrease of approximately 41% in relation to the control. In +NB plant roots treated with 50 mM NaCl, an increase of 80% DM content was observed in relation to the control (Table 1).

The effects of salinity on dry matter production in -NB treatments may not only be related to the osmotic component due to the unavailability of water to the plant, but also to the ionic component of salt stress through the accumulation of ions in plant tissues. Both components reduce cell turgor and consequently impair growth due to a reduction in cell division and stretching (MACÊDO et al., 2005 CLAEYS; INZÉ, 2013). Under the effect of salinity, the reduced DM may also be related to energy for growth being deviated to maintenance and activation of other metabolic activities, such as maintaining membrane integrity, synthesizing organic solutes for osmoregulation and/or protecting macromolecules, among others (MUNNS et al., 2002; FLOWERS et al., 2014).

The effect of salinity on dry matter reduction observed in this study is known and has already been demonstrated in other species, such as *Solanum lycopersicum* (L.) (BABU et al., 2012), *Musa* spp. (JUNIOR et al., 2012) and *Jatropha curcas* (L.) (DÍAZ-LÓPEZ et al., 2012). In general, most plants submitted to salt stress present drastic changes in the plant water condition (osmotic salinity effect), increased concentration of toxic ions leading to physiological and biochemical disturbances and oxidative stress, and a nutritional imbalance caused by changes in the absorption of essential nutrients, which reduce and/or inhibit plant growth (DÍAZ-LÓPEZ et al., 2012).

Seedlings treated with phytohormones and cultivated in the presence of NaCl did not show significant reductions in DM, either in leaves or roots. Thus, pre-treatment with phytohormones may minimize the effects of NaCl on plant biomass production. Akbari et al. (2007) evidenced that despite increasing concentrations of NaCl reducing the length and DM of shoot and root systems, the addition of auxin indoleacetic acid (AIA) during the germination period promoted an increase in shoot length and dry matter in three *Triticum aestivum* (L.) cultivars. According to Hamdia and Shaddad (2010), cytokinins such as kinetin (KIN) and benzylaminopurine (BAP) could also contribute to increasing salinity tolerance in *Triticum aestivum* (L.), especially when these phytohormones interact with other plant hormones (mainly auxins), as it was also found in this study.

Contrary to what occurred with DM, no drastic interference by phytohormones in the water accumulation of leaves and roots was found. There were no differences observed in leaf and root RWC between -NB and +NB plants, except in leaves of -NB plants grown in the presence of 150 mM NaCl. In this treatment, RWC was 16% lower than +NB plants (Table 1). Increasing salt concentration caused a progressive decrease in RWC of leaves and roots of both plant groups (+NB and -NB), except in roots of +NB plants that did not have their water content affected by salt. However, this effect was more significant in plants of the -NB group and at 100 and 150 mM NaCl concentrations. In the presence of 150 mM NaCl, a reduction of RWC in phytohormone-treated (+NB) and non-treated (-NB) leaves was 15 and 27%, respectively, when compared to their respective controls. In the roots of the non-treated plants (-NB) and in the presence of the highest salt concentration (150 mM), the RWC reduction was 10% when compared to the control (Table 1).

The increase of salt doses caused a progressive decrease in leaf moisture in both groups (+NB and -NB), however root moisture was not affected by salt. The decrease observed in leaf Mo was more expressive at the concentration of 150 mM of NaCl. In the presence of 150 mM NaCl, the reduction of leaf Mo in +NB and -NB plants was approximately 2 and 4%, respectively, when compared to their respective controls (Table 1).

In cv. MD Gold pineapple plants, it is possible that water deficiency caused by the osmotic effect of salinity has made water and nutrients unavailable for plants, leading to serious consequences to the plant growth, especially limiting biomass accumulation (MAIA et al., 2007; ALVES et al., 2011). Thus, it is possible that the DM reduction observed in leaves and roots of the -NB treatment is related to the water deficiency caused by the addition of NaCl to the medium, as observed by RWC and MP reduction in leaves and by RWC (reduction) in roots. Moreover, it is also possible that the maintenance of DM values in roots in the +NB treatment, and even the increase of this variable at the concentration of 50 mM NaCl can be partly attributed to the combined action of cytokinin and auxin, considering that under salinity conditions these phytohormones can stimulate cellular elongation and increase biomass allocation into the roots (ALBACETE et al., 2008).

Under salt stress, the RWC and MP maintenance observed in +NB treatment roots and the reduced effects of salinity on leaf RWC in +NB treatment (compared to the -NB treatment) may be related to water conduct maintenance through an increased stomatal opening and transpiration, partly attributed to an exogenous incremental action of some phytohormones, such as abscisic acid in *Solanum lycopersicum* (AROCA et al., 2012) and cytokinins in *Phaseolus vulgaris* (L.), *Beta vulgaris* (L.), *Nicotiana tabacum* (L.) and *Zea mays* (L.) (POSPISILOVA et al., 2005).

Damage to membranes evaluated through electrolyte leakage (EL) were an average of 50% higher in -NB plants in comparison to +NB plants (Table 1). Pre-treatment with hormones in the control treatment (0 mM NaCl) reduced electrolyte leakage of leaves and roots by up to 50%. However, in the presence of salt, a progressive increase in membrane damage proportional to the salt dose was observed for both leaves and roots in both groups of plants (+NB and -NB), except in leaves of -NB plants. In the leaves of seedlings from *in vitro* +NB culture, an increase of approximately 200% EL in the 150 mM NaCl dose was observed (Table 1). In the roots, the effect of EL was observed starting at 50 mM NaCl. In the presence of 150 mM NaCl, the increase of EL in treated (+NB) and non-treated (-NB) leaves was approximately 200 and 140%, respectively, when compared to their respective controls (Table 1).

The EL observed in leaves may be more related to osmotic stress than to ionic stress, since as may be seen in Table 2, no ionic toxicity (represented by the K^+/Na^+ ratio) was found. The K^+/Na^+ ratio in the roots indicated a possible toxic effect caused by Na^+ in detriment of K^+, mainly at 150 mM NaCl (Table 2). In this organ, the ionic component of saline stress may be related to the increase in EL, considering that the excess of Na^+ and Cl^- in the protoplasm causes toxicity and leads to ionic balance disturbances, which consequently causes changes to the membrane structure (SHABALA et al., 2012). The highest EL indexes observed in roots were probably a result of the direct contact of this organ with the saline solution (LOPES; MACEDO, 2008). Additionally, it is possible that reduced EL in the +NB compared to -NB treatments is related to phytohormone action. In a study with *Zea mays* (L.) subjected to saline conditions, Kaya et al. (2010) observed that foliar application of cytokinin and auxin minimized the effects of salt on electrolyte leakage, similar to what was observed in the present study. For Kaya et al. (2010), exogenous cytokinin use was crucial for maintenance of cell membrane permeability, which facilitated the transport of solutes through this structure and improved the tolerance of maize plants to salinity.

In the presence of NaCl, the accumulation of Na^+ in -NB and +NB plants increased significantly both in foliar and root tissues. Such increases were directly proportional to the increase of NaCl dose and more expressive in +NB plants (Table 2). In the presence of 150 mM NaCl, the Na^+ increase in the leaves of +NB and -NB plants was approximately 50 and 30 times higher, respectively, in relation to their respective controls (Table 2). Na^+ concentration was higher in the roots of both plant groups (+NB and -NB) than in their leaves. In the presence of 150 mM NaCl, the increase of Na^+ in the roots of treated (+NB) and non-treated (-NB) plants was respectively about 88 and 23 times higher than their respective controls (Table 2).

The increase in NaCl concentration in the solution led to an increase in Na^+ levels, both in leaves and roots of the studied plants (Table 2). Such an increase may be related to the similarity between Na^+ and K^+ charges which allows passive entry through low or high affinity carriers common to K^+ (TESTER; DEVENPORT, 2003; FARIAS et al., 2009). High concentrations of specific ions, especially of Na^+, can lead to a nutritional imbalance in plants with frequent ion reduction, especially potassium (LI et al., 2010), as observed in leaves and roots of plants in the -NB treatment, and in the roots of +NB treatment. The antagonistic effect between the increase of Na^+ levels in detriment of the reduced K^+ levels observed in the present study suggests an increase in K^+ efflux with increasing salt concentrations in the nutrient solution, as was evident in leaves of the -NB treatment and in roots of -NB and +NB treatments. Alterations in Ca^{2+} uptake and metabolism were possibly induced as a consequence of Na^+ accumulation in compartments such as the cytosol of cells (CABOT et al., 2014). Ca^{+2} replacement by Na^+ in cell membranes exposed to salinity affect membrane permeability, reducing its selectivity and leading to damage to its structure and fluidity (FARIAS et al., 2009; WILLADINO; CAMARA, 2010), which could have occurred in the present study as a result of the increase in EL observed in the leaves and roots of cv. MD Gold pineapples.

A higher accumulation of Na^+ was observed in the roots than the leaves. Babu et al. (2012) and Hasegawa et al. (2000) emphasized that the distribution of ionic accumulation can vary between plant organs. In this experiment, it is possible that the accumulation of Na^+ in the roots may have helped to maintain the water status of this organ, thus minimizing root water deficiency as represented by RWC and MP levels. Some plants have the ability to establish osmotic equilibrium even under low water potential in the soil; and the roots play an important role, as they maintain the Na^+ and Cl^- amounts at adequate and constant levels (TESTER; DAVENPORT, 2003). Na^+ retention in the roots represented by higher levels than those found in cv. MD Gold Pineapple leaves suggest that this mechanism has restricted Na^+ movement to the shoot system, compartmentalizing this ion in the root system (CAVALCANTI et al., 2004; PRAXEDES et al., 2010).

In general, pre-treatment with phytohormones interfered with the accumulation of K^+ both in roots and leaves. Significant differences in K^+ concentrations were observed between -NB and +NB plants. The accumulation of this ion was slightly higher (approximately 1.1 fold) in the leaves of non-treated plants, while treated plants accumulated approximately four times more K^+ in their roots (Table 2). Salt treatment induced a decrease in K^+ concentration in the leaves of -NB plants proportional to the NaCl dose. In the presence of 150 mM NaCl, there was a reduction of almost 40% in relation to the control. However, in the leaves of +NB plants, NaCl caused a 12% increase in K^+ concentration, starting from the treatment with 50 mM NaCl, and remaining stable up to the 150 mM NaCl concentration. An increase in K^+ concentration of 76 and 27%, respectively, was observed in the roots of -NB plants for 50 and 100 mM treatments, and a reduction of almost 60% in the highest concentration of NaCl (150 mM). In the roots of plants pre-treated with phytoregulators (+NB) and in the presence of 150 mM, the NaCl reduced K^+ concentration almost nine times in relation to control.

The antagonistic effect between the increase in Na^+ levels and the reduction of K^+ levels observed in leaves of the -NB treatment and in the roots for both -NB treatment (only at 150 mM NaCl) and in the +NB treatment of this study were also shown in species such as *Plantago coronopus* (L.), (KOYRO, 2006), *Daucus carota* (L.) (INAL et al., 2009) and *Zea mays* (L.) (KAYA et al., 2010). According to the results obtained by Kaya et al. (2010), the exogenous addition of cytokinin and auxin caused a reduction in Na^+ concentration and an increase in K^+ concentration when compared to plants under salt stress. In this study, pre-treatment with phytohormones did not reduce intracellular Na^+ levels; however, a significant increase of K^+ levels in the +NB treatment compared to the -NB treatment can be attributed to pretreatment with phytohormones.

The presence of NaCl in the nutrient solution led to a significant reduction in the K^+/Na^+ both in leaves and in roots of both groups of plants (+NB and -NB). This effect was proportional to the increase of the NaCl concentration and was more intense in +NB plants. In the leaves of -NB and +NB plants treated with 150 mM NaCl, the K^+/Na^+ ratio was reduced about 50-fold less than controls. In roots, the reduction in the K^+/Na^+ ratio of -NB plants treated with 150 mM NaCl was approximately 38 times less than its control, while such a reduction in +NB plants was almost 800 times less than its control (Table 3). At the highest salt dose, the K^+/Na^+ ratio (which indicates ionic toxicity) was less than 1.0 in the roots of both groups (Table 3).

A reduction of the K^+/Na^+ ratio was observed in both leaves and plant roots in the -NB and +NB treatments with increasing salt concentration. However, toxic effects caused by the accumulation of Na^+ in the studied tissues were not observed, except in the roots of the plants submitted to 150 mM NaCl of the -NB and +NB treatments. The K^+/Na^+ ratio is considered a good indicator of ionic toxicity, where values higher than 1.0 indicate no effect of the ionic component of saline stress on the plant (MAATHUIS; AMTMANN, 1999). Despite this ratio not being a conclusive parameter for salt tolerance discrimination among some species (WEI et al., 2003), the values obtained for the K^+/Na^+ ratio in this work were higher than 1.0, evidencing that there was probably no toxic effect of ions on cv. MD Gold pineapples. This reinforces that the deleterious effects of Na^+ were not confirmed by the K^+/Na^+ ratio, suggesting maintenance of K^+ concentration at acceptable levels. This effect is particularly important for salt tolerance because maintaining cytoplasmic K^+ levels is essential for survival in saline environments (KAYA et al., 2010).

Leaf total soluble sugars (TSS), total free amino acids (TFAA) and proline (PRO) concentrations were also evaluated in our study (Table 4). In general, the pre-treatment with phytohormones interfered in the accumulation of total soluble sugars (TSS) in the leaves. Significant differences in TSS concentrations were observed between -NB and +NB plants. The accumulation of this organic solute was seven times higher in the leaves of plants treated with phytohormones (Table 4). TSS content in -NB plants was on average 84% lower than that observed in +NB plants. A gradual reduction of sugar content with increasing salt dose was also found in both groups of plants. In the leaves of -NB and +NB plants treated with 150 mM NaCl, a reduction of more than 40% was observed in the sugar content in relation to their respective controls (Table 4).

A reduction in TSS concentration was observed in leaves of cv. MD Gold pineapples as the

NaCl concentration in the nutrient solution increased. In this case, it is possible that the release of hexoses from the hydrolysis of sucrose has been exploited by anabolic or catabolic growth and/or storage processes, as suggested by the maintenance of DM in plants of the +NB treatment (CHAVES FILHO; STACCIARINI-SERAPHIN, 2001). An association of TSS accumulation in leaves of different plant species with possible osmotic adjustment by plants has been commonly discussed (MUNNS, 2005; NIO et al., 2011). However, in this study, the reduction of TSS levels in both organs studied, and especially in the -NB treatment subjected to salt treatment, can be related to disturbances caused by ionic and osmotic components of salinity to the photosynthetic apparatus of pineapple plants. In fact, considering that when photosynthetic production becomes insufficient to meet the demand, the catabolism of soluble carbohydrates sustains plant metabolism (JIE et al., 2010). TSS accumulation with increasing NaCl concentrations was not observed, suggesting that TSS does not participate in the osmotic adjustment mechanism in cv. MD Gold pineapple.

The highest TSS concentrations in cv. MD Gold pineapple observed in +NB plants when compared to -NB treatment indicate that TSS accumulation may be partly due to its direct involvement in the cell division and cell growth processes, thus leading to DM maintenance even in salt treatments. Bhatia and Singh (2002) reported that adding auxin to the nutrient solution increases starch levels in grains of *Sorghum vulgare* (L.). On the other hand, exogenous cytokinin application (ASTHIR et al., 1998) can promote an accumulation of sucrose in *Triticum aestivum* (L.). LeClere et al. (2010) found a correlation between sugar content, auxin levels and the expression of an auxin biosynthesis gene in *Zea mays*. Although a link between sugars and phytohormone participation has been suggested in this paper, the mechanism of this relationship still remains unclear.

The presence of NaCl in the nutrient solution caused a significant increase in the concentration of total free amino acids (TFAA) in leaves of both plant groups (-NB and +NB). This effect was proportional to the increase in NaCl concentration and was more intense in -NB plants. In presence of 150 mM NaCl and in leaves of -NB and +NB plants, increases in TFAA concentration of 140 and 95%, respectively, were observed in relation to their controls (Table 4). These results indicated that TFAA, especially proline, had their synthesis increased and/or their metabolic uses decreased. Another cause of increased concentrations of these osmolytes may be justified by disorders in protein metabolism with occurrence of proteolysis, for example. This type of response is commonly related to a strategy of plants to combat stressful saline conditions (PÉREZ-PÉREZ et al., 2009; HAMDIA; SHADDAD, 2010). Authors such as Tester and Davenport (2003) and Munns and Tester (2008) suggest that these solutes play an important role in minimizing salt entry into the plant, or even in regulating salt concentration in the cytoplasm.

In this study, the minimization of salt effects was not proven due to the accumulation of Na^+ in the foliar tissues. However, Cuin and Shabala (2005; 2007) proposed that amino acids can substantially mitigate the effects of salt stress on potassium (K^+) homeostasis, enhancing plants adaptation to salinity, and reducing the amplitude of K^+ efflux induced by NaCl, as seen in our study in leaves from +NB plants.

In general, pre-treatment with phytohormones interfered with the accumulation of proline (PRO) in leaves. Significant differences in the concentrations of PRO were observed between -NB and +NB plants. Accumulation of this organic solute was 2.5 times greater in leaves of the plants that were not treated with phytohormones (Table 4). In -NB plants, the content of PRO was on average 60% higher than that observed in +NB plants. The presence of NaCl in the nutrient solution caused a significant increase in PRO concentration in leaves of both groups of plants (-NB and +NB). This effect was proportional to the increase of the NaCl concentration and more intense in -NB plants. An increase in the concentration of PRO above 90% was observed in presence of 150 mM salt and in the -NB and +NB plant leaves in relation to its controls (Table 4).

In this experiment, we observed increases in PRO concentrations proportional to the increase of NaCl in the nutrient solution. However, the observed values may not be sufficient to configure an osmotic adjustment, since plants treated with NaCl both from the -NB and the +NB treatments presented water deficiency under salt stress conditions. This fact suggests that the increase in concentrations of this amino acid is merely a disturbance to the metabolic pathways caused by salt stress (for example), rather than an actual tolerance mechanism (ERRABII et al., 2007). It is also possible that increases in the levels of PRO, since they are also a TFAA, are also a consequence of an increase in protein catabolism (SILVEIRA et al., 2003). Moreover, proline accumulation in many plants in general represents a response to water stress, and along with compounds containing nitrogen (such as is the case of amino acids), they are among the major solutes that accumulate in response to salinity (PARIDA; DAS, 2005; GARCÍA-SÁNCHEZ et al., 2007).

In the present experiment, plants from the +NB treatment had reduced PRO levels in cv. MD Gold pineapple leaves for all salt treatments when compared to -NB treatment. In general, this result reinforces the previous findings by Das et al. (2002) and Kaya et al. (2010), where a reduction in proline accumulation was also observed in *Morus alba* (L.) and *Zea mays* (L.), respectively, treated with phytohormones and submitted to salinity. According to these authors, the results obtained in plants treated with phytohormones indicate a partial exemption of the unfavorable conditions imposed by the saline treatment, since plants from the +NB group did not show alteration in DM production. On the other hand, PRO concentrations in cv. MD Gold pineapples from -NB treatment were significantly higher than those from +NB treatment. In this group of plants, maintenance of EL rates regardless of saline concentration suggests the important role of PRO in favoring the stability of cell membranes, thereby minimizing the damage caused by salt stress to thes structures (BABU et al., 2012).

TABLE 1- Mean values of dry mass (DM), relative water content (RWC), moisture percentage (MP) and electrolyte leakage (EL) in leaves and roots of 'Gold' pineapple seedlings from *in vitro* saline treatments in absence (-NB) and presence (+NB) of phytohormones (6-benzylaminopurine + naphthaleneacetic acid) submitted to different concentrations of NaCl (control – 0; 50; 100 and 150 mM) in hydroponic conditions for 10 days.

Parameter	NaCl (mM)	Leaves		Roots	
		Phytohormones		Phytohormones	
		-NB	+NB	-NB	+NB
$DM_{(g)}$	0	0,214 Aa	0,160 Aa	0,017 Aa	0,005 Bb
	50	0,158 Ab	0,176 Aa	0,010 Ab	0,009 Aa
	100	0,110 Abc	0,119 Aa	0,014 Aab	0,005 Bb
	150	0,080 Ac	0,146 Aa	0,014 Aab	0,005 Bb
$RWC_{(\%)}$	0	89,5 Aa	91,3 Aa	93,1 Aa	89,9 Aa
	50	83,3 Ab	85,8 Aa	92,2 Aa	89,2 Aa
	100	75,9 Ac	77,9 Ab	85,5 Ab	89,1 Aa
	150	64,7 Bd	77,4 Ab	84,2 Ab	94,7 Aa
$MP_{(\%)}$	0	92,5 Aa	91,6 Aa	92,7 Aa	91,4 Aa
	50	92,1 Aab	91,1 Bab	94,8 Aa	92,5 Ba
	100	91,3 Ab	90,0 Bbc	93,7 Aa	91,2 Ba
	150	89,7 Abc	89,6 Ac	93,5 Aa	91,5 Ba
$EL_{(\%)}$	0	32,6 Aa	15,2 Bc	30,0 Ac	16,4 Bc
	50	30,1 Aa	16,2 Bbc	51,2 Ab	36,4 Bb
	100	30,4 Aa	20,2 Bb	51,4 Ab	44,5 Bab
	150	34,2 Ba	45,3 Aa	70,8 Aa	51,1 Ba

Means followed by the same capital letter in a row and the same lowercase letter in a column do not differ statistically for each organ studied by the Tukey averages test at 5% probability.

TABLE 2 - Mean values of sodium (Na$^+$) and potassium (K$^+$) concentrations and K$^+$/Na$^+$ ratio in leaves and roots of 'Gold' pineapple seedlings from *in vitro* saline treatments in absence (-NB) and presence (+NB) of phytohormones (6-benzylaminopurine + naphthaleneacetic acid) submitted to different concentrations of NaCl (control – 0; 50; 100 and 150 mM) in hydroponic conditions for 10 days.

Parameters	NaCl (mM)	Leaves		Roots	
		Phytohormones		Phytohormones	
		-NB	+NB	-NB	+NB
Na$^+$ (μmol g^{-1} DM)	0	0,84 Ad	0,88 Ac	2,75 Ad	0,88 Bc
	50	13,64 Bc	16,26 Ab	28,07 Bc	64,14 Ab
	100	22,42 Ab	21,82 Ab	49,29 Bb	93,97 Aa
	150	27,19 Ba	54,43 Aa	62,76 Aa	78,16 Aab
K$^+$ (μmol g^{-1} DM)	0	1794,39 Aa	1699,29 Bb	94,36 Bc	379,13 Aa
	50	1680,94 Bab	1900,02 Aa	166,09 Ba	277,05 Ab
	100	1522,48 Bb	1936,96 Aa	116,48 Bb	273,41 Ab
	150	1154 Bc	1920,77 Aa	55,99 Ad	44,75 Ac
K$^+$/Na$^+$	0	2131,11 Aa	1924,46 Ba	34,22 Ba	429,36 Aa
	50	123,20 Ab	116,84 Ab	5,91 Ab	4,31 Ab
	100	67,90 Bc	88,75 Ab	2,36 Ac	2,90 Ac
	150	42,43 Ac	35,28 Ac	0,89 Ad	0,57 Bd

Means followed by the same capital letter in a row and the same lowercase letter in a column do not differ statistically for each organ studied by the Tukey averages test at 5% probability.

TABLE 3- Mean values of total soluble sugars (TSS), total free amino acids (TFAA) and proline (PRO) concentrations in leaves of 'Gold' pineapple seedlings from *in vitro* saline treatments in absence (-NB) and presence (+NB) of phytohormones (6-benzylaminopurine + naphthaleneacetic acid) submitted to different concentrations of NaCl (control – 0; 50; 100 and 150 mM) in hydroponic conditions for 10 days.

Parameters	NaCl (mM)	Phytohormones	
		-NB	+NB
TSS (μmol.g^{-1}DM)	0	147,80 Ba	1045,51 Aa
	50	157,95 Ba	875,51 Aab
	100	131,74 Bab	745,04 Abc
	150	98,88 Bb	636,18 Ac
TFAA (μmol . g^{-1}DM)	0	249,21 Ad	191,41 Ab
	50	327,82 Ac	183,08 Bb
	100	497,42 Ab	366,92 Aa
	150	598,02 Aa	374,55 Ba
PRO (μmol . g^{-1}DM)	0	11,79 Ac	4,65 Bd
	50	13,43 Abc	5,48 Bc
	100	16,55 Ab	6,20 Bb
	150	22,93 Aa	9,14 Ba

Means followed by the same capital letter in a row and the same lowercase letter in a column do not differ statistically for each organ studied by the Tukey averages test at 5% probability.

CONCLUSIONS

Pre-treatment with phytohormones (NAA and BAP) in cv. MD Gold pineapple minimized the effects of NaCl concentrations added to the nutrient solution, especially in biomass maintenance, in reducing damage to cell membranes, increasing potassium and in increasing total soluble sugars levels.

High NaCl concentrations in the nutrient solution affected the water status of cv. MD Gold pineapple, especially in leaves, increased damage to leaf and root membranes, increased Na^+ levels in detriment of K^+ levels and interfered in the metabolism of total soluble sugars, total free amino acids and proline, more expressively in the plants of the -NB group.

Osmotic effect of salt stress was predominant over ionic stress, considering the K^+/Na^+ ratio was higher than 1.0, with the exception of roots submitted to 150 mM NaCl.

Both leaves and roots presented different responses regarding pre-treatment with phytohormones, and to increasing NaCl added to the nutrient solution.

ACKNOWLEDGMENTS

The financial support received from the Empresa de Pesquisa Agropecuária do Rio Grande do Norte (EMPARN), Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

REFERENCES

ADEM, G. D.; ROY, S. J.; ZHOU M.; BOWMAN, J. P.; SHABALA, S. B. M. C. Evaluating contribution of ionic, osmotic and oxidative stress components towards salinity tolerance in barley. **Plant Biology**, Stuttgart, v.14, n.1, p.113, 2014.

AKBARI, G.; SANAVY, S.A.; YOUSEFZADEH, S. Effect of auxin and salt stress (NaCl) on seed germination of wheat cultivars (*Triticum aestivum* L.). **Pakistan Journal of Biological Science**, Pakistan, v.10, n.1, p.2557-61, 2007.

ALBACETE, A.; GHANEM, M.E.; MARTÍNEZ-ANDÚJAR, C.; ACOSTA, M.; SÁNCHEZ-BRAVO, J.; MARTÍNEZ, V.; LUTTS, S.; DODD, I.C.; PÉREZ-ALFOCEA, F. Hormonal changes in relation to biomass partitioning and shoot growth impairment in salinized tomato (*Solanum lycopersicum* L.) plants. **Journal of Experimental Botany**, Oxford, v.59, n.15, p.4119-31, 2008.

ALVES, F.A.L.; FERREIRA-SILVA, S.L.; SILVEIRA, J.A.G.; PEREIRA, V.L.A. Efeito do Ca^{2+} externo no conteúdo de Na^+ e K^+ em cajueiros expostos a salinidade. **Revista Brasileira de Ciências Agrárias**, Recife, v.6, n.4, p.602-8, 2011.

AROCA, R.; PORCEL, R.; RUÍZ-LOZANO, J.M. Regulation of root water uptake under abiotic stress conditions. **Journal of Experimental Botany,** Oxford, v.63, n.1, p.43-57, 2012.

ASTHIR, B.; KAUR, A.; BASRA, A.S. Do phytohormones influence the uptake and metabolism of sucrose in spikelets of wheat? **Phyton**, Horn, v.38, n.1, p.293-299, 1998.

BABU, M.A.; SINGH, D.; GOTHANDAM, K.M. The effect of salinity on growth, hormones and mineral elements in leaf and fruit of tomato cultivar PKM1. **Journal of Animal & Plant Sciences**, Nairobi, v.22, n.1, p.159-64, 2012.

BARREIRO NETO, M.; LACERDA, J.F. de; CARVALHO, R.A.; OLIVEIRA, E.F. de. **Paraíba-Rubi**: cultivar de abacaxi resistente à fusariose. João Pessoa: Emepa, 2007. 4p.

BARTHOLOMEW, D.P. MD-2 pineapple transform the world`s pineapple fresh fruit export industry. **Pineapples News**, Honolulu, n.16. p 2-5, 2009.

BATES, L.S.; WALDREN, R.P.; TEARE, I.D. Rapid determination of free proline for water-stress studies. **Plant and Soil**, Dordrecht, v.39, p.205-7, 1973.

BENDERRADJI, L.; BRINI, F.; AMAR, S.B.; KELLOU, K.; AZAZA, J.; MASMOUDI, K.; HAMENNA, B.; HANIN, M. Sodium transport in the plants of two bread wheat (*Triticum aestivum* L.) genotypes showing contrasting salt stress tolerance. **Australian Journal of Crop Science**, Brisbane, v.5, n.1, p.233-41, 2011.

BHATIA, S.; SINGH, R. Phytohormonemediated transformation of sugars to starch in relation to the activities of amylases, sucrose-metabolising enzymes in sorghum grain. **Plant Growth Regulation**, Ames, v.1, n.1, p.1-8, 2002.

BLUM, A.; EBERCON, A. Cell membrane stability as a measure of drought and heat tolerance in wheat. **Crop Science,** Madison, v.21, n.1, p.43-7, 1981.

CABOT, C.; SIBOLE, J.V.; BARCELÓ, J.; POSCHENRIEDER, C. Lessons from crop plants struggling with salinity. **Plant Science**, New York, v.226, p.2-13, 2014.

CARVALHO, J.S.B.; WILLADINO, L.G.; CAMARA, T.R.; PORTO, A.L.F.; NOGUEIRA, R.J.M.C. Acúmulo de solutos orgânicos em genótipos de Bananeira (*musa* spp.) sob estresse salino *in vitro*. **Plant Cell Culture & Micropropagation**, Lavras, v.2, n.1, p.1-8, 2006.

CAVALCANTI, F.R.; OLIVEIRA, J.T.A.; MARTINS-MIRANDA, A.S.; VIÉGAS, R.A.; SILVEIRA, J.A.G. Superoxide dismutase, catalase and peroxidase activities do not confer protection against oxidative damage in salt-stressed cowpea leaves. **New Phytologist**, London, v.163, n.1, p.563-71, 2004.

CHAVES FILHO, J.T.; STACCIARINI-SERAPHIN, E. Alteração no potencial osmótico e teor de carboidratos solúveis em plantas jovens de lobeira (*Solanum lycocarpum* St.Hil.) em resposta ao estresse hídr**(1)**ico. **Revista Brasileira de Botânica**, São Paulo, v.24, n.2, p.199-204, 2001.

CLAEYS, H.; INZÉ, D. The agony of choice: How plants balance growth and survival under water-limiting conditions. **Plant Physiology**,Washington, v.162, p.1768-79, 2013.

CUIN, T.A.; SHABALA, S. Exogenously supplied compatible solutes rapidly ameliorate NaCl-induced potassium efflux from barley root. **Plant and Cell Physiology**, Oxford, v.46, n.1, p.1924-33, 2005.

CUIN, T.A.; SHABALA, S. Amino acids regulate salinity-induced potassium efflux in barley root epidermis. **Planta**, Hidelberger, v.225, n.1, p.753-61, 2007.

DAS, C.; SENGUPTA, T.; CHATTOPADHYAY, S.; SETUA, M.; DAS, N.K.; SARATCHANDRA, B. Involvement of kinetin and spermidine in controlling salinity stress in mulberry (*Morus alba* L. cv. S-1). **Acta Physiologiae Plantarum**, Warszawa, v.24, n.1, p.53-57, 2002.

DÍAZ-LÓPEZ, L.; GIMENO, V.; LIDÓN, V.; SIMÓN, I.; MARTÍNEZ, V.; GARCÍA- SÁNCHEZ, F. The tolerance of *Jatropha curcas* seedlings to NaCl: an ecophysiological analysis. **Plant Physiology and Biochemistry**, Kalyani, v.54, n.1, p.34-42, 2012.

DUBOIS, M.; GILLES, K.A.; HAMILTON, J.K.; REBERS, P.A.; SMITH, F. Colorimetric Method for determination of sugars and related compounds. **Analytical Chemistry**, Washington, v.28, n.3, p.350-6, 1956.

ERRABII, T.; GANDONOU, C.B.; ESSALMANI, H.; ABRINI, J.; IDAOMAR, M.; SENHAJI, N.S. Effects of NaCl and mannitol induced stress on sugarcane (*Saccharum sp*.) callus cultures. **Acta Physiologiae Plantarum**, Berlin, v.29, n.1, p.95-102, 2007.

FAO - Food and Agriculture Organization. **Soil salinity management**. 2014. Disponível em: <http://www.fao.org/tc/exact/sustainable-agricultureplatform-pilot-website/soil-salinity-management/en/>. Acesso em: 20 jan. 2014.

FARIAS, S. G. G.; SANTOS, D. R.; FREIRE, A. L. O.; SILVA, R. B. Estresse salino no crescimento inicial e nutrição mineral de Gliricídia (*Gliricidia sepium* (Jacq.) Kunt ex Steud) em solução nutritiva. **Revista Brasileira de Ciência do Solo**, Viçosa, MG, v. 33, p. 1499-505, 2009.

FLOWERS, T.J.; MUNN, R.; COLMER, T.D. Sodium chloride toxicity and the cellular basis of salt tolerance in halophytes. **Annals of Botany**, London, v.115, n.3, p.419-31, 2014.

GARCÍA-SÁNCHEZ, F.; SYVERTSEN, J.P.; GIMENO, V.; BOTÍA, P.; PEREZ-PEREZ, J.G. Responses to flooding and drought stress by two citrus rootstock seedlings with different water use efficiency. **Physiologia Plantarum**, Copanhagen, v.130, n.1, p.532-42, 2007.

HAMDIA, M.A.; SHADDAD, M. A. K. Salt tolerance of crop plants. **Journal of Stress Physiology & Biochemistry**, Irkutsk, v.6, n.3, p.64-90, 2010.

HASEGAWA, P.M.; BRESSAN, R.A.; ZHU, J.K.; BOHNERT, H.J. Plant cellular and molecular responses to high salinity. **Annual Review of Plant Physiology and Plant Molecular Biology**, Palo alto, v.51, n.1, p.463-99, 2000.

HOAGLAND, D.R.; ARNON, D.I. **The water culture method for growing plants without soils**. Berkeley: California Agricultural Experimental Station, 1950. 347p.

IBGE – Instituto Brasileiro de Geografia e Estatística. **Levantamento Sistemático da Produção Agrícola,** Rio de Janeiro, v.9, n.4, p.1-81, 2015.

INAL, A.; GUNES, A.; PILBEAM, D.J.; KADIOGLU, Y.K.; YUSUF, K.; ERASLAN, F. Concentrations of essential and nonessential elements in shoots and storage roots of carrot grown in NaCl and Na_2SO_4 salinity. **X-Ray Spectrometry**, New York, v.38, n.1, p.45-51, 2009.

IRIGOYEN, J.J.; EMERICH, D.W; SÁNCHEZ-DÍAZ, M. Water stress induced changes in concentrations of proline and total soluble sugars in nodulated alfafa (*Medicago sativa*) plants. **Physiologia Plantarum**, København, v.84, n.1, p.55-60, 1992.

JIE, Z.; YUNCONG, Y.; STREETER, J.G.; FERREE, D.C. Influence of soil drought stress on photosynthesis, carbohydrates and the nitrogen and phophorus absorb in different section of leaves and stem of Fugi/M.9EML, a young apple seedling. **African Journal of Biotechnology**, Nairobi, v.9, n.33, p.5320-5, 2010.

JOSEPH, E.A.; RADHAKRISHNAN, V.V.; MOHANAN, K.V. A Study on the Accumulation of Proline - An Osmoprotectant Amino Acid under Salt Stress in Some Native Rice Cultivars of North Kerala, India. **Universal Journal of Agricultural Research**, Alhambra, v.3, n.1, p.15-22, 2015.

JULKOWSKA, M.M.; TESTERINK, C. Tuning plant signaling and growth to survive salt. **Trends in Plant Science**, Cambridge, v.20, n.9, p.586-94, 2015.

JUNIOR, G.S.E S.; MORAIS, M.B.; CAMARA, T.R.; WILLADINO, L. Crescimento de genótipos diplóides de bananeira submetidos ao estresse salino. **Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v.16, n.11, p.1145-51, 2012.

KAYA, C.; TUNA, A. L.; OKANT, A. M. Effect of foliar applied kinetin and indole acetic acid on maize plants grown under saline conditions. **Turkish Journal of Agriculture and Forestry**, Ankara, v.34, n.1, p.529-38, 2010.

KOYRO, H. W. Effect of salinity on growth, photosynthesis, water relations and solute composition of the potential cash crop halophyte *Plantago coronopus* (L.). **Environmental and Experimental Botany**, Amsterdam, v.56, n.2, p.136-46, 2006.

LECLERE, S.; SCHMELZ, E.A.; CHOUREY, P.S. Sugar levels regulate tryptophan-dependent auxin biosynthesis in developing maize kernels. **Plant Physiology**, Rockville, v.153, n.1, p.306-18, 2010.

LI, R.; SHI, F.; FUKUDA, K. E YANG, Y. Effects of salt and alkali stresses on germination, growth, photosynthesis and ion accumulation in alfalfa (*Medicago sativa* L.). **Soil Science and Plant Nutrition**, Tokyo, v.56, n.1, p.725–33, 2010.

LOPES, J. C.; MACEDO, C. M. P. Germinação de sementes de sob influência do teor de substrato e estresse salino. **Revista Brasileira de Sementes**, Londrina, v.30, p.79-85, 2008.

MAATHUIS, F. J. M.; AMTMANN, A. K^+ Nutrition and Na^+ Toxicity: The Basis of Cellular K^+/Na^+ Ratios. **Annals of Botany**, London, v.84, n.1, p.123-33, 1999.

MACÊDO, C.E.C.; BARROSO, P.A.V.; MOURA, G.E.D.D.; ALLOUFA, A.I.A. Efeito do NaCl sobre o crescimento e a multiplicação *in vitro* de bananeira. **Revista Brasileira de Fruticultura***,* Jaboticabal, v.27, n.2, p.194-7, 2005.

MAIA, P.S.P.; OLIVEIRA NETO, C.F.; CASTRO, D.S.; FREITAS, J.M.N.; LOBATO, A.K.S.; COSTA, R.C.L. Conteúdo relativo de água, teor de prolina e carboidratos solúveis totais em folhas de duas cultivares de milho submetidas a estresse hídrico. **Revista Brasileira de Biociências**, Porto Alegre, v.5, n.2, p.918-20, 2007.

MENDES, B.S.S.; WILLADINO L.; CUNHA, P.C.; OLIVEIRA FILHO, R.A.; CAMARA, T.R. Mecanismo fisiológicos e bioquímicos do abacaxi ornamental sob estresse salino. **Revista Caatinga**, Mossoró, v.24, n.3, p.71-7, 2011.

MELO, Y.L.; GOMES, I.A.; DANTAS, C.V.S.; BRITO, L.K.F.; OLIVEIRA, M.D.M.; MACÊDO, C.E.C. Indicadores de estresse salino em abacaxizeiro cultivado na ausência e presença de fitorreguladores. **Revista Brasileira de Fruticultura**, Jaboticabal, v.33, n.3, p.698-705, 2011.

MORAES, A.M.; ALMEIDA, F.A.C.; BRUNO, R.L.A.; FILHO, J.C.; NUNES, S.T.; GOMES, J.P. Micropropagação de abacaxizeiro cv.Emepa 1.**Revista Brasileira de Engenharia Agrícola e Ambiental**, Campina Grande, v.14, n.9, p.932-6, 2010.

MUNNS, R. Comparative physiology of salt and water stress. **Plant, Cell and Environment**, Oxford, v.25, n.1, p.239-50, 2002.

MUNNS, R. Genes and Salt Tolerance: Bringing Them Together. **New Phytologist**, Cambridge, v.167, p.645-63, 2005.

MUNNS, R.; TESTER, M. Mechanisms of salinity tolerance. **Annual Review of Plant Biology**, Stuttgart, v. 59, n.1, p.651-81, 2008.

NASSAR, A.M.K.; KUBOW, S.; DONNELLY, D.J. High-throughput screening of sensory and nutritional characteristics for cultivar selection in commercial hydroponic greenhouse crop production. **International Journal of Agronomy**, New York, p.1-28, 2015.

NIO, S.A.; CAWTHRAY, G.R.; WADE, L.J.; COLMER, T.D. Pattern of solutes accumulated during leaf osmotic adjustment as related to duration of water deficit for wheat at the reproductive stage. **Plant Physiology and Biochemistry**, Kalyani, v.49, p.1126-37, 2011.

OLIVEIRA, L.A.A.; BARRETO, L.P.; NETO, E.B.; SANTOS, M.V.F.; COSTA, J.C.A. Solutos orgânicos em genótipos de sorgo forrageiro sob estresse salino. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.41, n.1, p.31-5, 2006.

PARIDA, A.K.; DAS, A.B. Salt tolerance and salinity effects on plants: a review. **Ecotoxicology and Environmental Safety**, Orlando, v.60, n.1, p.324-49, 2005.

PEOPLES, M.B.; FAIZAH, A.W.; REAKASEM, B.; HERRIDGE, D.F. **Methods for evaluating nitrogen fixation by nodulated legumes in the field**. Canberra: Australian International Center of Agricultural Research, 1989. 76p.

PÉREZ-PÉREZ, J.G.; ROBLES, J.M.; TOVAR, J.C.; BOTÍA, P. Response to drought and salt stress of lemon 'Fino 49' under field conditions: water relations, osmotic adjustment and gas exchange. **Scientia Horticulturae**, New York, v.122, n.1, p.83-90, 2009.

POSPISILOVA, J.; VAGNER, M.; MALBECK, J.; TRAVNICKOVA, A.; BATKOVA, P. Interactions between abscisic acid and cytokinins during water stress and subsequent rehydration. **Biologia Plantarum**, Dordrecht, v.49, n.1, p.533-40, 2005.

PRAXEDES, S.C.; LACERDA, C.F. de; DAMATTA, F.M.; PRISCO, J.T.; GOMES-FILHO, E. Salt tolerance is associated with differences in ion accumulation, biomass allocation and photosynthesis in cowpea cultivars. **Journal of Agronomy and Crop Science**, v.196, n.1, p.193-204, 2010.

SÁNCHEZ, A.S.; NOGUEIRA, I.B.R.; KALID, R.A. Uses of the reject brine from inland desalination for fish farming, Spirulina cultivation, and irrigation of forage shrub and crops. **Desalination**, Amsterdam, v.364, n.1, p.96-107, 2015.

SARRUGE, J. R. S.; HAAG, H. P. **Análises químicas em plantas**. Piracicaba: USP-ESALQ, 1974. 56p.

SHABALA, L.; MACKAY, A.; TIAN, Y.; JACOBSEN, S.; ZHOU, D.; SHABALA, S. Oxidative stress protection and stomatal patterning as components of salinity tolerance mechanism in quinoa (*Chenopodium quinoa*). **Physiologia Plantarum**, Kabenhavn, v.146, n.1, p.26-38, 2012.

SHABALA, S.; MUNNS, R. **Salinity stress**: physiological constraints and adaptive mechanisms. London: CAB, 2012. 291 p.

SILVA, E.N.; SILVEIRA, J.A.G.; RODRIGUES, C.R.F.; LIMA, C.S.; VIÉGAS, R.A. Contribuição de solutos inorgânicos no ajustamento osmótico de pinhão-manso submetido à salinidade. **Pesquisa Agropecuária Brasileira**, Brasília, DF, v.44, n.5, p.437-45, 2009a.

SILVA, R.L.O.; MARTINS, L.S.S.; GOMES, E.W.F.; FERRAZ, G.M.G.; SILVA, S.O.; WILLADINO, L. Avaliação de diplóides de bananeira (*Musa* spp.) quanto à tolerância a salinidade. **Revista Brasileira de Fruticultura**, Jaboticabal, v.31, n.1, p.1084-91, 2009b.

SILVEIRA, J.A.G.; VIÉGAS, R.A.; ROCHA, I.M.A; MONTEIRO-MOREIRA, A.C.O; MOREIRA, R.M.; OLIVEIRA, J.T.A. Proline accumulation and glutamine synthetase are increased by salt induced proteolysis in cashew leaves. **Journal of Plant Physiology**, Rockville, v. 160, p. 115-23, 2003.

SLAVICK, B. **Methods of studying plant water relations**. New York: Springer Verlag, 1974. 449 p.

TESTER, M.; DAVENPORT, R. Na^+ tolerance and Na^+ transport in higher plants. **Annals of Botany**, London, v.91, n.1, p.503-27, 2003.

WEI, W.; BILSBORROW, P.E.; HOOLEY, P.; FINCHAM, D.; LOMBI, A.E.; FORSTER, B.P. Salinity induced differences in growth, ion distribution and partitioning in barley between the cultivar Maythorpe and its derived mutant Golden Promise. **Plant and Soil**, The Hague, v.250, n.2, p.183-91, 2003.

WILLADINO, L.; CAMARA, T.R. Tolerância das plantas à salinidade: aspectos fisiológicos e bioquímicos. **Enciclopédia Biosfera**, Goiânia, v.6, n.11, p.1-23, 2010.

ZHANG, J.L.; FLOWERS, T.J.; WANG, S.M. Mechanisms of sodium uptake by roots of higher plants. **Plant Soil**, The Hague, v.326, n.1, p.45-60, 2010.