Journal of Recent Advances in Applied Sciences

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Research Article

EFFECT OF SALINITY ON SOME MORPHOPHYSIOLOGICAL CHARACTERS OF SOMATIC EMBRYOGENESIS-REGENERATED PLANTS OF PINEAPPLE [*Ananas comosus* (L.) MERR. CV. SMOOTH CAYENNE]

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Received January 03, 2018; Accepted March 02, 2018; Published March 29, 2018;

Cite This Article: Samuel, K., Salomé, Y., Souleymane, C., Hilaire, K.(2018). Effect of salinity on some morphophysiological characters of somatic embryogenesis-regenerated plants of pineapple [*Ananas comosus* (L.) Merr. cv. Smooth Cayenne]. Journal of Recent Advances in Applied Sciences, 31(1). 1-9

ABSTRACT

Pineapple in Côte d'Ivoire is going through problems of various origins. Reviving this state imperatively requires both to clean up the plant material and to select varieties which easily adapt to the current pedoclimatic conditions of growing areas. This work assesses the impact of salinity on regenerated pineapple plants by somatic embryogenesis. The tests were carried out in polyethylene bags filled with sea sand, under semi-controlled conditions. Regenerated pineapple plants were watered with six saline solutions (2, 4, 6, 8, 10 and 14 g/L). Control plants were watered with water. The results revealed that salt stress reduces the studied morphophysiological parameters (number of leaves and roots, length of leaves and roots, chlorophyll a, chlorophyll b and total chlorophyll content, the carotenoids content and the relative water content). Pineapple tolerates low salt levels (2 and 4 g/L). However, important disturbances were observed in the metabolism of stressed plants with high levels of NaCl (6 and 10 g/L). As an adaptation strategy to maintain its metabolism from this stress, pineapple responded by accumulating proline. The highest saline content (14 g/L) was found to be lethal for pineapple plants.

KEYWORDS: Acclimation, *Ananas comosus,* pineapple, sodium chloride, smooth cayenne.

INTRODUCTION

Pineapple (*Ananas comosus* L.) holds an important place in the economy of many developing countries. Indeed, it constitutes a livelihood for a great deal of farmers, as well as a major source of foreign currency earnings. The cultivation of pineapple is therefore of a considerable economic and social importance. In Côte d'Ivoire, for example, pineapple represents 1.2 % of exports and generates an estimated annual revenue of about 34 billion FCFA [1]. In this regard, Côte d'Ivoire has long been the leading supplier of pineapples of the European Union (97 % of the market). However, this prominent place has been delighted by Costa Rica. This decline is linked to many problems (problems of pedoclimatic nature) that undermine this sector. Indeed, the soils degradation, linked to climate change, has favored soil salinization. Acid rains and the uncontrolled use of crop protection products have also accentuated soil salinization. According to FAO [2], salinization affects more than 800 million hectares of farmlands. It is one of the major factors causing the decrease in agricultural productivity in the world. Salinity also affects the physiological processes (perspiration,

photosynthesis, translocation and respiration). In addition, it causes water and/or ionic imbalance in plants [3, 4]. Several studies revealed that some species use strategies to mitigate the impact of salinity. The synthesis of organic compounds called osmoticum and osmoprotectors, such as proteins, soluble sugars [5], amino acids [6], and more specifically proline, is one of the strategies that plants develop to minimize the impact of salinity [7]. Thus, a thorough knowledge of the physiological, chemical, biochemical and genetic mechanisms of salinity tolerance would help to select a variety of pineapple that adapts to pedoclimatic conditions. The objective of this study is to evaluate the impact of salinity on the pineapple vitroplants regenerated through somatic embryogenesis.

The aim of this study was to examine the behavior of pineapple plants from vitroculture in response to NaCl stress after acclimation.

MATERIAL & METHODS

The experiment was carried out at the Plant Tissue Culture Laboratories, Plant Production Research Pole, Nangui Abrogoua University, Abidjan, Côte d'Ivoire.

Plant material

Plant material was consisted to 4-month old acclimated pineapple plants (*Ananas comosus* L. var. Smooth Cayenne, cv. CI 16) from *in vitro* regeneration by somatic embryogenesis.

Application of saline stress

Acclimated plants were transplanted into black polythene bags (10 cm x 20 cm). These bags were pre-filled with sea sand and perforated to prevent water excess and roots asphyxiation. The bags containing the seedlings were kept in a tunnel at a temperature ranging between 28 and 42°C and a relative humidity oscillating between 92 and 95%. These plants were watered with six concentration of NaCl (2, 4, 6, 8, 10 and 14 g/L). Control plants were sprayed with distilled water. Bags were kept under field capacity moisture by regular watering with the different solutions of NaCl and water at a rate of 30 mL per plant every two days. Experimental design was completely random block. Four tests with two repetitions were used. Each saline concentration was one test. For each test, thirty seedlings were used.

Morphophysiological parameters

Pineapple plants were removed from their bags after two months of saline treatments. Then, their leaves and roots were counted and measured.

Determination of chlorophyll pigments and carotenoids

The leaf pigments (chlorophylls and carotenoids) were extracted according to the method described by Dekok *et al*. [8]. This method was adapted to our plant material. The extraction was carried out on the upper third of the leaf D (the rosette longest leaf). Approximately, 100 mg of cut leaves were milled with a pinch of Fontainebleau sand and calcium carbonate in 10 mL of 20 % acetone. The ground material was centrifuged at 5000 rpm during 15 min at 4 °C. The resulted supernatant constituted the crude extract of leaf pigments. The determination of chlorophyll pigments and carotenoids was carried out on the basis of the method described by Lichtenthaler [9]. The different concentrations were calculated according to the following formulas:

Chl a (µg/g FM) = [(12.25 x OD_{663} – 2.79 x OD_{647}) x V]/m
Chl b (µg/g FM) = [(21.5 x OD_{647} – 5.10 x OD_{663}) x V]/m
Chl tot (µg/g FM) = [(7.15 x OD_{663} + 18.71x OD_{647}) x V]/m
Car (µg/g FM) = [((1000 x OD_{470} – 1.82 x Chl a – 85.02 x Chl b) / 198) x V]/m

With, V= final volume of crude extract, m= mass of milled leaves, FM= fresh material, Chl= chlorophyll, Car= carotenoids and OD= Optical density.

Determination of proline

Samples extraction and determination were carried out according to the method of Dreir and Goring (1974) [10]. 100 mg of fresh material (FM) from the leaf D were weighed and then homogenized in 3 mL of 40 % of methanol. The whole was heated in a bain-marie (85 °C) during 30 min. After cooling in melting ice, the mixture was centrifuged at 4000 rpm during 10 minutes and the resulted supernatant constituted the proline extract to be determined. Approximately, 1 mL of collected extract was added to 1mL of glacial acetic acid, 25 mg of ninhydrin and 1 mL of the mixture I (prepared from 120 mL of distilled water, 300 mL of acetic acid (CH_3COOH) and 80 mL of phosphoric acid (H_3PO_4)). The mixture was heated to boiling (100 °C, during 45 min) until it turns red. After cooling, 5 mL of toluene were added to the solution, stirred during about 15 to 20 seconds and then left standing for 30 minutes. The toluene phase was sampled and its absorbance is read at 528 nm with the spectrophotometer (A & ELAB UV/VIS SPECTROPHOTOMETER) using toluene as a reference. The amount of proline is calculated using the extinction coefficient by the below formula:

$$C = \frac{OD}{\varepsilon . d}$$

With, ε= 0.9986 mM/L/cm, d (cm) = Diameter of the spectrophotometer tube and C= concentration of proline (mg/g MF), OD= Optical density.

Relative leaf water content

Relative leaf water content was determined by the method of Clarke and McCaig [11]. The leaves per plant and per bag were weighed in order to determine their fresh weight. Subsequently, the leaves in jars with distilled water were placed in a dark place at 4°C during 24 hours. Then, the turgor weight was determined. The samples were oven dried at 80°C during 48 h to determine dry weight of leaves. The relative content was calculated according to the following formula:

$$RWC = \frac{FW - DW}{TW - DW} \times 100$$

With, FW= fresh weight of the leaves, DW= dry weight of the leaves, TW= turgor weight and RWC= relative water content.

Statistical analysis

Completely randomized design was used with five replicates. The data were subjected to analysis of variances (ANOVA) using the STATISTICA 7.1 software and mean values were compared using Newman-Keuls. For percentages that are non-parametric values, the data was first transformed by Arcsin (√x) before any statistical comparison.

RESULTS

Effect of NaCl on the morphological parameters of pineapple

Leaf length of pineapple plants

The growth in length of leaves is significantly ($p < 0.0001$) influenced by salt stress. The saline concentration of 2 g/L did not considerably influence the leaf length growth (14.02 cm) as compared to the control treatment (15.41 cm); approximately only 9.02 % reduction . However, saline concentrations at a high level (4, 6 and 10 g/L) have inhibited growth in plant length (Table 1). This inhibition is stronger as the concentration of NaCl is high. Thus, 10 g/L was the saline concentration that induced the lowest leaf growth (01.63 cm) as compared to the control concentration (15.41 cm), a reduction of about 89.42 %. This saline concentration caused a crumbling of old leaves as well as a slight yellowing of the leaves. These signs of chlorosis are very often followed by the death of those leaves.

Root length of pineapple plants

The growth in root length is strongly slowed down in the presence of high saline concentrations (4 - 6 and 10 g/L) (Figure 1). Thus, the saline concentration of 4 g/L induced a growth of 6.89 cm (48.85 % reduction) whereas 6 g/L NaCl strongly inhibits growth in root length (4.05 cm or 70.15 % reduction). Saline concentration 10 g/L induced the lowest root growth (0.75 cm), or 94.43 % reduction. In contrast, treatment with 2 g/L NaCl did not significantly influence root growth (11.95 cm) compared to the control treatment (only a reduction of 11.28 %) (Table 1).

Number of pineapple leaves

The results show a decrease in the phyllogenesis of the treated plants as compared to those induced by the control treatment (14.33 leaves), when the concentration of NaCl in the acclimation substrate is greater than 2 g/L. Thus, the lowest numbers of leaves emitted were obtained with the concentrations of 4, 6 and 10 g/L of NaCl; producing respectively 08.33; 05.67 and 02.25 leaves. The saline concentration of 2 g/L allowed the production of the largest number of leaves (13.67 leaves); statistically identical to that of the control (14.33 leaves) (Table 1).

Number of pineapple roots

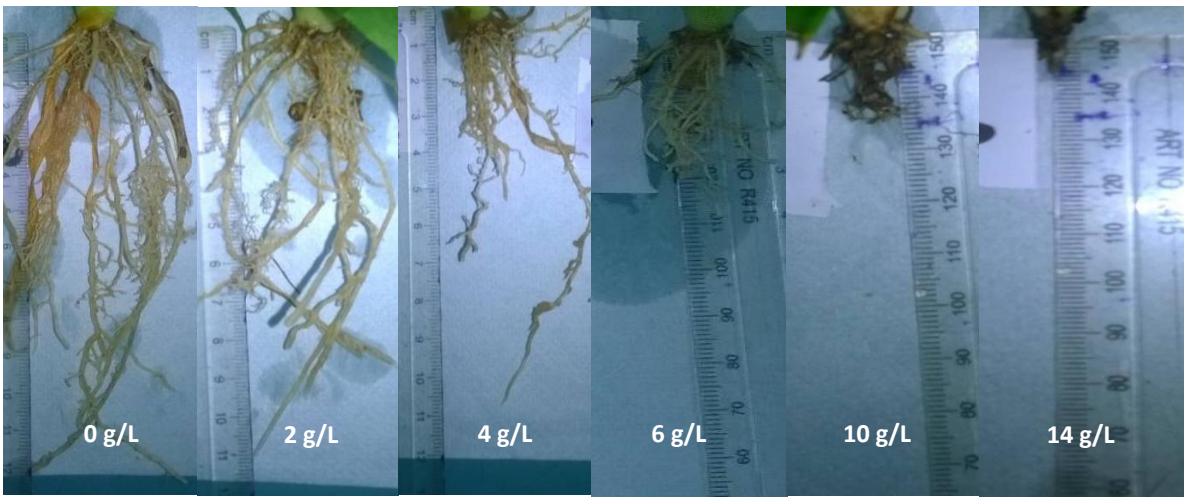
Induction of roots in pineapple plants was significantly influenced ($p<0.0001$) by salt stress (Table 1). Indeed, rhizogenesis decreases with increasing NaCl concentration of the acclimation substrate. Thus, salt concentrations of 4-6 and 10 g/L negatively affected the number of the generated roots (06.33, 03.97 and 01.17 roots, respectively). However, the content of 2 g/L salt did not statistically influence rhizogenesis (10.94 roots) as compared to control treatment (12.21 roots). NaCl concentration of 14 g/L was lethal for all pineapple plants. The results from the salt stress application on the morphological parameters revealed that pineapple tolerates low salt contents (2 g/L) whose effects are statistically identical to those of the control treatment (0 g/L NaCl). On the other hand, all these parameters are strongly influenced as soon as the salt content reaches 4 g/L.

Table 1: Effect of NaCl on vegetative characteristics of pineapple plant

	Morphological parameters			
NaCl (g/L)	**Mean length of leaves (cm)**	**Mean length of roots (cm)**	**Mean number of leaves (no)**	**Mean number of roots (no)**
0	15.41 ± 1.67a	13.47 ± 0.27a	14.33 ± 0.10a	12.21 ± 0.16a
2	14.02 ± 0.91a	11.95 ± 2.07a	13.67 ± 1.47a	10.94 ± 0.09a
4	09.63 ± 1.02b	06.89 ± 2.54b	08.33 ± 1.15b	06.33 ± 1.23b
6	06.02 ± 0.17c	04.02 ± 0.98c	05.67 ± 0.28c	03.97 ± 0.67c
10	01.63 ± 0.21d	0.75 ± 0.36d	02.25 ± 0.81d	01.17 ± 0.19d
14	0 ± 0e	0 ± 0e	0 ± 0e	0 ± 0e

± s : standard error; means with the same letter in a column are not significantly different (test of Newman-Keuls at 5%); data are the mean of triplicates.

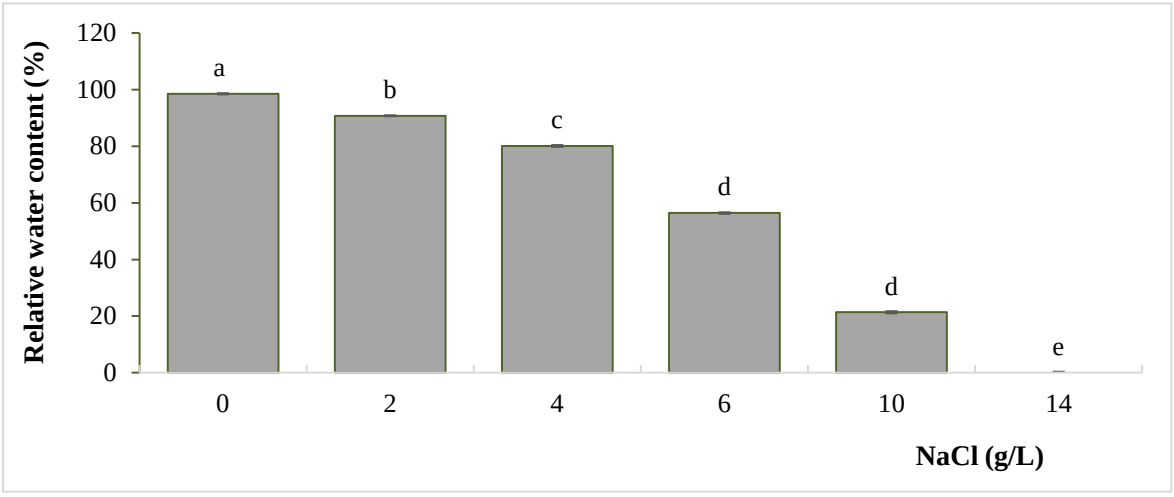
Figure 1: Effect of NaCl concentration on root length of pineapple plants.



Effect of NaCl on pineapple relative leaf water content

The analysis of variance revealed a highly significant negative correlation (r= 0.92; p <0.0001) between salinity and RWC. Thus, the pineapple leaves RWC decreases as the concentration of NaCl in the acclimation substrate increases. From the control plants to those treated with 4 g/L of NaCl, the leaf water content remained slightly affected. However, from a treatment of 6 g/L of NaCl, the RWC experienced a significant drop: 56.42%, a reduction rate of 43.58%). The plants RWC is strongly reduced in the presence of 10 g/L, a reduction of 78.31%. Since the 14 g/L saline concentration was lethal to the tested plants, the RWC has not been determined (Figure 2).

Figure 2: Effect of NaCl concentration on relative water content in pineapple leaves



Values followed of a same letter are not statistically different (test of Newman-keuls at 5%).

Effect of NaCl on leaves pigments content

Effect of NaCl on chlorophyll pigments content

Salt stress has significantly influenced the chlorophyllogenesis (p <0.0001). Indeed, total chlorophyll progressively deteriorates as salt stress intensifies. As far as the treated plants are concerned, the highest rate of total chlorophyll was obtained with the 2 g/L treatment (158,74 µg/g FM), followed by the 4 g/L (118.51 µg/g FM), 6 g/L (98.76 µg/g FM) and 10 g/L treatment (30.56 µg/g FM). The plants did not survive the 14 g/L treatment. As for the Chl a/Chl b report, it is statistically optimal and less than 1 (Chl a/Chl b < 1) in all pineapple plants, whatever the saline concentration used during the stress (Table 2). Chl b level is therefore higher than that of Chl a.

Table 2 : Effect of NaCl concentration on chlorophyll content.

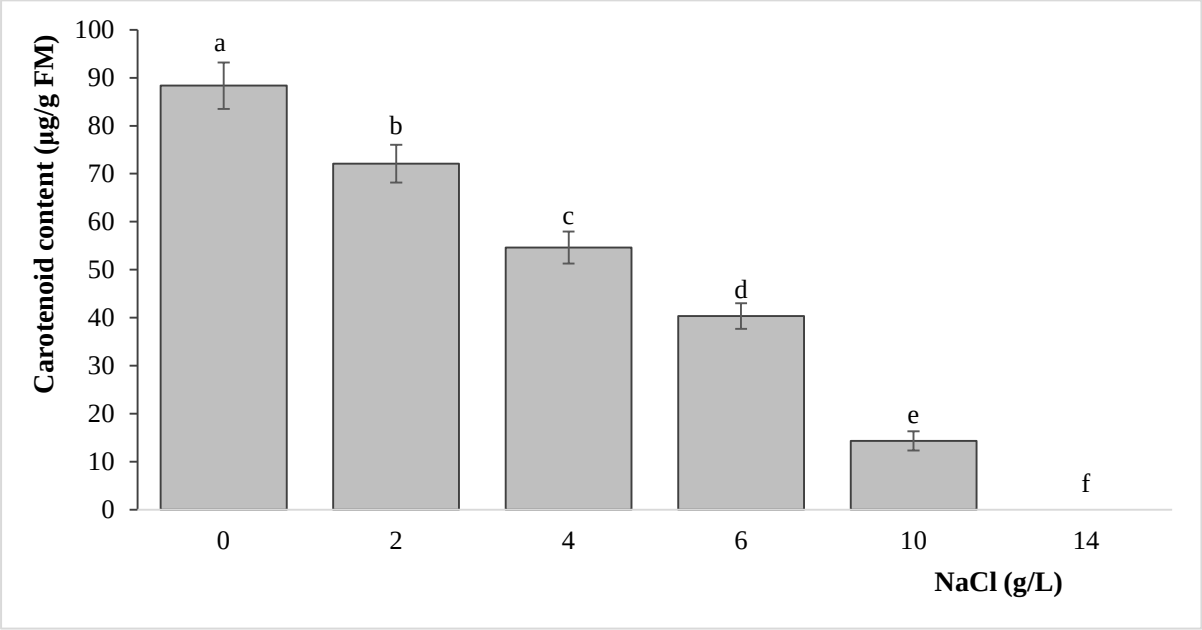
	Chlorophyll content (µg/g FM)			
NaCl (g/L)	**Chl a**	**Chl b**	**Chl a/ Chl b**	**Chl T**
0	56.60 ± 4.01a	125.60 ± 8.62a	0.45 ± 0.001a	182.11 ± 11.45a
2	47.47 ± 4.33b	111.27 ± 4.31b	0.43 ± 0.002a	158.74 ± 8.33b
4	35.67 ± 2.62c	83.05 ± 5.01c	0.43 ± 0.010a	118.51 ± 7.10c
6	28.91 ± 2.58d	69.70 ± 3.64d	0.41 ± 0.023a	98.76 ± 5.97d
10	8.97 ± 2.02e	21.16 ± 4.42e	0.42 ± 0.058e	30.56 ± 2.56e
14	0 ± 0f	0 ± 0f	0 ± 0f	0 ± 0f

Chl a: chlorophyll a; Chl b: chlorophyll b; Chl T: total chlorophyll; FM : fresh material; ± s : standard error; means with the same letter in a column are not significantly different (test of Newman-Keuls at 5 %); data are the mean of triplicates.

Effect of NaCl on carotenoid content

Salt stress significantly influenced the carotenoid content ($p<0.0001$). According to the results shown in Figure 3, the increase in saline concentration is accompanied by a very significant decrease of carotenoid content in the leaves. This content, from 88.35 µg/g FM in control (0 g/L of NaCl) reached the value of 14.35 µg/g FM in the seedlings exposed to the salt concentration of 10 g/L; displaying thus a decrease of more than 83.76 %. This reduction is less severe in seedlings stressed with 2 g/L of NaCl (72.08 µg/g FM, a reduction of about 18.41 %). Plants treated with 4 g/L NaCl have a carotenoid content of 54.63 µg/g FM, a reduction of approximately 38.17 % as compared to that of the control treatment. The maximum saline concentration (14 g/L) caused pineapple plants death.

Figure 3:Effect of NaCl concentrations on carotenoid content in pineapple leaves.

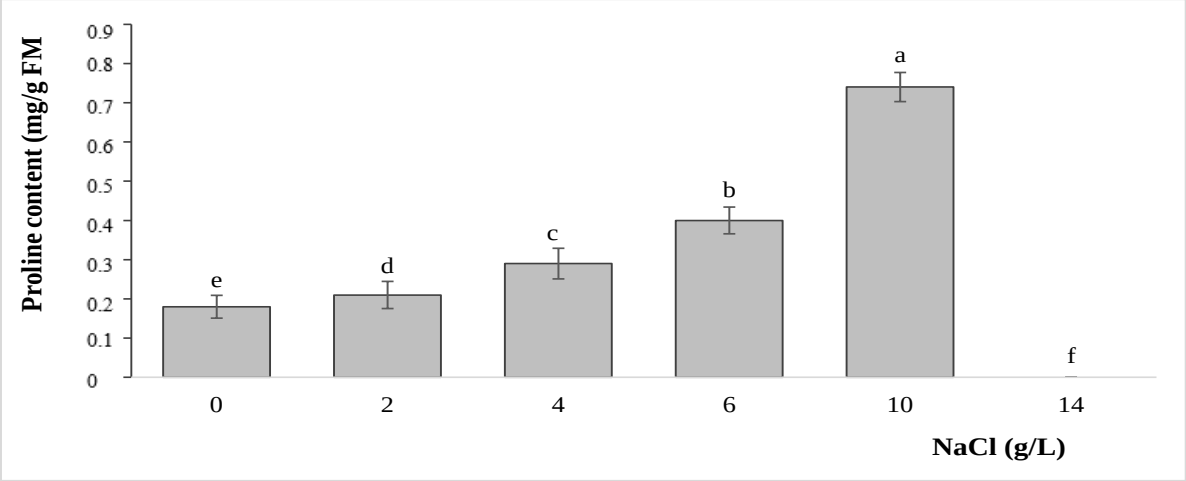


Values followed of a same letter are not statistically different (test of Newman-keuls at 5%)

Effect of NaCl on proline content variation

Salt considerably impacts on the leaf accumulation of proline ($p < 0.001$). The analysis of variances revealed that the proline content in the leaves increases with the saline concentration of the medium. Proline therefore accumulates more in the leaves when the NaCl concentration of the culture substrate increases (Figure 4). However, this proline accumulation is low in the leaves of plants treated with saline solutions of 2 and 4 g/L (respectively 0.21 and 0.29 mg/g FM) as compared to the control accumulation. The highest leaf accumulation of proline is recorded in plants treated with 10 g/L NaCl (0.74 mg/g FM, an increase of 311.11 % as compared to the control content (0.18 mg/g FM).

Figure 4: Effect of NaCl concentrations on proline content in pineapple leaves.



Values followed of a same letter are not statistically different (test of Newman-Keuls at 5%

DISCUSSION

The results of our study showed that pineapple tolerates low salt levels (2 g/L). On the other hand, the high levels (4, 6 and 10 g/L) induced a significant reduction in above-ground and root biomass. This reduction in biomass would probably be due to an accumulation of mineral ions in different parts in the plant. In fact, the Na^+ and Cl- ions, accumulated in the tissues of the plants, would cause a toxicity which affects plants development and growth [12]. In addition, the works of Benmahioul *et al.* [13] showed that the repressive effect of salt on the vegetative tract growth is due to an increase in the osmotic pressure of the medium.

This fact inhibits the root absorption of water. Thus, the plant metabolism would be affected and this results in a decrease in cell division, hence the observed slowdown in growth. The high salt level affects the leaves call for water, necessary for photosynthesis. There would be a disturbance in the carbon nutrition of the plant and consequently a decrease in cell division resulting in a decrease in growth. Similar results have been reported by Achour *et al.* [14] in okra and Koutoua *et al.* [15] as for tomato. Therefore, salt stress strongly affects plants morphophysiological functions. According to our results, the depressive action of salt stress on the morphological parameters has often been accompanied by symptoms such as chlorosis, followed by necrosis in some leaves. These toxicity symptoms reduced the active area for photosynthesis and caused a considerable decrease in growth. These mechanisms which cause the reported growth decrease would be the adaption strategies of plants as a response to the stress [15].

Salt stress induces an increasing decrease of relative water content of pineapple leaves as the level of stress increases. Indeed, according to Rochdi *et al.* [16], salt affects all the plant parts. However it is more noticeable on the leaves with the intensification of salt stress. It consequently induces a decrease in tissue hydration, increases the concentration of the internal medium and consequently prevents the release of water from the plants [17]. Similar results have been reported in maize [18], okra [14] and tomato [15]. Other authors argue that the decrease in relative water content under salt stress is related to the toxicity of Na^+ and/or Cl- accumulated in the cytoplasm to levels exceeding the compartmentalization capacity in the vacuole [16]. The apoplasmic accumulation of Na^+ and Cl^- ions is the parameter the most implicated in fading in some species [19]. The results also showed that despite the intensification of saline treatment, the decrease in the level of hydration remains tolerable; except for a lethal level. This high relative water content maintaining under salt stress is a remarkable form of resistance [20, 15]. The relative water content is therefore generally considered as a sensitivity index for plants under stress.

Regarding the physiological parameters in this study, it is reported that the levels of total chlorophyll and carotenoid are negatively influenced by the saline treatments. The contents of these leaves pigments recorded a significant decrease under stress intensification. Salinity therefore has a depressive impact through a reduction in the content of chlorophyll a, chlorophyll b and total chlorophyll [21, 22, 23]. This is because; under stress, plants reduce all metabolic activities that can induce excessive energy expenditure. Thus, the reduction of the leaf area causes a decrease in the absorption of CO_2 and consequently a reduction in photosynthetic activities [15]. Similar results have been reported by other authors [24, 25]. Furthermore, Levitt [26] reported that the degradation of chlorophylls in leaves under salt stress is due to the instability of the pigmented protein complex, which is disturbed by the excess of Na^+ and Cl^- ions, as well as the breakdown of bonds chelated magnesium. The works of [14] on okra helped to infer that the decrease in pigments synthesis is related to the inhibition of the synthesis of the 5-aminolevulinic acid, a chlorophyll precursor [27, 28]. Indeed, under stress, the disturbance of photosynthetic photochemical reactions blocks electrons transfer between LHC II and PSII [29]. Also, salinity damages the PSII and photosynthetic enzymes [30] and

also inhibits chlorophyllogenesis. In addition, our results show that the chlorophyll a content is more sensitive to the impact of salt stress than chlorophyll b. According to El Iklil *et al.* [31], the reduction in chlorophyll may be related to the sensitivity of one of the stages of its sodium chloride biosynthesis.

This study also showed that the leaf proline content increases with the saline concentration of the culture substrate. Proline therefore accumulates more in the leaves when the NaCl concentration in the medium increases. This accumulation of proline is a protective strategy [32], from the protein metabolism disturbance due to salt stress [21]. Indeed, the synthesis of this amino acid from L-glutamate is catalyzed by an enzyme: Δ1- pyrroline-5-carboxylate synthase (P5C synthase) whose synthesis is induced by salt stress [33, 34]. Many studies have already argued that proline is certainly one of the most common osmotica that plants synthesize when exposed to water or salt stress [12, 14]. According to these authors, the cytoplasmic accumulation of proline permits to neutralize the ionic and osmotic effects of salt accumulation in the vacuole. This proline accumulation is therefore an adaptation phenomenon to salinity; allowing plants to maintain their turgescence by reducing the water potential. It is a form of adjustment of its osmotic potential [35]. In light of the literature, the role attributed to proline in plants response to stress is sometimes controversial [36, 37]. Lemzeri [38] reported that the most morphophysiologically sensitive species react by accumulating proline more rapidly. On the other hand, those that are tolerant have a relative stability or a low accumulation of their proline content as compared to those which are sensitive. Other authors such as argue that a slight accumulation of proline is an adaptation reaction of plants to stress whereas a high accumulation of this amino acid is a sign of metabolic disturbance [39]. In our study, the high proline accumulation observed in plants with reduced growth and exhibiting signs of chlorosis under extreme salt stress, allows us to think that a high accumulation of this amino acid is a sign of metabolic disturbance. As for pineapple, a low accumulation of leaf proline would therefore be an adaptation response to salinity. However, the high levels of proline would reflect a state of distress and sensitivity to salt. The mechanism of proline accumulation permits to think of the presence of sites of resistance to stress in the plant. Indeed, the proline transportation from the source (site of synthesis) to the site of the resistance has long been recognized as an important parameter in the acquisition of this resistance [40]. In addition, the variances analysis of salt stress impact on the chlorophyll pigments content and the proline content of the leaves in this study, revealed that the high salt contents induce both a decrease in total chlorophyll, chlorophyll a and chlorophyll b pigments, and a strong accumulation of proline in the leaves. Tahri *et al.* [41] showed similar results in wheat. These results suggest the existence of a probable connection between the biosynthetic pathways of chlorophyll pigments and proline [41]. A competition between these two compounds on their common precursor, glutamate, can be at the origin of this evolution [42, 43]. It appears that the stimulation of proline synthesis is parallel to an overall activation of a metabolic pathway from the semi-aldehyde glutamate to proline [44]. Proline may also intervene in the regulation of the cytoplasmic pH or constitute a reserve of nitrogen used by the plant after the period of stress [45]. The death of all the vitroplants treated with 14 g/L helps to suggest that this NaCl concentration is a lethal dose [46].

CONCLUSION

Pineapple tolerates low saline concentrations. On the other hand, high concentrations disturb the development of the plant. Morphologically, the toxicity effect due to high levels of salt in the medium induced a reduction in above-ground and root biomass. These concentrations of NaCl also disrupted the plant photosynthetic metabolism. However, pineapple has responded to high saline concentrations by proline accumulation as an adaptation strategy. This proline would be involved in osmotic adjustment mechanisms and would also serve as osmoprotective agents. This made it possible to maintain a suitable cell turgor in young pineapple plants. However, the accumulation of very high levels of proline would reflect a state of distress and sensitivity to salt. Maintaining moderate relative water content, under salt stress, is a remarkable form of resistance. Pineapple was therefore tolerant to saline concentrations of less than or equal to 4 g/L, because despite these levels the vital parameters were maintained at acceptable values. Concentrations above 4 g/L induce a disturbance of the metabolism of the plant. In pineapple, 14 g/L NaCl is the lethal dose.

CONFLICT OF INTEREST STATEMENT

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

REFERENCES

[1]. Kouadio O, Yapo E, Kouassi K, Silue O, Koffi E, Kouakou T. Improved callogenesis and somatic embryogenesis using amino acids and plant growth regulators combination in pineapple [*Ananas comosus* (L.) merr. (Bromeliaceae)]. Eur. J. Biotechnol. Biosci. 2017, 5 (5) : 06-16.

[2]. FAO. FAO Statistic division, Annuaire de production, www.fao.org. 31/03/2017.

[3]. Richards R. Increasing salinity tolerance of grain crops: is it worthwhile? Plant and Soil 1992, 146: 89-98.

[4]. Bohra J, Doerffling K. Potassium nutrition of rice (*Oryza sativa* L.) varieties under NaCl salinity. Plant and Soil. 1993, 152: 299-303.

[5]. Boriboonkaset T, Theerawitaya C, Yamada N, Pichakum A, Supaibulwatana L, Chaum S. *et al.* Regulation of some carbohydrate metabolism-related genes, starch and soluble sugar contents, photosynthetic activities and yield attributes of two constrasting rice genotypes subjected to salt stress. Protoplasma. 2013, 250(5): 1157-1167.

[6]. Slama I, Ben Rejeb K, Rouached A, Jdey A, Rabhi M, Talbi O. *et al.* Presence of proline in salinized nutrient solution re-enforces the role of this amino acid in osmoregulation and protects lipid membrane peroxidation in *Arabidopsis thaliana.* Aust. J. Crop Sci. 2014, 8(10): 1367-1372.

[7]. Mohamdi M, Bouya D, Salem A. Etude de l'effet du stress salin (NaCl) chez deux variétés de tomate

(Campbell 33 et Mongal).Inter. J. Biol. Chem. Sci. 2011, 5(3) : 860-900.

[8]. Dekok L, Graham M. Levels of pigments, soluble proteins, aminoacids and sulfhydryl compounds infoliar tissue of *Arabidopsis thaliana* during dark-induced and natural senescence. Plant Physiol. and Biochem. 1989, 27: 203-209.

[9]. Lichtenthaler H. Chlorophyll and carotenoids: Pigments of photosyntetic biomembranes. Met. in Enzymol. 1987, 148: 350-382.

[10]. Dreier W, Göring M. Der einfluss hoher salzkonzentration auf verschieden physiollogische parameter von maiswurzeln. Win Z. der HU Berlin, Nath. Naturwiss. R. 1974, 23 : 641-644.

[11]. Clarke J, McCaig T. Evaluation of Techniques for Screening for Drought Resistance in Wheat, Crop Sci. 1982, 22 (3) 503-506.

[12]. Ould Mohamdi M, Bouya D, Ould Mohamed S. Etude de l'effet du stress salin (NaCl) chez deux variétés de tomate (Campbell 33 et Mongal). Int. J. Biol. Chem. Sci. 2011, 5(3): 860-900.

[13]. Benmahioul B, Daguin F, Kaid-Harche M. Effet du stress salin sur la germination et la croissance in vitro du pistachier (*Pistacia vera* L.). C. R. Biol. 2009, 332:164-170.

[14]. Achour A, Bidai Y, Belkhodja M. L'impact de la salinité sur le comportement hydrique et métabolique d'une variété de Gombo (*Abelmoschus esculentus* L.). Int. J. Innov. Appl. Stud. 2015, 12 (4): 943-953.

[15]. Koutoua A, Kouassi N, Yapo E, N'guessan A, Kouakou T, Kouadio Yatty. Evaluations morphological, physiological and biochemical of three tomato varieties (*Lycopersicum esculentum*) under salt stress at the University Jean Lorougnon Guede (Côte d'Ivoire). Int. J. Biosci. 2016, 9(3) :99-107.

[16]. Rochdi A, Lemsellek J, Bousarhal A, Abdellatif R. Evaluation sous serre de la tolérance à la salinité de quelques porte-greffes d'agrumes : *Citrus aurantium* et deux hybrides de *Poncirus trifoliata* (Poncirus x Citrus sinensis et Poncirus x Mandarinier sunki). Biotechnol. Agron. Soc. Environ. 2005, 9(1): 65-73.

[17]. Hamrouni G, Hamed R, Abddellaoui K, Kadri M, Ben Naceur S, Bel Hadj. Évaluation de la tolérance au stress salin de quelque accession d'orge (Hordeum vulgare L.) Cultivées en Tunisie : Sci. Tech. 2008, 28 : 30 -37.

[18]. Cui M, Van der Beek J, Ltifi A. Evidence for salt tolerance in pepper varieties (*Capsicum annuum* L.) in Tunisia. Euphytica. 2015, 57: 51-56.

[19]. Hasanuzzaman, Nahar M, Fujita K, Ahmad M, Chanda P, Prasad R, Ozturk M. Enhancing plant productivity under salt stress: Relevance of polyomics, in: P. Ahmad, M.M. Azooz and M.N.V. Prasad (Eds.), Salt Stress in Plants: Signalling, Omics and Adaptations, New York, NY: Springer New York. 2013, pp.113-56.

[20]. Kara K, Brinis L. Réponse Physiologique au Stress Hydrique de Variétés de Blé Tendre (*Triticum Aestivum* L.) Cultivées en Algérie, Eur.J. Sci. Res. 2012, 81(4) : 524-532.

[21]. Lepengue A, Mouaragadja I, Ibrahim B, Ake S, M'Batchi B. Réponse du maïs (*Zea mays* var. LG 60) au stress salin: étude de la synthèse de quelques composes biochimiques. J Anim. Plant Sci. 2012, 14 (1): 1866-1872.

[22]. R'him T, Tlili I, Hnan I, Ilahy R, Benali A, Jebari H. Effet du stress salin sur le comportement physiologique et métabolique de trois variétés de piment (*Capsicum annuum* L.). J. Appl. Biosci. 2013, 66: 5060-5069.

[23]. Rohanipoor A, Norouzi M, Moezzi A, Hassibi P. Effect of Silicon on Some Physiological Properties of Maize (*Zea mays*) under Salt Stress, J. Biol. Environ. Sci. 2013, 7(20): 71-79.

[24]. Saha P, Chatterjee P, Biswas A. NaCl pretreatment alleviates salt stress by enhancement of antioxidant defense system and osmolyte accumulation in mungbean (*Vigna radiata* L. Wilczek). Ind. J. Exp. Bio. 2010, 48(6): 593-600.

[25]. Baghizadeh A, Salarizadeh M, Abaasi F. Effects of Salicylic acid on some physiological and biochemical parameters of *Brassica napus* L. (Canola) under salt stress. Int. J. Agri. Sci. 2014, 4 (2):147-152.

[26]. Levitt J. Response oh plants to environnemental stress: water, radiation, salt and other stresses. Academic Press. 1980; 607p.

[27]. Santos C. Regulation of chlorophyll biosynthesis and degradation by salt stress in sunflower leaves. Sci. Horticult. 2004, 103 (1): 93-99.

[28]. Nunkaew T, Kantachote D, Kanzaki H, Nitoda T, Ritchie R. Effects of 5 aminolevulinic acid (ALA)-containing supernatants from selected Rhodopseudomonas palustris strains on rice growth under NaCl stress, with mediating effects on chlorophyll, photosynthetic electrontransport and antioxidative enzymes. Elec. J. Biotechnol. 2014, 17(1): 19-26.

[29]. O'Neill P, Shanahan J, Schepers J. Use of chlorophyll fluorescence assessments to differentiate corn hybrid response to variable water conditions. Crop Sci. 2006, 46(2): 681-687.

[30]. Youssef T, Awad M. Mechanisms of enhancing photosynthetic gas exchange in date palm seedlings (*Phoenix dactylifera* L.) under salinity stress by a 5-aminolevulinic acid-based fertilizer. J. Plant Growth Regul. 2008, 27(1): 1-9.

[31]. El Iklil Y, Karrou M, Mrabet R, Benichou M. Effet du stress salin sur la variation de certains métabolites chez *Lycopersicon esculentum* et *Lycopersicon sheesmanii*. Can. J. Plant Sci. 2002, 82: 177-183.

[32]. Verbruggen N, Hermans C. Proline accumulation in plants: a review. Amino Acids. 2008, 35(4): 753-759.

[33]. KaviKishor P, Sangam S, Amrutha R, Sri Laxmi P, Naidu K, Rao K. *et al.* Regulation of proline biosynthesis, degradation, uptake and transport in higher plants: Its implications in plant growth and abiotic stress tolerance. Curr. Sci. 2005, 88 (3): 424-438.

[34]. Szabados L, Savoure A. Proline: a multifunctional amino acid. Trends in plant sci. 2010, 15(2): 89-97.

[35]. Demir Y. Growth and proline content of germinating wheat genotypes under ultraviolet light Turk. J. Bot. 2000, 24: 67-70.

[36]. Feitosa L, Cambraia J, OlivoCano M, Ruiz H. Plant growth and solute accumulation and distribution in two sorghum genotypes under NaCl stress. Braz. J. Plant. Physiol. 2001, 13: 270-284.

[37]. Meloni D, Gulotta M, Martinez C, Oliva M. The effects of salt stress on growth, nitrate reduction and proline and glycinebetaine accumulation in Prosopis alba. Braz. J. Plant Physiol. 2004, 16(1): 1-8.

[38]. Lemzeri H. Réponses écophysiologiques de trois espèces forestières du genre Acacia, Eucalyptus et Schinus (*A. cyanophylla, E. gomphocephala et S. mölle*) soumises à un stress salin. Thèse Université Mentouri Constantine, Algérie. 2007, 132 p.

[39]. Cheikh M'hamed H, Abdellaoui R, Kadri K, Ben Naceur, Bel hadj S. Evaluation de la tolérance au stress salin de quelques accessions d'orge (*Hordium vulgare* L.) cultivées en Tunisie: Approche physiologique. Sci. Tech. 2008, 28: 30 -37.

[40]. Belkhodja M, Bidai Y. Analyse de la proline pour l'étude de la résistance d'une halophyte *Atriplex halimus* L. à la salinité. Note technnique. Laboratoire de Physiologie Végétale, Faculté des Sciences, Université d'Oran, Algérie. 2007, 8 p.

[41]. Tahri E, Belabed A, Sadki K. Effet d'un stress osmotique sur l'accumulation de proline, de chlorophylle et des ARNm codant pour la glutamine synthétase chez trois

variétés de blé dur (*Triticum durum*). Bull. de l'Institut Sci. 1998, 21: 81-87.

[42]. Reddy P, Veeranjaneyulu K. Proline metabolism in senescing leaves of horsgram (*Macrotyloma uniflorum* Lam.). J. Plant Physiol. 1991, 137(3): 381-383.

[43]. Grennan A. High Impact Abiotic Stress in Rice. Plant Physiol. 2006, 140(4): 1139-1141.

[44]. Levigneron A, Lopez F, Vansuyt G, Berthomieu P, Fourcroy P, Casse-Delbart F. Les plantes face au stress salin. Cah. Agri. 1995, 4(4): 263-273.

[45]. Hassani A, Seddiki D, Kouadria M, Bounaceur F, Sehari N, Belkhodja M. Impact of salt stress on morphological and physical behavior of the foliar system by cultivating olive and wild olive trees. Int. J. Curr. Sci. 2014, 12: 161-169.

[46]. Majid A. I. Effect of NaCl Stress on Pineapple Plant (*Ananas comosus* Merr. (L.) cv. Del Monte) *In Vitro*. Int. J Farm. Allied Sci. 2013, 2(9): 206-210.