Original Research Paper

Effect of NaCl salt stress on growth, ions and organic solutes contents in a local cultivar of African eggplant (*Solanum macrocarpon* L.), an important traditional leafy vegetable in the Republic of Benin.

ABSTRACT

Aims: This research has as objective to evaluate the effect of NaCl salt stress on African eggplant plant growth and to determine the implication of the accumulation of Na^+, proline and soluble sugars and the reduction of K^+ in the detrimental effect of NaCl in the growth of this plant species.

Study design: The experiment was laid out in a Completely Randomized Design (CRD) having five treatments and three replications.

Place and duration of study: The experiment was carried out in screening house under natural conditions at the International Institute of Tropical Agriculture, Commune of Abomey-Calavi, Republic of Benin from june to august 2022.

Methodology: Five NaCl concentrations (0, 30, 60, 90 and 120 mM) were used to irrigate three weeks old plants for two weeks. Plant growth, sodium (Na), potassium (K), proline, and soluble sugars contents of leaves and roots were determined at the end of the experiment.

Results: Salt stress induced a significant reduction (*P* = .001) in shoot growth from 30 ; 60 or 90 mM NaCl according to the growth parameter but had no impact on shoot water content. Leaf and roots Na^+ contents significantly increased (*P* = .001) under salt stress whereas K^+ content decreased significantly (*P* = .05) only in root. No change was observed for proline and soluble sugars contents in both leaf and root.

Conclusion: Salt stress reduces the growth of plants of African eggplant due mainly to Na^+ ion toxicity. The ionic selectivity ratio (K^+/Na^+) rather than the K^+ ion content plays an important role in the response of plants of African eggplant to salt stress. Proline and soluble sugars accumulation appeared not to intervene.

Key words: salt stress, Benin Republic, ions contents, proline, soluble sugars, *Gboma*, young plants

Introduction

Salt stress is known as one of the most important abiotic stresses that reduce plant growth, yield and fruit quality worldwide [1]. ~~According to [2], it~~It is one of the most severe abiotic factors in many agronomic and horticultural crops [2]. It is well known that the detrimental effect of salinity on plants can be due to three main factors: (i) the reduction of water absorption by the plant after high osmotic pressure, known as osmotic effect; (ii) the transport and accumulation of excessive amounts of certain ions, in particular Na^+ in the aerial parts, known as toxic effect; and (iii) the deficiency in the absorption of certain essential ions, such as K^+ and Ca^{++} known as nutritional effect [3]. Under this stress, plants generally respond by excluding toxic ions (Na^+) from their leaves, by maintaining high absorption of K^+ and/or Ca^{++} ions, and /or by increasing their internal osmotic pressure via the accumulation of some organic solutes known as osmolytes [Reference]. These responses generally permit to plants to survive and continue to growth (and produce) in this harmful environment. In Benin, vegetables occupy an important place in the food production and are mainly cultivated in the coastal zone where they are exposed to salt stress, phenomenon which tends to be aggravated by climate change [4]. African eggplant (*Solanum macrocarpon*) is an important tropical perennial vegetable belonging from the family *Solanaceae*. In West Africa, leaves of this plant are a regular part of the diet [5]. In Benin, this plant is consumed as leafy vegetable very important for its high nutritional value due to its high protein, fat, ash, crude fiber and moisture [6].In a recent study it has been reported that in some vegetables production zones of the southern of Benin where this plant is more produced, the irrigation water used by producers has salinity higher than the threshold accepted for vegetables [7]. Moreover, data related to the response of this plant to salt stress is very scarce and except our previous studies on the effect of salt stress on germination [8] and on growth of young plant [9], practically no other study was addressed this subject. In our study, we have reported that NaCl salt stress reduced plant growth of some African eggplant cultivars produced in Benin and that cultivar *Kpinman*, the most produced and appreciated by farmers and consumers, was one of the most salt sensitive cultivars [9]. However, this study did not address the effect of salt stress on ions and osmolytes contents and the way in which these compounds intervene in the response of plants of African eggplant to salt stress. This study aimed to fill this gap by determining the implication of Na^+, K^+, proline and soluble sugars in the

response of plants of cultivar *Kpinman* to NaCl salt stress.

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2. MATERIAL AND METHODS

2.1. Plant Material

The plant material used in this study consists of seeds of the local cultivar of African eggplant *Solanum macrocarpon* L.) called *Kpinman* in the local language Fongbé and catalogued in Benin as *BenGbo-01*. This cultivar is identified as salt sensitive at young plant level [9]. Seeds of this cultivar was provided by the National Institute of Agricultural Research of Benin (INRAB), Republic of Benin, located in Abomey-Calavi.

2.2. Methodology

2.2.1. Experimental conditions

The experiment was carried out in a screening house at the International Institute of Tropical Agriculture (IITA, Republic of Benin) in the commune of Abomey-Calavi. This commune is located in the Guinean ecological zone characterized by a subequatorial bimodal climate with two dry seasons and two rainy seasons [10]. The annual rainfall varies between 1200 and 1500 mm/year and the temperature ranges from 24 to 30 °C [10]. The experiment design and conditions were the same as that described by [11]. Plants were irrigated every two days with 100 ml / pot of 0-120 mM NaCl with an increment of 30. The experiment was evaluated after two weeks exposure to salt stress.

Insert tables of the climate and soil analysis

2.2.2. Growth and water content determination

At the end of the experiment, plant height (PH), shoot fresh mass (SFM), shoot dry mass (SDM), root fresh mass (RFM), and root dry mass (RDM) were determined.

Shoot water content was calculated as [shoot fresh mass – shoot dry mass) / shoot fresh mass] x 100.

2.2.3. Extraction and measurement of ion concentrations

Na^+ and K^+ extraction and quantification were done from leaf and root dry matters as described by [12] using a flame spectrophotometer (Sherwood Model 360). Ions were expressed in mg g^{-1} dry matter (dm).

2.2.4. Extraction and determination of organic solutes concentrations

Proline extraction and quantification were done from leaf and root fresh matters as described by [12] using an UV-visible spectrophotometer (Jenway 7305). Proline was expressed in nmole g^{-1} fresh matter (fm) and soluble sugars in mg g^{-1} fresh matter (fm).

2.2.5. Statistical analysis

For all parameters, means were calculated using an Excel spreadsheet with four replications. The analysis of the effect of salt stress was based on the one-way analysis of variance (ANOVA). Means were compared with Tukey-Kramer test. Statistical analyses were performed using JMP Pro 12 software [13].

~~Résultats~~ Results

Effets of salt stress on plant height

NaCl effect results in a reduction of plant height which decreased from 40.1 cm in the control to 35.87 cm; 31.82 cm; 27.47 and 25.9 cm respectively with 30; 60; 90 and 120 mM NaCl after two weeks of stress (fig. 1). The reduction was significant (p = .001) from 90 mM NaCl.

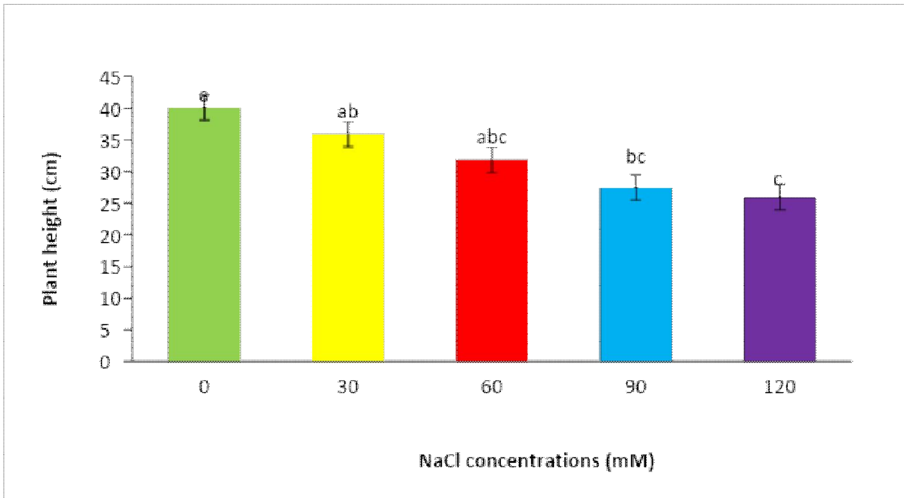

Figure 1 : Effect of different NaCl concentrations on plant height (PH) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at p =.001.

Effets of salt stress on shoot fresh mass

NaCl induced a reduction of shoot fresh mass which decreased from 33.30 g in the control to 20.53 g; 15.02 g; 9.65 g and 8.81 respectively with 30; 60; 90; 120 mM NaCl after two weeks of stress (fig. 2). The reduction was significant (*p* =0.001) from 30 mM NaCl.

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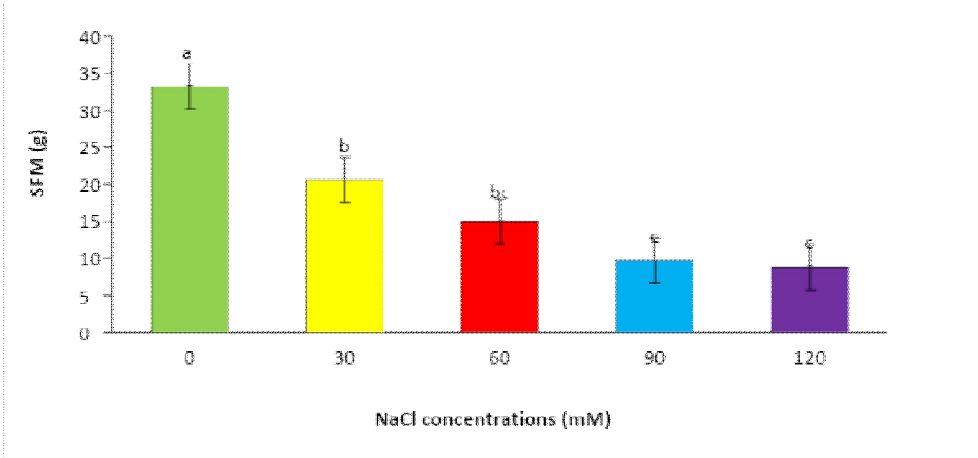

Figure 2 : Effect of different NaCl concentrations on shoot fresh mass (SFM) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at *p* = 0.001.

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Effets of salt stress on shoot dry mass

NaCl induced a reduction of shoot dry mass which decreased from 2.67 g in the control to 1.61 g; 1.04 g; 0.68 g and 0.82 g respectively with 30; 60; 90; 120 mM NaCl after two weeks of stress (fig. 3). The reduction was significant (*p*= 0.001) from 30 mM NaCl.

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Effets of salt stress on root fresh mass

NaCl induced a reduction of shoot fresh mass which decreased from 5.82 g in the control to 3.59 g; 2.40 g; 1.53 g and 1.48 respectively with 30; 60; 90; 120 mM NaCl after two weeks of stress (fig. 4). The reduction was significant (*p* = 0.001) from 60 mM NaCl.

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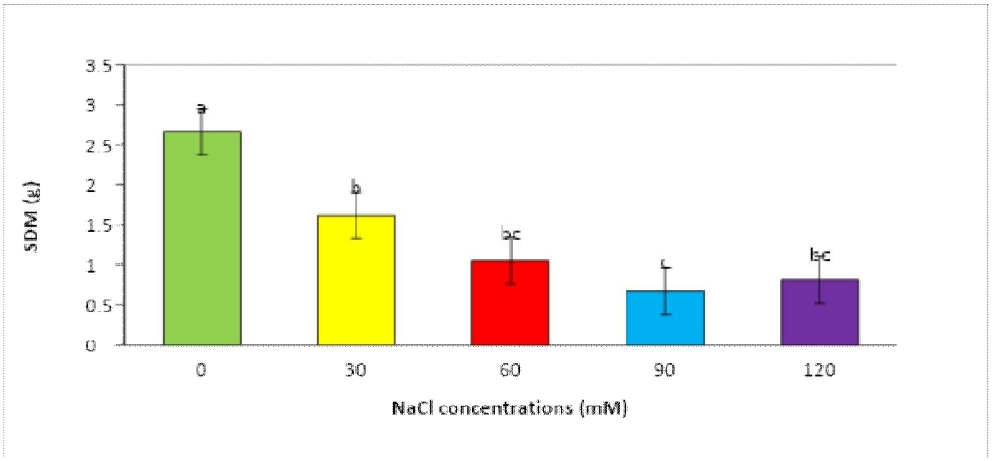

Figure 3 : Effect of different NaCl concentrations on shoot dry mass (SFM) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at *p* = 0.001.

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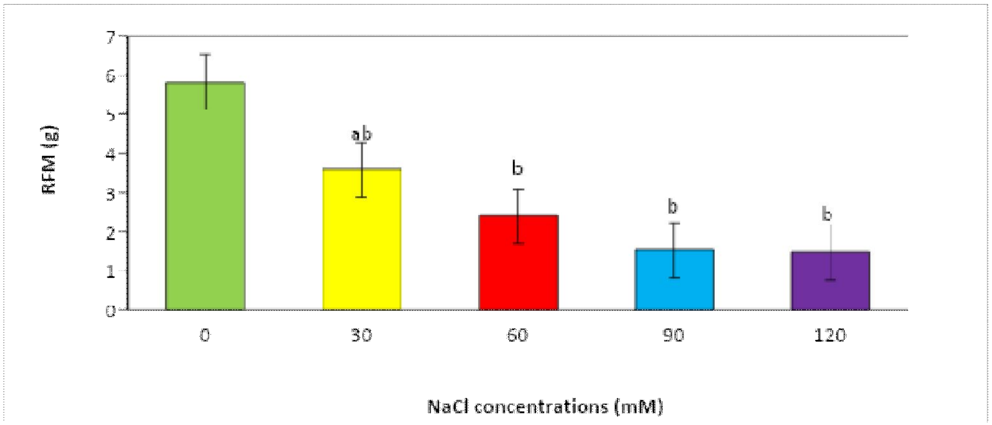

Figure 4: Effect of different NaCl concentrations on root fresh mass (RFM) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at *p* = 0.001.

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Effets of salt stress on root dry mass

NaCl induced a reduction of root dry mass which decreased from 0.492 g in the control to 0.288 g; 0.141 g; 0.09 g and 0.102 respectively with 30; 60; 90; 120 mM NaCl after two weeks of stress (fig. 5). The reduction was significant (*p* = 0.001) from 30 mM NaCl.

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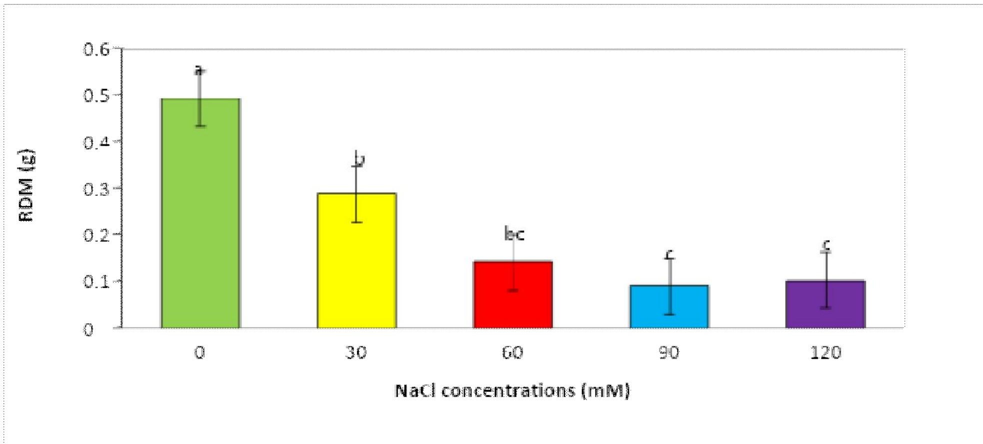

Figure 5 : Effect of different NaCl concentrations on root dry mass (RDM) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at *p* = 0.001.

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Globally salt stress effect resulted in a significant plant growth reduction but the extend of this reduction varied greatly according to the growth parameter condidered. Plant heigth was the least affected followed by root fresh mass.

Effets of salt stress on shoot water content

Salt stress has no significant effect on shoot water content (Table 1). However, shoot water content at 120 mM NaCl was weaker than that at 60 and 90 mM NaCl.

Table 1. Shoot water content of African eggplant plants after two weeks of exposure to different NaCl concentrations (n=3 ; values are means ± standard error).

NaCl concentrations (mM)				
0	**30**	**60**	**90**	**120**
91.94±0.55[ab]	92.14±0.26[ab]	93.01±0.65[a]	93.03±0.32[a]	90.52±0.31[b]

Means with different letters within line differ significantly at *P* = .05.

Effets of salt stress on plant ion contents

NaCl induced a significant (p= 0.001) increase in leaf and root Na^+ content from 60 mM NaCl which increased from 0.026 mg g^{-1} dm in leaf of the control plants to 0.035 mg g^{-1} dm with 60 mM NaCl and 0.043 mg g^{-1} with 120 mM NaCl. The values corresponded respectively to an increase of 34.61% and 65.38% of that of the control. In root, Na^+ content decreased from 0.032 mg g^{-1} dm in the control to 0.045 mg g^{-1} dm with 60 mM NaCl and 0.044 mg g^{-1} dm with 120 mM NaCl. The values corresponded respectively to an increase of 40.62% and 37.50% of that of the control. However, values in root were higher than that in leaf at each NaCl concentration.

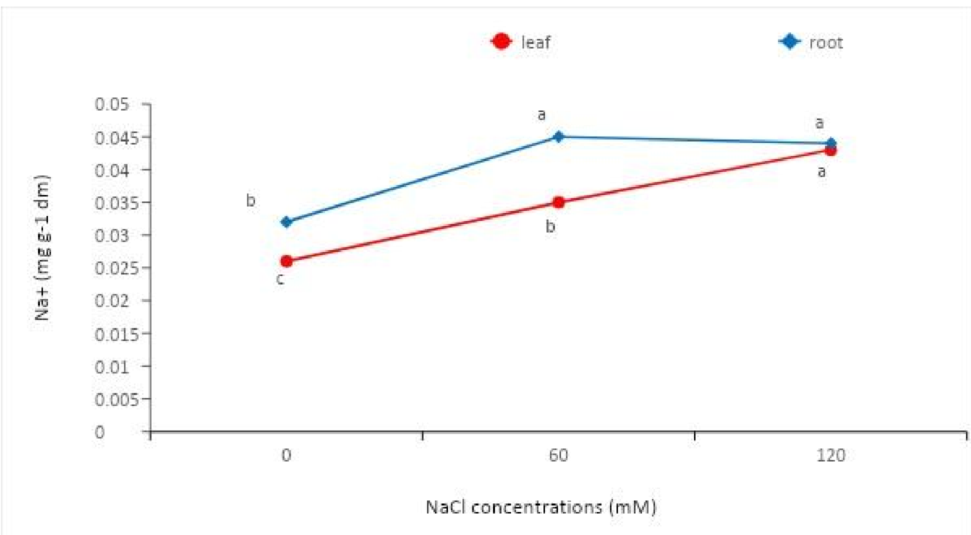

Figure 6 : Effect of different NaCl concentrations on leaf and root Na^+ content (mg g^{-1} dm) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at p= 0.001.

Effets of salt stress on plant ion contents

NaCl induced a significant (p= 0.001) decrease only in root K^+ content only at 120 mM NaCl. No change was observed in leaf K^+ content under salt stress. However, values in leaf were higher than that in root at each NaCl concentration.

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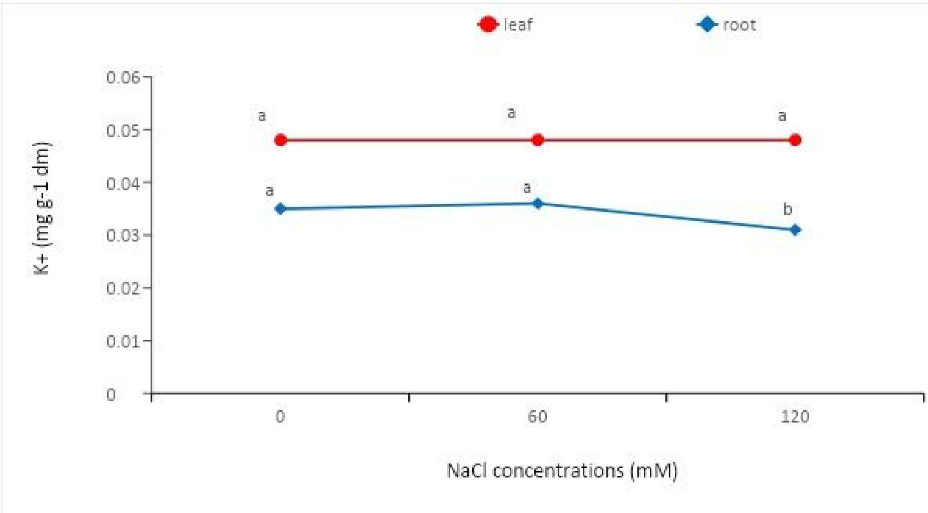

Figure 7 : Effect of different NaCl concentrations on leaf and root K^+ content (mg g^{-1} dm) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at *p*= 0.001.

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Effets of salt stress on plant ionic selectivity ratio K^+/Na^+

NaCl induced a significant (p = .001) decrease in leaf and root K/Na ration from 60 mM NaCl (table 2). It decreased from 1.829 in the control to 1.38 and 1.139 in leaf, respectively with 60 and 120 mM NaCl after two weeks. In root, It decreased from 1.105 in the control to 0.809 and 0.715, respectively with 60 and 120 mM NaCl after two weeks. However, values in leaf were higher than that in root at each NaCl concentration.

Table 2 : Effect of different NaCl concentrations on leaf and root K/Na ratio of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors).

	NaCl concentrations (mM)		
	00	**60**	**120**
Leaf	1.829±0.00[a]	1.380±0.06[b]	1.139±0.02[c]
Root	1.105±0.02[a]	0.809±0.02[b]	0.715±0.01[c]

Values within lines with different letters are significantly different at *p*= 0.001.

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Effets of salt stress on proline contents

NaCl induced no significant effect on leaf and root proline content in African eggplant plants after two weeks. A non-significant decrease was observed in leaf whereas a non-significant increase followed by a non-significant decrease was observed in root. However, values in leaf were higher than that in root at each NaCl concentration.

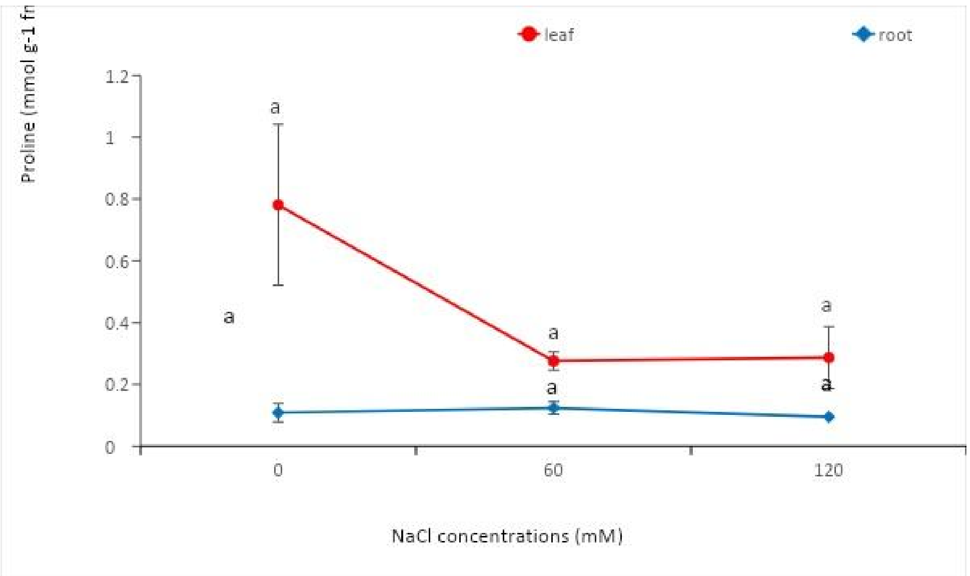

Figure 8 : Effect of different NaCl concentrations on leaf and root proline content (nmole g^{-1} fm) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at *p*= 0.001.

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Effets of salt stress on soluble sugars contents

NaCl induced no significant effect on leaf and root soluble sugars content in African eggplant plants after two weeks. A non-significant increase was observed in leaf whereas a non-significant decrease followed by a non-significant decrease was observed in root. However, values in leaf were higher than that in root in the presence of NaCl.

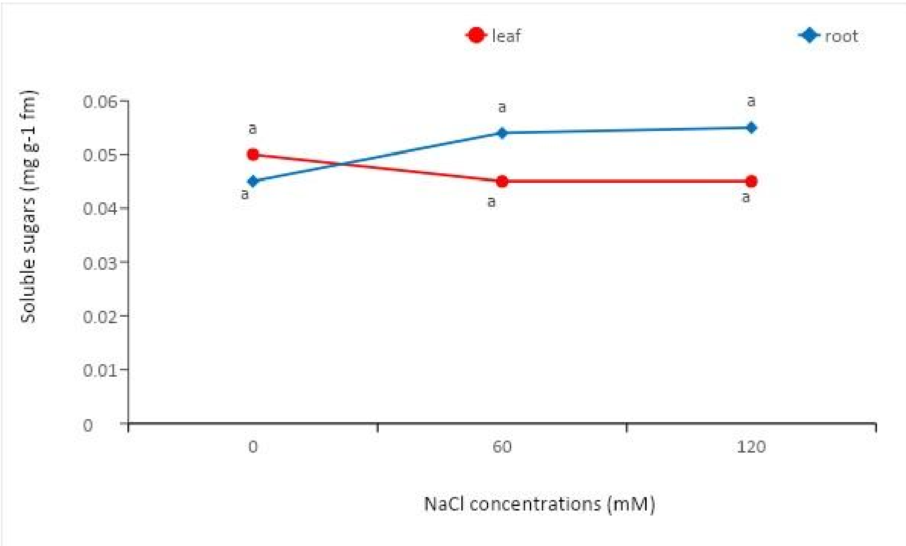

Figure 9 : Effect of different NaCl concentrations on leaf and root soluble sugars content (mg g^{-1} fm) of African eggplant cultivar *Kpinman* after two weeks (n = 3; vertical bars are standard errors). Values with different letters are significantly different at *p*= 0.001.

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DISCUSSION

Results of this study revealed that salt stress induced a reduction in plant growth and that this reduction was as NaCl concentration dependent whatever the growth parameter taken into account. These data confirmed our previous findings in the same African eggplant cultivar [9]. The reduction of plant growth under salt stress is a common phenomenon as reported in other plant species including vegetables [14, 15, 16, 17, 18]. According to [3], the detrimental effects of salinity on plants can be osmotic in nature (reduction of water absorption by the plant after high osmotic pressure), toxic (transport and accumulation of excessive amounts of certain ions, in particular Na^+ in the aerial parts) or nutritional (deficiency in the absorption of certain essential ions, K^+ and Ca^{++} in particular). This study revealed that NaCl induced an increase of Na^+ content in both leaf and root of plants and that values in root were higher than that in leaf indicating that African eggplant prefers to accumulate this ions in root excluding them from leaves. This behavior is typical of glycophytes which thus protect their leaves, which are the seat of photosynthetic phenomena, from the toxic effect of salt stress. This kind of behavior was reported in several plant species [19, 20, 21]. These findings suggested that Na^+ toxicity plays an important role in the detrimental effect of NaCl on African eggplant growth.

No change was observed in leaf and root K^+ content under salt stress despite a significant reduction in plant growth at the NaCl concentrations used. These data seemed to indicate that salt detrimental effect in African eggplant growth was not mediated by deficiency in the absorption of K^+ ion. The same tendency was reported in leaves of African basil plants [11]. However, salt stress effect generally resulted in a reduction in K^+ content as reported in several vegetable species such as sweet basil [22], eggplant [23] ; okra [24, 25, 26, 27] ; African basil [11] ; tomato [12] and tossa jute [28].

Salt stress induced a decrease in K/Na ratio in both leaf and root at 60 and 120 mM NaCl as for plant growth. Similar resulted were obtained in other vegetables species [29, 27]. This ratio known as ionic selectivity ratio revealed the ability of plant to absorbe and use K^+ in the presence of the excess of Na^+ [30]. The reduction of this ratio followed the same tendency with plant growth indicating that this ratio is important in expressing salt detrimental effect in plants of African eggplant.

No change was observed in proline and soluble sugars content in plants of African eggplant under salt stress. Generally, salt stress effect resulted in an increase in both proline and soluble sugars content in leaves and roots as reported in several vegetable species [31, 32, 27, 11, 28]. These organic solutes are known to play a key role in plant osmotic adjustment [33, 34] as well as in the stabilization of certain proteins for proline [33]. The fact that the NaCl concentrations used, that induced a significant reduction in plant growth, did not induce any change in proline and soluble sugars contents in the same plants indicated that these organic solutes did not play an important role in the response of plants of African eggplant to salt stress. Further studies are needed to evaluate the implication of other osmolytes such as trehalose, mannitol, polyamines and glycine betaine in the response of African eggplant to salt stress.

5. CONCLUSION

The study found that salt stress reduces the growth of African eggplant (*Solanum macrocarpon*) and induced an accumulation of Na^+ ion in both leaves and roots, and no change in K^+ ion, proline and soluble sugars in both leaf and root. Results revealed that the toxicity of the Na^+ ion is implicated in African eggplant plants growth reduction and that the ionic selectivity ratio (K/Na) rather than the K^+ ion content plays an important role in the response of plants of African eggplant to salt stress. Proline and soluble sugars accumulation seemed not to play an important role in the response of these plants to salt stress.

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