Original Research Article

Response of African basil (*Ocimum gratissimum* L.) to salt stress: Growth, ions and organic solutes accumulation

ABSTRACT

Aims: This research aims at evaluating the effect of salt stress on plant growth, Na^+, K^+, proline and soluble sugars contents in leaves and roots of the local cultivar of African basil cultivated in Republic of Benin.

Study design: The experiment was laid out as a Completely Randomized Design (RCD) with five treatments and three replications.

Place and duration of study: The experiment was carried out in screening house at Center of Agricultural Research of Agonkanmey, Commune of Abomey-Calavi, Republic of Benin from january to february 2020.

Methodology: Five salt concentrations (0, 30, 60, 90 and 120 mM) were used to irrigate three weeks old plants for two weeks. The experiment was conducted in screening house with natural conditions. Plants growth, sodium (Na) and potassium (K), proline and soluble sugars contents of leaves and roots were determined after two weeks.

Results: Salt stress induced a significant reduction ($p=.05$) in shoot growth from 60 mM NaCl but had no impact on the number of leaves and shoot water content. Root growth was significantly reduced ($p=.05$) already at 30 mM NaCl. Leaf and roots Na^+, proline and soluble sugars contents significantly increased ($p=.05$) under salt stress whereas K^+ content decreased significantly ($p=0.05$) only in root.

Conclusions: Salt stress reduces the growth of African basil plants 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 basil plants to salt stress while both proline and soluble sugars accumulation may

Key words: Adaptation to salinity, Benin Republic, ionic selectivity ratio, osmotic adjustment, proline, soluble sugars, *Tchiayo*, young plants

1. INTRODUCTION

In several areas of the earth, salinization is the major process of land degradation. Globally, it is estimated that almost 800 million hectares of land are affected by salt, either by salinity (397 million ha) or by sodization conditions associated with sodium levels (434 million ha) [1]. This phenomenon is becoming increasingly worrying as salinity reduces the area of cultivable land and threatens food security. Soil salinization is not only linked to climatic conditions, but also to the poorly controlled use of irrigation water and its poor quality. Salt stress may induce a water deficit in the plant resulting in a physiological drought [2]. This osmotic stress is the consequence of a high external concentration of ions which decrease the soil water potential and hampers water absorption by the roots [3] . In addition, the reduction in growth due to salinity is also attributable to ion toxicity and nutrient imbalance. This state not only leads to increased accumulation of sodium (Na^+) and chloride (Cl^-) in plants, but also affects the absorption of essential elements like potassium (K^+), calcium (Ca^{++}) and magnesium (Mg^{++}) in competition with Na^+ and nitrates (NO_3^-) in contrast with Cl^- [4]. Salt stress influences growth through many facets of metabolism, such as absorption of nutrients and their distribution within the plant, alteration of photosynthesis [5], decreases in proteosynthesis, accumulation of organic solutes, hormonal imbalance and decrease in water availability [6].

According to [7], market gardening is an activity that responds to preferences and urban food demand. It is an important source of income and employment [8]. In South Benin, it enables several thousand families to meet their needs [9]. However, this important activity faces many constraints that hamper productivity in relation to land tenure, lack of pest and diseases, soil salinity, poor water management, post-harvest problems and low level of industry players organization [10]. African basil (*Ocimum gratissimum* L.) is a perennial herb of the family *Lamiaceae*, commonly called *Tchiayo* in the local language Fongbé. It is a pantropical species native to Asia, India and Vietnam [11]. It is a traditional vegetable [12] that is one of the most cultivated market garden crops in Benin, especially in urban and peri-urban areas because of its nutritional, aromatic and medicinal properties [13]. In Nigeria and other parts of the world, it is used as a traditional vegetable condiment and oral care products [14]. This plant is also known to possess numerous pharmacological properties such as antioxidant, anti-anemic, antidiarrheal effects, protective effects on hepato-renal indices and erectile function [14 and references therein]. Furthermore, it has been shown that this species is efficient in controlling pests of harvest products and the phytosanitary treatment of crops in the field [15, 16]. It is now well known that the southern Benin where is located an important African basil production area is strongly affected by poor irrigation water quality resulting in soil salinization which hampers crop production [17]. Some studies were focused on the effect of salt stress on sweet basil (*Ocimum basilicum*) seed germination, plant growth, yield and physiology [18, 19, 20, 21, 22] as well as essential oil and pigments accumulation [18, 22]. However, data related to salt stress effect on plant growth and physiology in African basil are extremely scarce. Thus, the current study is designed to fill this gap by evaluating the effect of salt stress on the growth, Na^+ and K^+ ions, proline and soluble sugars contents in leaves and roots of the local cultivar of African basil produced in Republic of Benin. The tested hypotheses are (i) salt stress reduces African basil plants growth as for other plants, (ii) basil plants respond to salt stress by accumulating Na^+ ion in both roots and leaves and by reducing K^+ ion content in leaves and roots; (iii) basil plants respond to salt by accumulating proline and soluble sugars in leaves and roots.

2. MATERIAL AND METHODS

2.1. Plant Material

The plant material used in this study consists of seeds of the local cultivar of African basil (*O. gratissimum* L.) which 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 National Institute of Agricultural Research of Benin (INRAB, Republic of Benin). The experiment was laid out as a completely randomized design with three replications (three pots) and three plants per replication (per pot) (9 plants per treatment). Pots (11.3 cm diameter and 14 cm) were filled with 3 kg mixture of potting soil and sandy loam soil (composition in table 1) 50:50. The experiment was conducted as described by [23]. Plants were irrigated every two days with 100 ml / pot of 0-120 mM NaCl (CAS n°7647-14-5) with an increment of 30. The experiment was evaluated after two weeks exposure to salt stress.

Table 1 : Composition of the sandy loam soil used for plant culture

		Component						
Year	Depth	pH (H_2O)	C (%)	N (%)	C/N	Organic matter (%)	K exch. (meq/100g)	P avail. (ppm)
2017	0-40 cm	5.74	0.58	0.05	8.14	0.79	0.18	64.25

(Data from the characterization of soils of the National Institute of Agricultural Research of Benin done by the laboratory of the Soil Sciences, Water and Environment in 2017).

2.2.2. Growth and water content determination

The effect of salt stress on plant growth was determined after two weeks exposition to salt. Growth parameters taking into account were Plant height (PH), leaf number (LN), Shoot fresh mass (SFM), shoot dry mass (SDM), root length (RL), root fresh mass (RFM) and root dry mass (RDM).

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

2.2.3. Extraction and measurements of ion concentrations

Roots and leaves samples were used for ions concentrations determination according to [24] with three replications (one plant per pot). “20 mg of the leaf and root powders were placed in 10 ml jars and digested in nitric acid (68%) at room temperature” according to the previous authors. After filtration through Whatman paper (85 mm, Grade 1), solution was used to quantify Na^+ and K^+ using a flame spectrophotometer (Sherwood Model 360).

2.2.4. Quantification of organic solutes

Proline was extracted from the youngest fully expanded leaf with three replications (one plant per pot). Proline concentration was quantified using an UV-visible spectrophotometer (Jenway 7305). with the ninhydrin acid (CAS n°486-47-2) method of Bates *et al.* [25]. Results were presented as nmole proline g^{-1} fresh matter (fm) and L proline (CAS n° 147-85-3) was used as standard.

Total soluble sugars were extracted from the youngest fully expanded leaf with three replications (one plant per pot). Total soluble sugars were determined using the anthrone (CAS n°90-44-8) reagent method as used by Manaa *et al.* [26] with an UV-visible spectrophotometer (Jenway 7305). Soluble sugars quantity was presented as µg soluble sugars g^{-1} fresh matter (fm). D Glucose (CAS n°50-99-7) was used as standard.

2.2.5. Statistical analysis

Statistical analyses were performed using JMP Pro 12 software [27]. The analysis of the main effects of salt stress was based on the variance analysis. Means were compared utilizing Tukey-Kramer test.

3. RESULTS

3.1. Effect of salt stress effects on plant growth

Salinity symptoms were characterized by leaf chlorosis and necrosis (Fig**ure** 1).

Control plants **Stressed plants (120 mM NaCl)**

Figure 1. Chlorosis (yellowing) and necrosis on leaves of African basil salt stressed plants (120 mM NaCl)

Salt stress induced a significant reduction (p = .001) from 90 mM NaCl in plant height (Figure 2). The reductions observed correspond to 8.77%; 13.76%; 31.27% and 43.12% of that of control, respectively *at 30; 60; 90 and 120 mM NaCl.

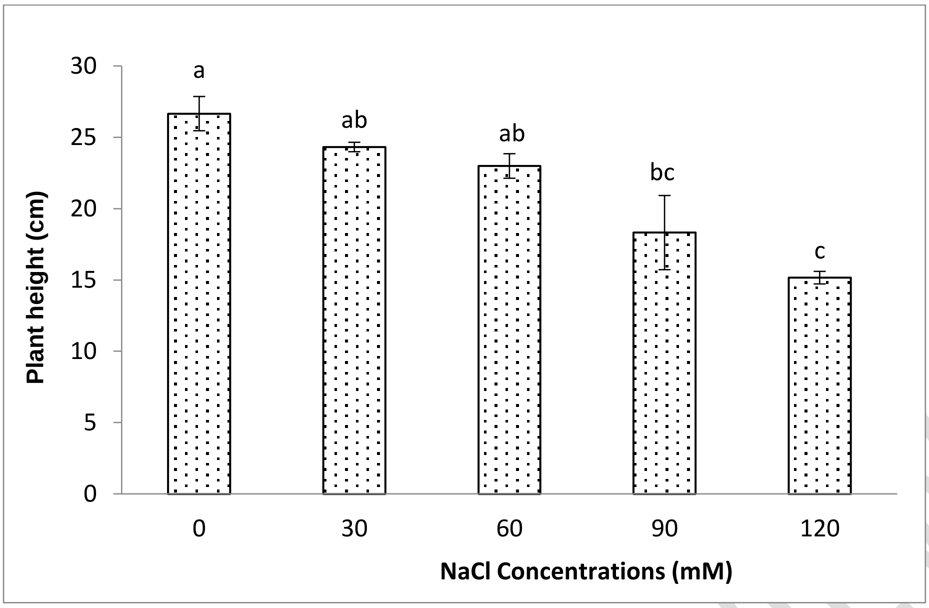

Figure 2. Effect of salt stress on Plant Height of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3 ; vertical bars are standard errors)

Means with different letters differ significantly at p=.001.

Salt stress has no significant (p = .16) effect on leaf number (Figure 3).

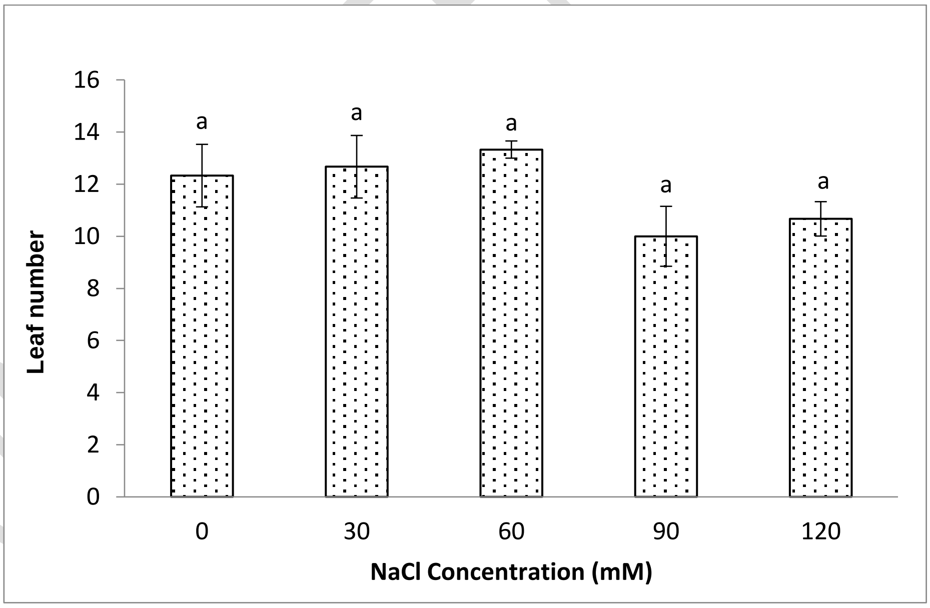

Figure 3. Effect of salt stress on leaf number of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3 ; vertical bars are standard errors).

Salt stress induced a significant reduction (p = .001) from 60 mM NaCl in shoot fresh mass (Figure 4). The reductions corresponded to 20.80%; 31.75%; 43.80% and 69.71% of that of the control respectively at 30; 60; 90 and 120 mM NaCl.

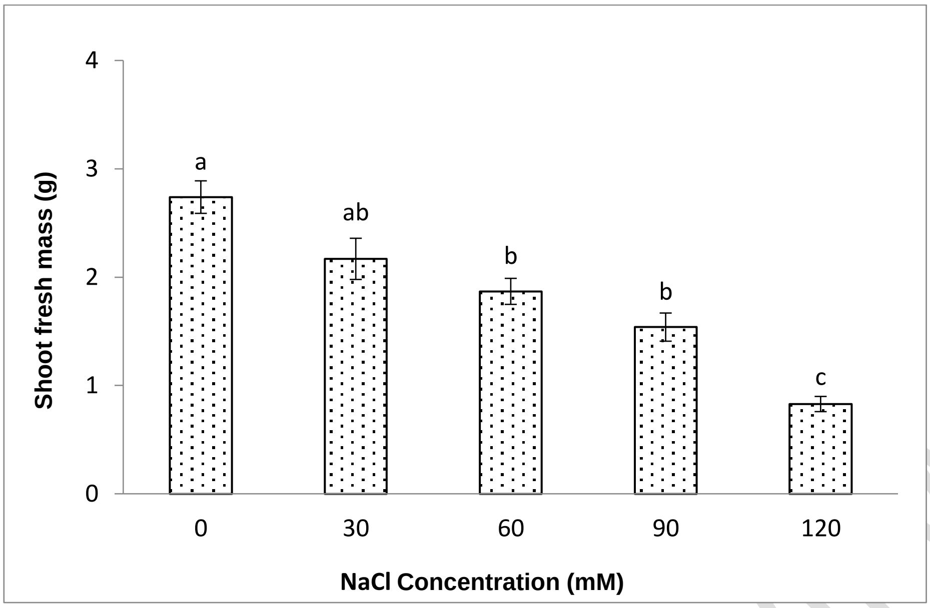

Figure 4. Effect of salt stress on Shoot Fresh Mass (SFM) of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3 ; vertical bars are standard errors).

Means with different letters differ significantly at p=.001.

Salt stress induced a significant reduction (p = .001) from 90 mM in shoot dry mass of African basil plants (Figure 5). These reductions correspond to 8.53% NaCl; 31.77%; 47.35% and 71.47% of that of the control respectively at 30; 60; 90 and 120 mM NaCl.

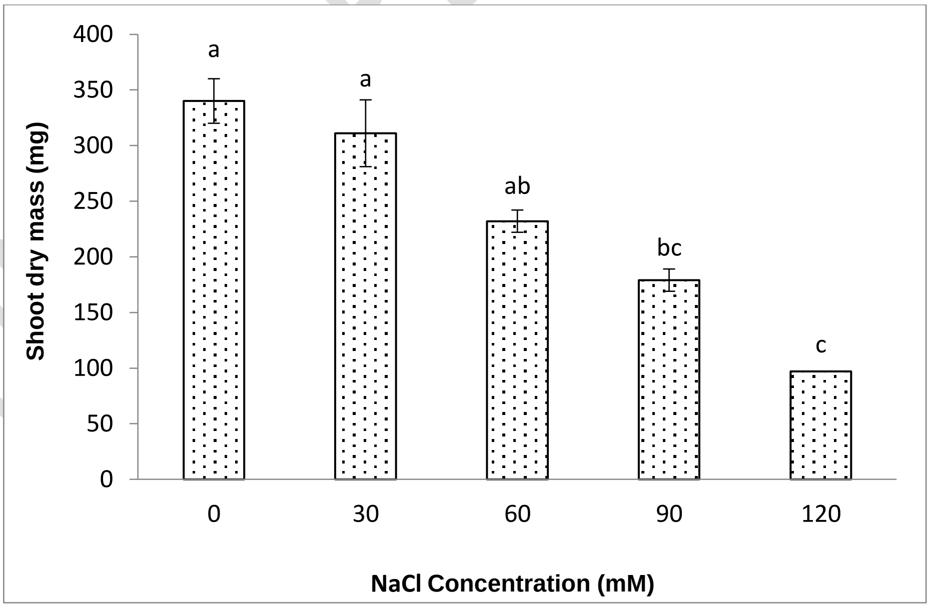

Figure 5. Effect of salt stress on Shoot Dry Mass (SDM) of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3 ; vertical bars are standard errors).

Means with different letters differ significantly at p=.001.

Table 2 showed the effect of NaCl on root growth of African basil plants. Salt stress induced a significant reduction (p = .05) in root length (RL) of African basil plants only at 120 mM NaCl. These reductions correspond to 12.63%; 20.52%; 9.2% and 48.68% of that of the control respectively at 30; 60; 90 and 120 mM NaCl. For root fresh mass (RFM), the growth reduction was significant (p = .001) from 30 mM NaCl. These reductions correspond to 30.16%; 55.83%; 68.33% and 69.58% of that of the control respectively at 30; 60; 90 and 120 mM NaCl whereas for root dry mass (RDM), the reduction was significant (p = .01) from 90 mM NaCl. These reductions correspond to 9.23%; 40%; 52.30% and 69.24% of that of the control respectively at 30; 60; 90 and 120 mM NaCl

Thus, salt stress effects resulted in both aerial part and roots growth reduction.

Table 2: Effect of salt stress on roots length (cm), root fresh mass (g) and root dry mass (mg) of African basil plants after two weeks of exposure to different NaCl concentrations (n=3; values are means ± standard error)

	NaCl concentrations (mM)				
Parameters	**0**	**30**	**60**	**90**	**120**
RL	12.67±1.45^{a}	11.07±0.63^{ab}	10.07±1.15^{ab}	11.50±1.44^{ab}	6.50±0.76^{b}
RFM	0.24±0.2^{a}	0.15±0.01^{b}	0.11±0.00^{bc}	0.08±0.00^{c}	0.07±0.00^{c}
RDM	43.33±6.64^{a}	39.33±6.33^{ab}	26.00±1.00^{abc}	20.67±3.71^{bc}	13.33±1.20^{c}

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

3.2. Effets of salt stress on shoot water content

No significant change (p = .102) of shoot water content was observed under salt stress (Table 3).

Table 3 : Shoot water content of African basil 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**
87.60±0.59^{a}	85.75±0.52^{a}	87.62±0.35^{a}	88.33±0.69^{a}	88.13±0.88^{a}

Means with different letters within line differ significantly (p = .05).

3.3. Effect of salt stress on ions contents

Na^+ concentration increased significantly (p = .05) in leaves at 120 mM NaCl and in roots (p = .001) from 30 mM NaCl (Figure 6). These increases correspond to 9.46%; 11.49%; 13.51% and 46.62% of that of the control respectively at 30; 60; 90 and 120 mM NaCl in leaves; and to 24.87%; 41.12%; 44.16% and 52.79% of that of the control in roots at the same NaCl concentrations.

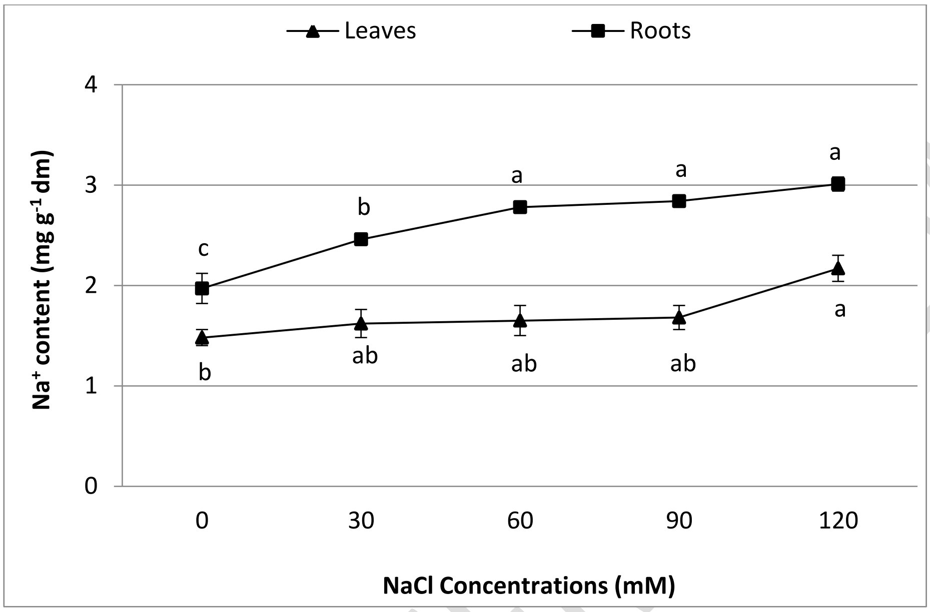

Figure 6. Effect of salt stress on sodium ion (Na^+) content in leaves and roots of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3; vertical bars are standard errors). Means with different letters for each organ differ significantly at p = .01.

For K^+, a significant reduction was observed from 30 mM NaCl only in the roots (p = .001) (Figure 7). The reductions correspond to 24.83%; 27.66%; 29.79% and 65.32% of that of the control respectively at 30; 60; 90 and 120 mM NaCl.

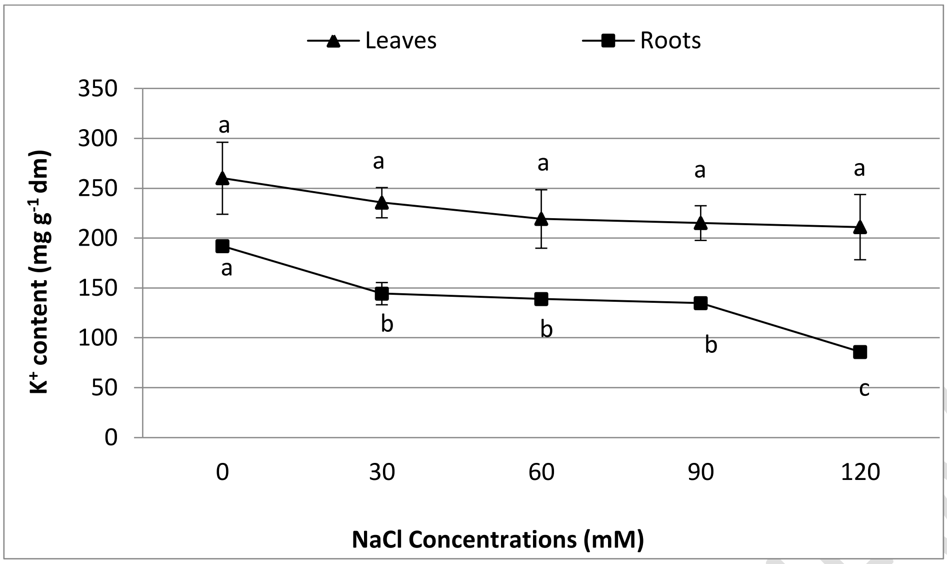

Figure 7. Effect of salt stress on potassium ion (K$^+$) content in leaves and roots of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3 ; vertical bars are standard errors) Means with different letters for each organ differ significantly at p= .01.

Consequently, salt stress induced a significant decrease in K$^+$/Na$^+$ selectivity ratio in leaves (p = .05) at 120 mM NaCl and from 30 mM NaCl in roots (p = .001) (Table 4).

Table 4. Effect of salt stress on ion selectivity ratio K$^+$/Na$^+$ in leaves and roots of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3).

	NaCl concentrations (mM)				
	0	**30**	**60**	**90**	**120**
Leaves	175.87±22.92^{a}	148.32±19.28^{ab}	134.84±20.25^{ab}	128.26±6.42^{ab}	96.06±8.92^{b}
Roots	97.43±2.44^{a}	58.74±5.07^{b}	49.93±0.84^{b}	47.51±0.95^{b}	28.48±1.48^{c}

Means with different letters within lines differ significantly at p= .01.

3.4. Effect of NaCl on proline and soluble sugars contents

Figure 8 (A and B) shows that the presence of NaCl in the plants culture medium resulted in a significant increase in proline content in leaves (p = .05) at 120 mM NaCl and from 90 mM NaCl in roots (p = .001). The increase was about 32.34%; 17.30%; 46.62% and 91.73 % of that of the control in leaves; and 50,01%; 63.53%; 147.32 and 179.76% of that of the control in roots, respectively at 30; 60; 90 and 120 mM NaCl.

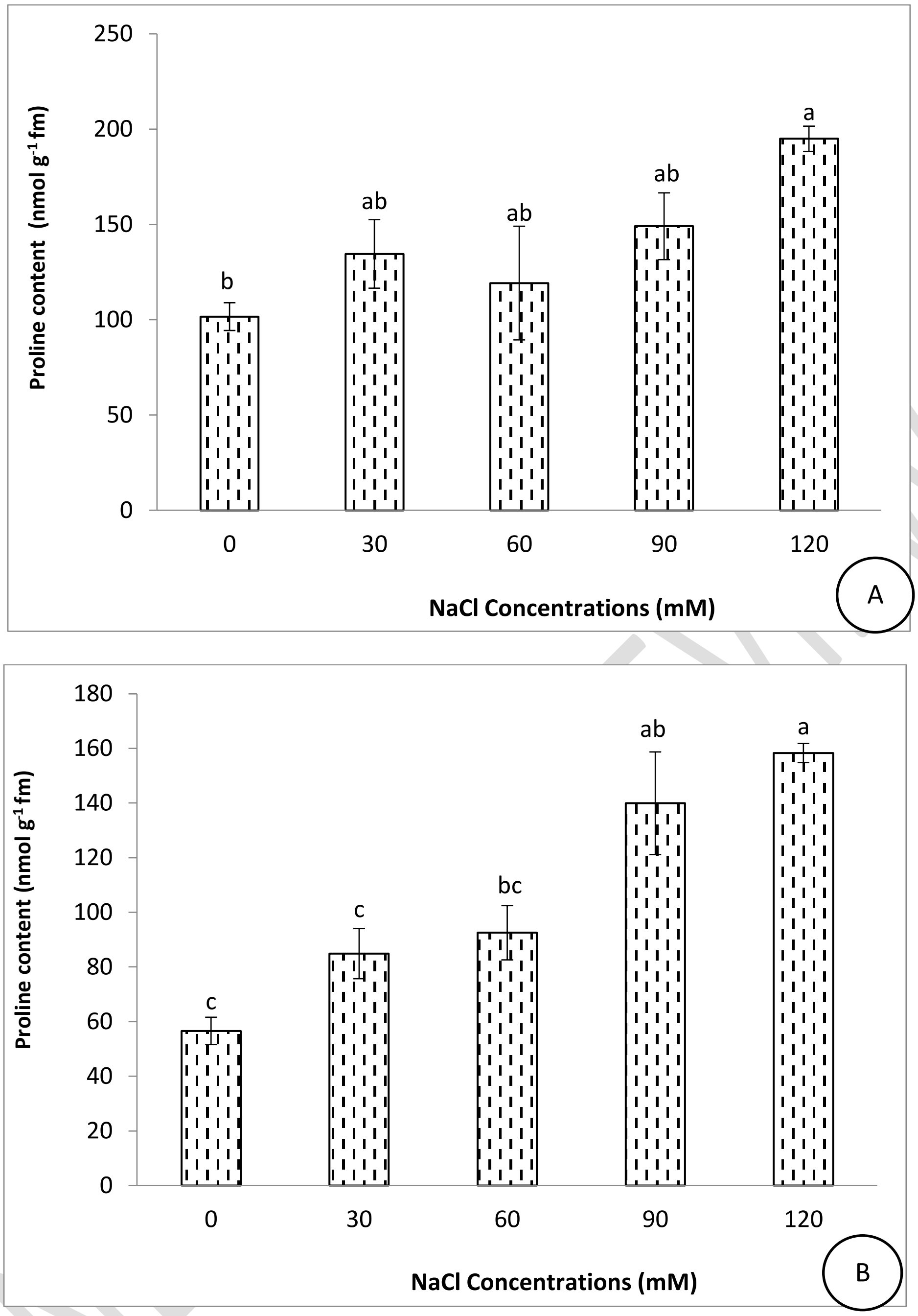

Figure 8. Effect of salt stress on proline content of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3; vertical bars are standard errors): (A) Leaves ; (B) Roots. Means with different letters differ significantly at p = .01.

Soluble sugars concentration increased significantly in both leaves (p = .05) and roots (p = .01) only at 120 mM NaCl (Figure 9). The increase was about 13.94%; 38.10%; 115.88% and 150.95% of that of the control in leaves; and 18.33%; 11.77%; 16.79 and 102.84% of that of the control in roots, respectively at 30; 60; 90 and 120 mM NaCl.

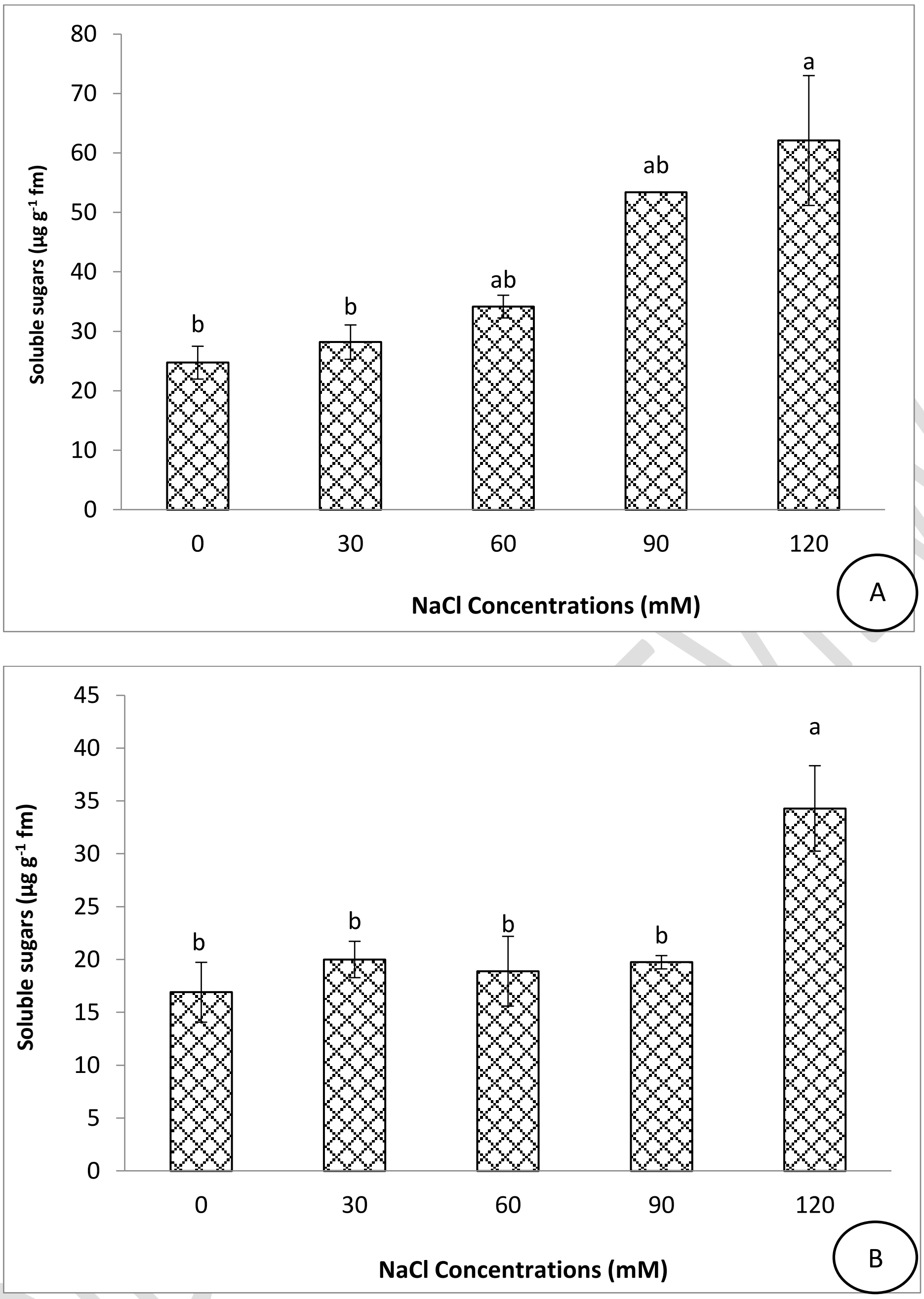

Figure 9. Effect of salt stress on soluble sugars content of African basil plants after two weeks of exposure to different NaCl concentrations (n= 3; vertical bars are standard errors): (A) Leaves ; (B) Roots

Means with different letters within lines are significantly different at p = .01.

4. DISCUSSION

4.1. Effects of salt stress on plant growth

Salinity produced visible chlorosis and necrosis at the tips of young leaves. Similar results have been reported by [28] on three pepper cultivars, and by [29] on sugarcane and could be related to a decrease in chlorophyll synthesis or to a degradation of chlorophyll in relation to hastening of senescence processes. Salt stress induced a significant reduction in plant growth for all growth parameters considered except for LN. Moreover, for the six growth parameters significantly affected by salt stress, there is a variability in the salt concentration from which salt effect was significant: roots fresh mass was significantly affected with the lowest NaCl concentration used (30 mM) whereas root length growth was significantly affected only at the highest NaCl concentrations used (120 mM). This suggest that salt stress induce a modification in root architecture. In addition, plant height, shoot dry mass and roots dry mass were significantly affected from 90 mM NaCl whereas shoot fresh mass was significantly affected from 60 mM NaCl. Thus, salinity effect on african basil plant growth depends on the growth parameter taken into account. This result corroborated those reported in other vegetable species including sweet basil [21, 19, 23, 22, 30]. According to the results, root fresh mass followed by shoot fresh mass appeared to be the most salt sensitive parameters in basil and could be validly used to characterize the response of other African basil cultivars to salinity. The results revealed that three growth parameters from the aerial part as well as three parameters from root part were significantly affected by the concentrations of NaCl used indicating that salinity effect was similar on growth of both aerial and root parts.

4.2. Effect of salt stress on plant ion content

According to Negrão *et al.* [31], Na^+ ions were accumulated to toxic levels before Cl^- which is also supposed to be less toxic than Na^+ [32]. For these reasons, our research was focused on Na^+. Results of this study showed that salt stress induced a significant increase in Na^+ content in both leaves and roots corroborating several previous reports on vegetable species [33, 24]. In some genotypes of sweet basil, similar result was reported [21, 19]. However, the increase was earlier in roots in comparison to leaves; moreover, the Na^+ accumulated in leaves are significantly lower than that accumulated in roots, even if growth of aerial part and that of root part were affected to similar extend. This result seems to indicate that African basil plants exclude Na^+ ions from leaves and accumulated them preferentially in roots to maintain in leaves, quantities compatible with plant metabolism. Thus, Na^+ toxicity appeared as one of physiological strategies explaining the detrimental effect of NaCl on African basil plants as reported in several other vegetable species [21, 33, 24].

It is well known that reducing Na^+ in the shoot, while maintaining K^+ homeostasis, is a key component of salinity tolerance in many crops [34]. Our results showed that salt stress caused a decrease in K^+ ion content in both leaves and roots of African basil plants. This observation is common in plants submitted to salt stress as reported in several vegetable species [33, 24] including *O. basilicum* [19]. According to [35], Na^+ and K^+ cations compete for uptake by membrane transporters; moreover, it is well known that K^+ deficiency has negative effect on plant productivity as it is involved in important physiological processes, such as osmotic adjustment during stress [36] and stomatal opening [37]. However, the decrease in K^+ ion content being significant only in roots as the growth reduction of shoot was significantly reduced by the salt concentrations used, we can suppose that the reduction of the African basil plants growth (in particular aerial part growth) observed under salt stress was not due mainly to K^+ content reduction. This growth reduction seems to be associated mainly to a reduction in K/Na selectivity ratio as this ratio was significantly affected by salt stress in both leaves and roots. The importance of the maintaining of high K/Na ratio in salt tolerance has been reported by several authors [38, 39].

4.3. Effect of NaCl on the content of organic solutes

Salt stress induced an increase in free proline content in both leaves and roots as previously reported [40, 41]. In one genotype of sweet basil (*O. basilicum* L.), [22] reported that NaCl concentrations of 4000 ppm induced higher increase in free proline content of plants than lowest NaCl concentrations. Proline is known as a compatible osmoregulator involved in salt tolerance [42]. However, the accumulation rate varied greatly depending on the organ and the NaCl concentration of the growing environment. Thus, results revealed that proline accumulation was early and more marked in roots in comparison with leaves. As African basil plant growth was significantly affected from 30 mM NaCl or concentration higher than it according to the growth parameter, it is logical to suppose that the role of proline accumulation in these plants was important at the highest NaCl concentration. Soluble sugars are known to be a key organic solute in plant response to salt stress. Our results showed an increase in the content of soluble sugars in both leaves and roots corroborating previous findings [42, 24]. The increasing rate was similar in leaves and roots except at the highest NaCl concentration where the accumulation was more accentuated in leaves in comparison with roots. As African basil plant growth was significantly affected from 30 mM NaCl or concentration higher than it according to the growth parameter, it is logical to suppose that the role of soluble sugars accumulation in these plants was important at the highest NaCl concentration. We have shown above that African basil plant excluded Na^+ from leaves, we can suppose that the high accumulation of proline and soluble sugars in leaves under salt stress contribute to ensure the osmotic adjustment by counterbalancing the exclusion of Na^+ from leaves.

5. CONCLUSION

The study found that salt stress reduces the growth of African basil (*O. gratissimum*) and induced an accumulation of Na^+ ion, proline and soluble sugars contents in both leaves and roots, and a reduction of K^+ ion content only in roots. Results revealed that the toxicity of the Na^+ ion is involved in African basil 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 basil plants to salt stress associated to proline and soluble sugars accumulation.

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