

*Full Length Research Paper*

# **Salinity resistance strategy of okra (*Abelmoschus esculentus* L. Moench) cultivars produced in Benin Republic**

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**Salinity negatively influences the physiology and productivity of plants which develop different strategies to resist to this stress. This study aims to evaluate the implication of sodium (Na), potassium (K), proline and soluble sugars accumulation in salt resistance of okra local cultivars after two weeks exposure to 0, 30, 60, 90 and 120 mM NaCl concentrations. Results revealed that the aerial part growth reduction under salt stress was more accentuated in the salt sensitive cultivar *Keleya* than the salt resistant *Yodana*. Na<sup>+</sup> accumulation in leaves was more accentuated in *Keleya* than *Yodana* whereas proline accumulation was more accentuated in both leaves and roots of *Yodana* than *Keleya*. K<sup>+</sup> content decrease was more accentuated both in leaves and roots of *Keleya* than *Yodana*. Consequently, the decrease in ionic selectivity ratio (K/Na) was more accentuated in the salt sensitive cultivar *Keleya* than the salt resistant *Yodana* in both leaves and roots. Soluble sugars accumulation in leaves depends on the NaCl concentration. Results indicated that the relative salinity resistance of cultivar *Yodana* is associated with sodium ions exclusion from leaves, the maintaining of good accumulation of potassium ions and a good K<sup>+</sup>/Na<sup>+</sup> selectivity ratio, and the accumulation of high amounts of proline.**

**Key words:** *Abelmoschus* species, NaCl, sodium, potassium, soluble sugars, proline.

## **INTRODUCTION**

Salt stress is one of the major environmental constraints limiting agricultural productivity (Wei et al., 2003). Salinity is the buildup of soluble salts by which saline soils are formed (Levy and Syvertsen, 2004). It was established in

several studies that plant growth is compromised by salinity at all stages of development, but sensitivity varies greatly at different stages (Akram et al., 2002; Akinci et al., 2004; Gandonou and Senhaji, 2015; Loko et al., 2020).

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Gama et al. (2007) reported that plants growing under saline conditions are affected in three ways: reduced water potential in root zone causing water deficit, phytotoxicity of ions such as  $\text{Na}^+$  and  $\text{Cl}^-$  and nutrient imbalance (Ashraf and Foolad, 2007) depressing uptake and transport of nutrients. In saline environment, plant growth is affected by complex interaction of hormones, osmotic effects, specific ion effects and nutritional imbalances, probably all occur simultaneously (Arbona et al., 2005). Although most, if not all crop plants are glycophyte species, their overall responses to increased sodium chloride ( $\text{NaCl}$ ) dose appear to be species specific (Levitt, 1980; Lutts et al., 1995; Karikalan et al., 1999; Lakra et al., 2006; Chukwu and Okpe, 2006; Gandonou et al., 2012). In addition, within the same given species, a substantial variation in salt sensitivity can appear in cultivars (Gandonou et al., 2012; Abbas et al., 2014; Sounou et al., 2021). This difference in species or cultivars' behavior is linked to a number of physiological and biochemical mechanisms developed by plants to survive, grow and produce in the presence of high salt concentrations including sodium ion exclusion, potassium ion maintenance and organic compounds accumulation. Among the compounds involved, there may be mentioned amino acids, in particular proline, soluble sugars, soluble proteins and quaternary ammonium compounds.

Okra (*Abelmoschus esculentus* L. Moench) is an annual vegetable of the tropical and subtropical areas belongs to family Malvaceae. It is a popular vegetable among both the consumers and farmers because it is rich in vitamins and minerals (Oyelade et al., 2003). Almost all parts of okra plant are consumed, like fresh okra fruits are used as vegetable, roots and stems are used for clearing the cane juice (Chauhan, 1972) and leaves and stems are used for making fiber and ropes (Jideani and Adetula, 1993). Being an excellent source of K, calcium (Ca) and unsaturated fatty acids for instance, linolenic and oleic acid (Arbona et al., 2005), okra is very essential for human nutrition (Khan et al., 2015). Although the area under okra has progressively increased during last few years, there is a decreasing trend in its yield per hectare (Haq et al., 2012). Among identified biotic and abiotic stresses, salinity has been the key factor responsible for yield reduction (Khan et al., 2002).

Despite a considerable amount of research work on plant responses to salt stress, data on okra salt tolerance strategy is scarce. In Asia, some research works studied the salt tolerance of some okra genotypes using several growth parameters (Haq et al., 2012 ; Abbas et al., 2014). Some other works studied the physiological strategy for salt tolerance in Asian okra genotypes (Shahid et al., 2011; Habib et al., 2012; Khan et al., 2015). In addition, there is hardly any work on the effect of salt stress on mineral nutrition, in particular on  $\text{Na}^+$  and  $\text{K}^+$  contents and on the accumulation of a number of organic compounds in okra cultivars grown in Benin Republic. In a previous study, it was found that an important variability exists

among okra cultivars produced in Benin in term of salinity resistance (Gouveitcha et al., 2021). The main objective of this study was to determine the physiological strategy developed by salt resistant okra cultivars grown in Benin to resist, to salt stress related to ion and organic solutes accumulation.

## MATERIAL AND METHODS

### Plant

The experiment focused on two cultivars of okra (*Abelmoschus esculentus* L. Moench) which exhibited contrasting behaviors towards salinity at the young plant stage. These are the cultivars *Yodana* which appeared to be a resistant cultivar and *Keleya* which appeared to be sensitive to  $\text{NaCl}$  according to Gouveitcha et al. (2021).

### Experimental conditions

The experiment was carried out in screening house of the National Institute of Agricultural Research of Benin (INRAB), Benin Republic. The seeds were germinated in tubs filled with potting soil for two weeks. The young plants were then transferred to small pots 5.8 cm in diameter and 6 cm in height containing a mixture of potting soil and sand (50:50) (one plant/pot) and grown for a week before application of the stress. Plants of the two cultivars were subjected to salt stress in large earthen pots (11.3 cm in diameter and 14 cm in height) filled with 3 kg of the same mixture as before. Treatments consisted of watering the plants every other day with 100 ml/pot of 0; 30; 60; 90 or 120 mM  $\text{NaCl}$ . The experimental set-up as a completely randomized design with two factors. The first factor represents the five (05) saline treatments ( $T_0 = 0$  mM;  $T_1 = 30$  mM;  $T_2 = 60$  mM ;  $T_3 = 90$  mM and  $T_4 = 120$  mM) and the 2<sup>nd</sup> is represented by the two (02) okra cultivars (*Yodana* and *Keleya*) with three replicates.

### Growth determination

Plant height (cm), number of leaves, root length (cm), fresh and dry mass (FM and DM) of the shoots and roots were first determined before application of the salt treatments ( $X_0$ ). They were determined again after 2 weeks of treatment ( $X_1$ ). Relative height growth of plants (RHG) was determined according to the formula:  $(X_1 - X_0) / X_0$ . The fresh mass of the aerial and roots parts was determined by weighing. The samples from each part were then transferred to an oven at 80°C for 72 h for the determination of the dry mass. Data in the presence of  $\text{NaCl}$  were expressed in percentage of that of the control.

### Extraction and estimation of ion concentrations

For the determination of the ions, the roots were quickly rinsed with distilled water to remove the ions fixed on them and those contained in the apoplast (Bourgeais-Chaillou and Guerrier, 1992). The leaves and roots were individually dried in an oven at 80°C for 72 h, ground in a mortar, and the powder was dried for 24 h. To determine the concentrations of  $\text{Na}^+$  and  $\text{K}^+$ , 20 mg of the leaf and root powders were placed in 10 ml jars and digested in nitric acid (68%) at room temperature. The solutions were filtered through Whatman paper (85 mm, Grade 1). The filtrate was used for the determination of cations ( $\text{Na}^+$  and  $\text{K}^+$ ) using a flame

**Table 1.** Effects of salt stress on some growth parameters of two okra cultivars after two weeks of treatment. Values are expressed as percentages (%) of means of control plants.

NaCl (mM)	Cv. <i>Keleya</i>					Cv. <i>Yodana</i>				
	RHG	RSFMG	RSDMG	RRFMG	RRDMG	RHG	RSFMG	RSDMG	RRFMG	RRDMG
30	94.63	81.00	78.60	83.04	75.78	95.94	92.02	93.31	62.83	72.57
60	81.30	69.92	75.66	69.68	75.06	93.07	84.87	77.05	59.71	66.71
90	75.31	53.47	65.37	56.89	59.92	87.93	66.41	69.95	44.90	47.43
120	68.10	16.19	13.71	39.18	47.05	70.35	59.10	54.21	38.98	40.02

spectrophotometer (Sherwood Model 360). The quantities of ions were expressed in mg g<sup>-1</sup> of dry matter (dm).

#### Extraction and determination of proline and soluble sugars

Proline concentration was determined spectrophotometrically using the method of Bates et al. (1973) and results were expressed as µg proline g<sup>-1</sup> FM (Fresh Mass).

Total soluble sugars were estimated by the anthrone reagent method using glucose as the standard accords to Yemm and Willis (1954) as used by Manaa et al. (2014) using an UV-visible spectrophotometer (Jenway 7305). Soluble sugars concentration was expressed as mg soluble sugars g<sup>-1</sup> FM (Fresh Mass).

#### Shoot water content

Shoot water content was determined according to the formula:

$$[(\text{Shoot fresh Mass} - \text{shoot dry Mass}) / \text{Shoot fresh Mass}] \times 100$$

#### Statistical analysis

The data collected was processed using descriptive statistics using an Excel spreadsheet and presented in the form of tables and graphs. Analysis of cultivar effects and stress intensity was based on one or two-ways analysis of variance (ANOVA) as appropriate. Means were compared using the Student, Newman and Keuls test. Statistical analyzes were performed using JMP Pro 12 software (JMP Pro SAS Institute, 2015).

## RESULTS

### Plant growth

NaCl stress significantly ( $p < 0.001$ ) reduced RHG in both cultivars from 90 mM NaCl but the reduction was more accentuated in the salt-sensitive *Keleya* (average of 20.16%) than the salt resistant *Yodana* (average of 12.95%) (Table 1). Salt effect induced a reduction in shoot fresh and dry mass in both cultivars but the reduction was more accentuated in the salt sensitive cultivar *keleya* than the salt resistance *Yodana*. The average reductions due to NaCl stress were 44.85, 44.16%, for cultivar *Keleya* and only 24.40 and 26.37% for cultivar *Yodana*, respectively for shoot fresh mass and shoot dry mass (Table 1). Roots growth also was

adversely affected by salt stress in both root fresh and dry mass in both cultivars. However, the reduction was less accentuated in the salt sensitive *Keleya* than the salt resistant *Yodana*. The average reductions due to NaCl stress were 37.80 and 35.55%, for cultivar *Keleya* and 48.39 and 43.32% for cultivar *Yodana*, respectively for roots fresh mass and roots dry mass (Table 1). Thus, plant aerial part growth reduction by NaCl stress was more accentuated in the salt-sensitive *Keleya* compared to the salt-resistant *Yodana*.

### Plant water content

NaCl stress induced similar effect on shoot water content in both cultivars characterized by slight non significant decrease (Table 2). Thus, shoot water content did not change significantly under salt stress in both cultivars.

### Effects of NaCl on ions accumulation

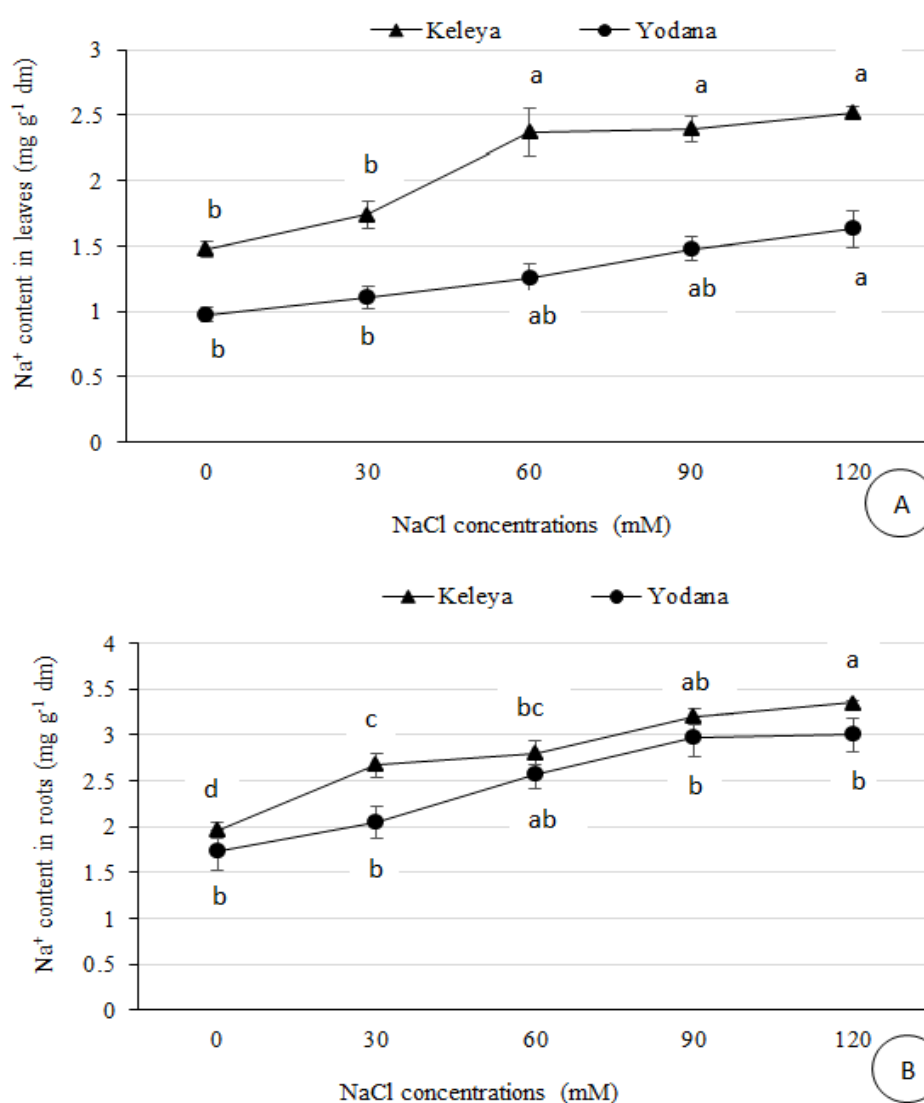
#### *Effect of NaCl on sodium ion (Na<sup>+</sup>) content*

Salt effect induced a significant ( $p < 0.001$ ) increase in the leaf Na<sup>+</sup> content of both cultivars but this increase was significant from 60 mM NaCl for the sensitive cultivar *Keleya* whereas it was significant only at 120 mM NaCl for the resistant cultivar *Yodana* (Figure 1A). Leaf Na<sup>+</sup> content passed from 1.478 mg g<sup>-1</sup> DM to 1.739, 2.369, 2.391 and 2.521 mg g<sup>-1</sup> DM for cultivar *Keleya*, respectively at 30, 60, 90 and 120 mM NaCl and from 0.978 mg g<sup>-1</sup> DM to 1.108, 1.26, 1.478 and 1.63 mg g<sup>-1</sup> DM for cultivar *Yodana* at 30, 60, 90 and 120 mM NaCl, respectively. Thus, salinity induced an increase in sodium content in the leaves in both cultivars but this increase is more marked in sensitive cultivar *Keleya* compared to the resistant cultivar *Yodana*. Likewise, Figure 1B shows a significant ( $P < 0.01$ ) increase in the roots Na<sup>+</sup> content of both cultivars but this increase was significant from 30 mM NaCl for the sensitive cultivar *Keleya* whereas it was significant from 90 mM NaCl for the resistant cultivar *Yodana*. Root Na<sup>+</sup> content passed from 1.956 mg g<sup>-1</sup> DM to 2.673, 2.804, 3.195 and 3.347 mg g<sup>-1</sup> DM for cultivar *Keleya*, respectively at 30, 60, 90 and 120 mM NaCl, and

**Table 2.** Effect of different NaCl concentrations on shoot water content (%) of two cultivars of *okra* after two weeks stress application.

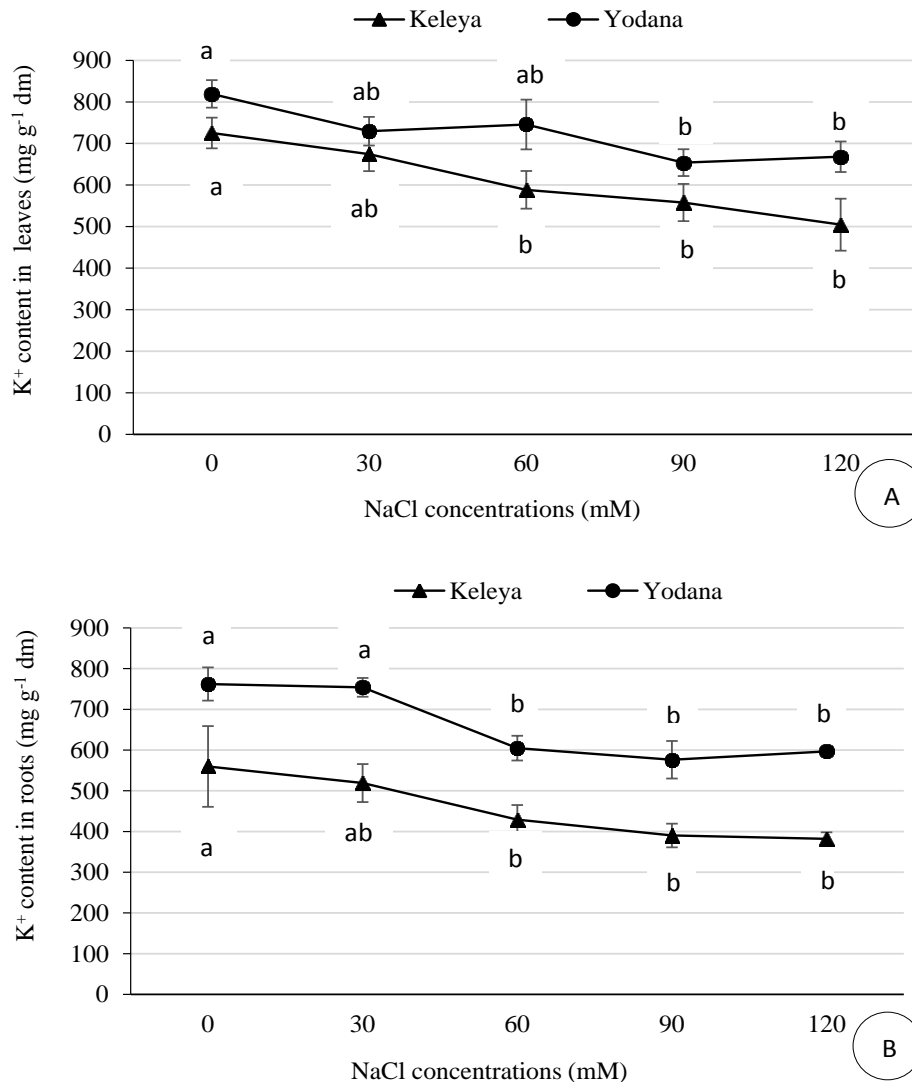
NaCl (mM)	Cv. <i>Keleya</i>	Cv. <i>Yodana</i>
0	93.532±0.65 <sup>a</sup>	94.198±0.42 <sup>a</sup>
30	92.994±0.28 <sup>a</sup>	93.860±0.27 <sup>a</sup>
60	92.785±0.16 <sup>a</sup>	93.901±0.27 <sup>a</sup>
90	92.497±0.38 <sup>a</sup>	93.731±0.30 <sup>a</sup>
120	92.850±0.25 <sup>a</sup>	93.468±0.24 <sup>a</sup>

Means with same letter within column did not differ significantly at P=0.05.

**Figure 1.** Effect of different NaCl concentrations on sodium ion content of two cultivars of *okra* after two weeks stress application (A) in leaves; (B) in roots (n= 3; vertical bars are standard errors). Means with different letters differ significantly at P=0.01.

from 1.739 to 2.043, 2.565, 2.978 and 3 mg g<sup>-1</sup> DM for cultivar *Yodana* at 30, 60, 90 and 120 mM NaCl, respectively. Thus, salinity induced an increase in sodium

content in roots in both cultivars but this increase is more marked in the sensitive cultivar *Keleya* compared to the resistant cultivar *Yodana*.



**Figure 2.** Effect of different NaCl concentrations on potassium ion content of two cultivars of okra after two weeks stress application (A) in leaves; (B) in roots (n= 3; vertical bars are standard errors). Means with different letters differ significantly at P=0.05.

### Effect of NaCl on potassium ion (K<sup>+</sup>) content

Salt effect induced a significant decrease in leaves K<sup>+</sup> content of both cultivars but this decrease was significant (P<0.05) from 60 mM NaCl for the sensitive cultivar *Keleya* whereas it was significant (P<0.01) from 90 mM NaCl for the resistant cultivar *Yodana* (Figure 2A). Leaf potassium content passed from 725.37 mg g<sup>-1</sup> DM to 674.29, 588.47, 557.82 and 504.7 mg g<sup>-1</sup> DM for cultivar *Keleya*, respectively at 30, 60, 90 and 120 mM NaCl, and from 819.37 mg g<sup>-1</sup> DM to 729.46, 745.81, 653.86, and 668.16 mg g<sup>-1</sup> DM for cultivar *Yodana*. Thus, salinity induced a decrease in potassium content in leaves in both cultivars but this decrease was more marked in sensitive cultivar *Keleya* compared to the resistant cultivar *Yodana*. Likewise, Figure 2B shows a significant

(p<0.05) decrease in roots potassium content from 60 mM NaCl in K<sup>+</sup> content of both cultivars. Roots potassium content passes from 559.86 mg g<sup>-1</sup> DM to 519, 429.10, 390.27, and 302.10 mg g<sup>-1</sup> DM for cultivar *Keleya* and 762.16 mg g<sup>-1</sup> DM to 753.98, 604.82, 576.22 and 596.65 mg g<sup>-1</sup> DM for cultivar *Yodana*. Thus, salinity induced a decrease in potassium ion content in roots in both cultivars but this decrease was more marked in the sensitive cultivar *Keleya* compared to the resistant cultivar *Yodana*.

The selectivity ratio decreased in both cultivars in leaves and roots as the NaCl concentration increased (Table 3). For the sensitive cultivar *Keleya*, the decrease was significant (P<0.001) from 60 mM NaCl. For the resistant cultivar *Yodana*, the decrease was significant (P<0.01) from 90 mM NaCl. Thus, salinity induced a

**Table 3.** Effect of different NaCl concentrations on selectivity ration (K/Na) in leaves and in roots of two cultivars of *okra* after two weeks stress application (n= 3; values are means  $\pm$  standard errors).

NaCl (mM)	Leaves (K/Na)		Roots (K/Na)	
	<i>Keleya</i>	<i>Yodana</i>	<i>Keleya</i>	<i>Yodana</i>
0	491.64 $\pm$ 23.50 <sup>a</sup>	844.63 $\pm$ 54.31 <sup>a</sup>	291.56 $\pm$ 57.62 <sup>a</sup>	456.29 $\pm$ 55.74 <sup>a</sup>
30	390.36 $\pm$ 24.91 <sup>a</sup>	666.90 $\pm$ 30.71 <sup>ab</sup>	195.99 $\pm$ 20.47 <sup>b</sup>	375.48 $\pm$ 24.11 <sup>ab</sup>
60	251.93 $\pm$ 24.91 <sup>b</sup>	615.80 $\pm$ 12.13 <sup>ab</sup>	154.69 $\pm$ 17.42 <sup>b</sup>	236.57 $\pm$ 5.43 <sup>b</sup>
90	234.64 $\pm$ 22.19 <sup>b</sup>	448.05 $\pm$ 18.98 <sup>b</sup>	122.52 $\pm$ 9.87 <sup>c</sup>	200.80 $\pm$ 34.13 <sup>c</sup>
120	200.41 $\pm$ 25.20 <sup>b</sup>	417.05 $\pm$ 12.50 <sup>b</sup>	114.08 $\pm$ 4.33 <sup>c</sup>	200.93 $\pm$ 11.58 <sup>c</sup>

Means with same letter within column did not differ significantly at P=0.05.

decrease in K<sup>+</sup>/Na<sup>+</sup> selectivity ratio of leaves for both cultivars but this decrease was more marked in a sensitive cultivar *Keleya* compared to the resistant cultivar *Yodana*. In roots, the same tendency was observed with a decrease significant (P<0.001) from 30 mM NaCl for the sensitive cultivar *Keleya*, and significant (P<0.01) from 60 mM NaCl for the resistant cultivar *Yodana*.

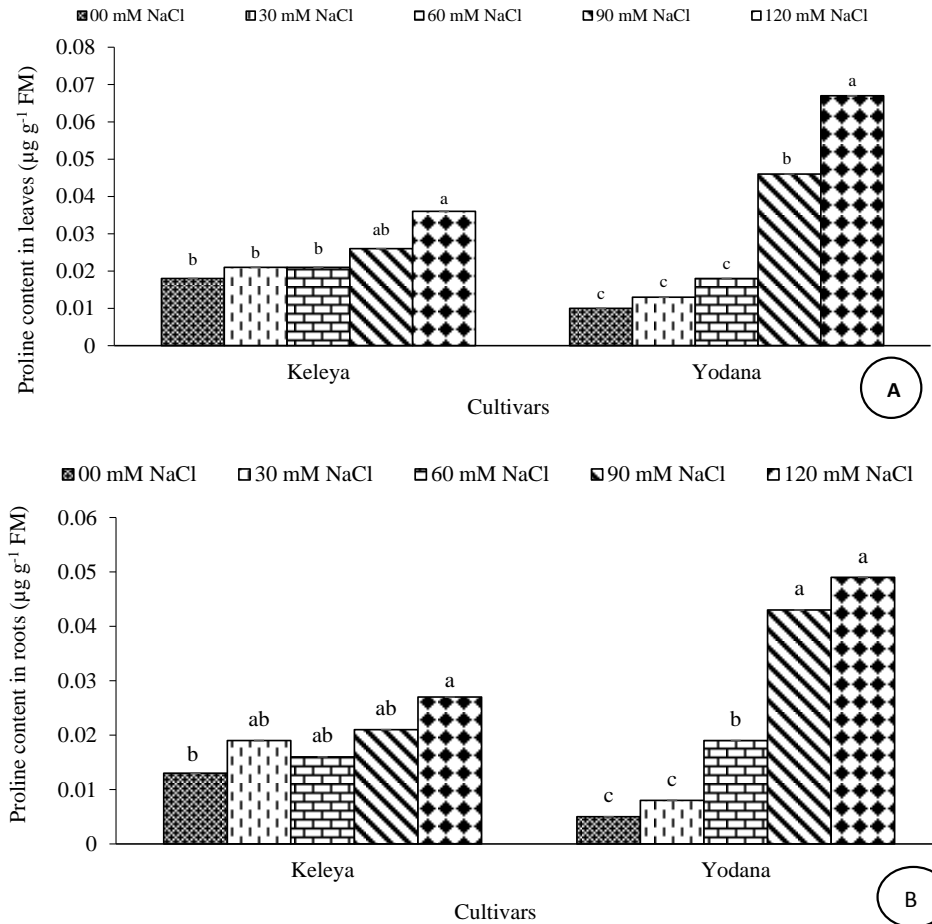
#### Effect of salt stress on proline content

Salt effect induced a significant (P< 0.001) increase in proline content in leaves of both cultivars but this increase was significant (P< 0.01) only at 120 mM NaCl for the sensitive cultivar *Keleya* whereas it was significant (p< 0.001) from 90 mM NaCl for the resistant cultivar *Yodana* (Figure 3A). Proline concentration passed from 0.018  $\mu\text{g g}^{-1}$  FM to 0.021, 0.021, 0.026, and 0.036  $\mu\text{g g}^{-1}$  FM for cultivar *Keleya*, respectively at 30, 60, 90, and 120 mM NaCl, and from 0.010  $\mu\text{g g}^{-1}$  FM to 0.013, 0.018, 0.046, and 0.067  $\mu\text{g g}^{-1}$  FM for cultivar *Yodana* at 30, 60, 90, and 120 mM NaCl, respectively. These increases correspond respectively to 16.66, 16.66, 44.44 and 100% compared to the control for the sensitive cultivar *Keleya*, and to 30, 80, 360, and 570% for the resistant cultivar *Yodana*. Thus, salinity induced an increase in proline content in leaves in both cultivars but this increase is much more marked in resistant cultivar *Yodana* compared to the sensitive cultivar *Keleya*. Likewise, Figure 3 shows a significant increase in roots proline content of both cultivars but this increase was significant (P< 0.01) only at 120 mM NaCl for the sensitive cultivar *Keleya* whereas it was significant (P< 0.001) from 60 mM NaCl for the resistant cultivar *Yodana*. Proline concentration passed from 0.013  $\mu\text{g g}^{-1}$  FM to 0.019, 0.016, 0.021 and 0.027  $\mu\text{g g}^{-1}$  FM for cultivar *Keleya*, respectively at 30, 60, 90, and 120 mM NaCl; and from 0.005  $\mu\text{g g}^{-1}$  FM to 0.008, 0.019, 0.043 and 0.049  $\mu\text{g g}^{-1}$  FM for cultivar *Yodana* at 30, 60, 90, and 120 mM NaCl, respectively. These increases correspond, respectively to 46.15, 23.07, 61.53, and 107.69% compared to the control for the sensitive cultivar

*Keleya*, and to 60, 280, 760, and 880% for the resistant cultivar *Yodana*. Thus, salinity induced an increase in proline content in roots in both cultivars but this increase is much more marked in the resistant cultivar *Yodana* compared to the sensitive cultivar *Keleya*. Moreover, proline accumulation was more marked in roots than leaves in both cultivars.

#### Effect of salt stress on soluble sugars content

Salt effect induced a significant (P< 0.001) increase in soluble sugars content in leaves of both cultivars but this increase was significant from 30 mM NaCl for the sensitive cultivar *Keleya* whereas it was significant from 60 mM NaCl for the resistant cultivar *Yodana* (Figure 4A). Soluble sugars content passed from 0.441 mg g<sup>-1</sup> FM to 0.73, 0.717, 1.059, and 1.804 mg g<sup>-1</sup> FM for cultivar *Keleya*, respectively at 30, 60, 90 and 120 mM NaCl, and from 0.291 mg g<sup>-1</sup> FM to 0.396, 0.529, 0.763, and 0.647 mg g<sup>-1</sup> FM for cultivar *Yodana* at 30, 60, 90, and 120 mM NaCl, respectively. These increases correspond, respectively to 65.53, 62.58, 140.13 and 309.07% compared to the control for the sensitive cultivar *Keleya*, and to 36.08, 81.78, 162.19, and 122.33% for the resistant cultivar *Yodana*. Thus, salinity induced an increase in soluble sugars content in leaves in both cultivars but this increase is more marked in the salt sensitive cultivar *Keleya* compared to the resistant cultivar *Yodana* mainly at 30 and 120 mM NaCl. Likewise, Figure 4B shows a significant (P< 0.001) increase in roots soluble sugars content of both cultivars but this increase was significant from 60 mM NaCl for the sensitive cultivar *Keleya* whereas it was significant from 90 mM NaCl for the resistant cultivar *Yodana*. Soluble sugars content passed from 0.215 mg g<sup>-1</sup> FM to 0.224, 0.514, 0.655, and 0.994 mg g<sup>-1</sup> FM for cultivar *Keleya*, respectively at 30, 60, 90 and 120 mM NaCl, and from 0.135 mg g<sup>-1</sup> FM to 0.180, 0.174, 0.190 and 0.310 mg g<sup>-1</sup> FM for cultivar *Yodana* at 30, 60, 90 and 120 mM NaCl, respectively. This increase corresponds, respectively to 4.18, 139.06, 204.65 and 362.32% compared to the control for the sensitive cultivar *Keleya*, and to 33.33,



**Figure 3.** Effect of different NaCl concentrations on proline content of two cultivars of *okra* after two weeks stress application (A) in leaves; (B) in roots ( $n=3$ ; vertical bars are standard errors). Means with different letters differ significantly at  $P=0.001$ .

28.88, 40.74 and 129.62% for the resistant cultivar *Yodana*. Thus, salinity induced an increase in soluble sugars content in roots in both cultivars but this increase is much more marked in the sensitive cultivar *Keleya* compared to the resistant cultivar *Yodana* except at 30 mM NaCl.

## DISCUSSION

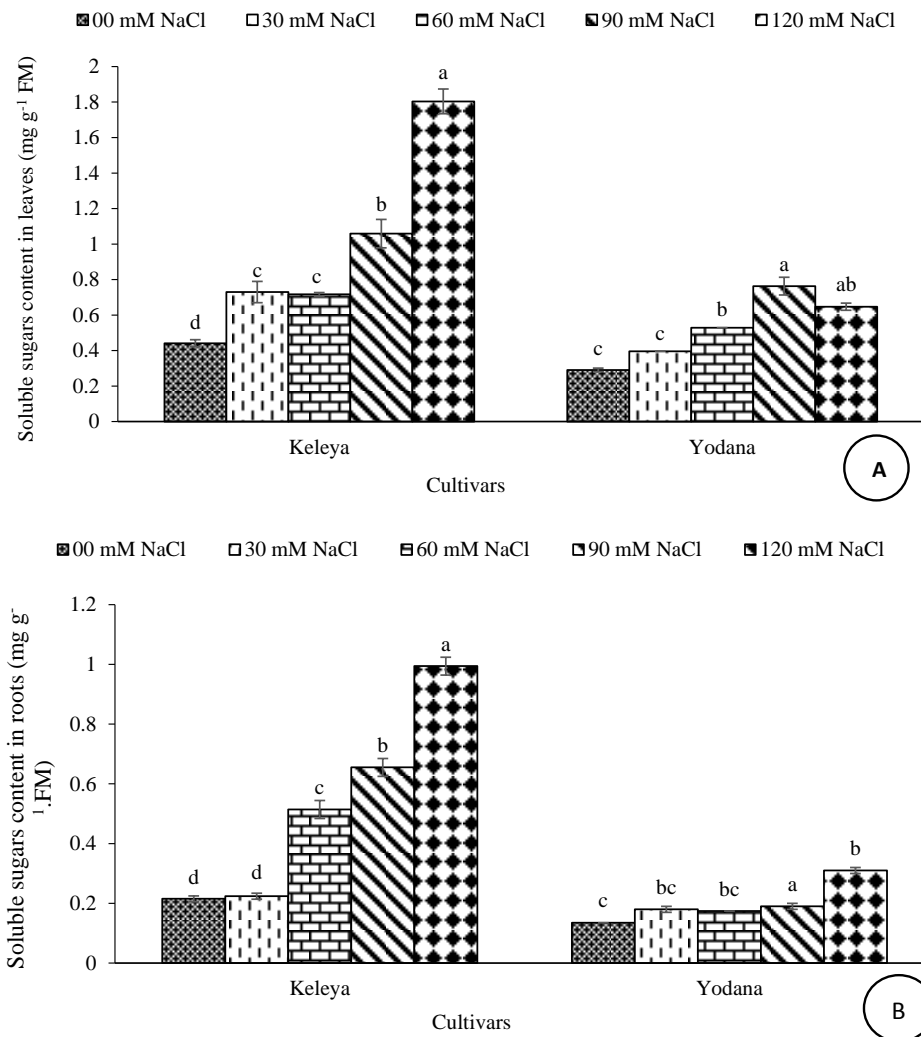
### Effect of NaCl on plant growth of okra cultivars

The results revealed that plant growth reduction due to NaCl stress was more accentuated in the salt-sensitive *Keleya* compared to the salt-resistant *Yodana* confirming the salt-resistance status of both cultivars as previously reported (Gouveitcha et al., 2021). In other okra genotypes, Abbas *et al.* (2014) used growth parameters to discriminate salt tolerant genotypes from the salt sensitive one. Salinity classically induced cell dehydration at low water potential, nutritional imbalance caused by

the interference of saline ions with essential nutrients in both uptake and translocation processes and toxicity due to the high accumulation of  $\text{Na}^+$  and  $\text{Cl}^-$  in the cytoplasm. No change in plant water content was observed in our cultivars indicating that water content parameter is not the main aspect of salt stress effect in these cultivars as previously reported in amaranth (Wouyou et al., 2019).

### Implications of ion accumulation in the salinity resistance of okra cultivars

The effect of salt stress on plants can induce the following three responses: dehydration of cells through low water potential; nutritional imbalance caused by the interference of salt ions with essential nutrients in both absorption and translocation processes; toxicity due to a high accumulation of  $\text{Na}^+$  in the cytoplasm and to a lesser extent of  $\text{Cl}^-$ . In most species,  $\text{Na}^+$  appears to accumulate to toxic levels before  $\text{Cl}^-$  does (Negrão et al., 2017) and  $\text{Cl}^-$  is considered less toxic than  $\text{Na}^+$  (Munns and Tester, 2008).



**Figure 4.** Effect of different NaCl concentrations on soluble sugars content of two cultivars of okra after two weeks stress application (A) in leaves; (B) in roots ( $n=3$ ; vertical bars are standard errors). Means with different letters differ significantly at  $P=0.05$ .

Thus, we focus here on  $\text{Na}^+$ , because reducing  $\text{Na}^+$  in the shoot, while maintaining  $\text{K}^+$  homeostasis, is a key component of salinity tolerance in many crops (Munns, 2011). Plants of both cultivars accumulated high amounts of  $\text{Na}^+$  in the leaves when subjected to NaCl in their growing medium. The same tendency was reported in other okra genotypes (Habib et al., 2012). The results revealed that the resistant cultivar *Yodana* accumulated less  $\text{Na}^+$  in the leaves and roots than the sensitive cultivar *Keleya*. However, it was observed that the harmful effects of  $\text{Na}^+$  on growth are more accentuated on the aerial part of plants of the sensitive cultivar *Keleya*, compared to those of the resistant cultivar *Yodana*. Thus, the detrimental effects of  $\text{Na}^+$  on growth are more accentuated in aerial part of the sensitive *Keleya* compared to cv. *Yodana* plants. This is a general trend in glycophytes in which salinity resistant varieties accumulate less  $\text{Na}^+$  and/or  $\text{Cl}^-$

in the leaves than susceptible varieties (Lutts et al., 1996; Almansouri et al., 1999; Akhtar et al., 2003; Wahid, 2004; Niu et al., 2010; Wouyou et al., 2019). The relative resistance of the cultivar *Yodana* to NaCl is therefore explained by its ability to exclude  $\text{Na}^+$  ions from the leaves leading to an avoidance or exclusion mechanism. In okra, Habib et al. (2012) have reported that foliar application of both pure glycine betaine and sugarbeet extract significantly reduced the adverse effects of salt stress in terms of plant growth, yield and leaf  $\text{Na}^+$  content indicating that the salt tolerance acquired following the foliar application of both pure glycine betaine and sugarbeet extract was due, at least partially, to  $\text{Na}^+$  exclusion from leaves. This result confirmed the importance of  $\text{Na}^+$  exclusion from leaves as part of okra plants salt tolerance strategy.

Salt stress caused a decrease in potassium ion in the



leaves and roots of the two cultivars tested. This observation is also made by Maggio et al. (2007) who proved that the presence of NaCl in the plant environment generally induces an increase in Na<sup>+</sup> and a decrease in K<sup>+</sup> in the various organs. Similar results were reported in other okra genotype (Shahid et al., 2011). Maintaining a good K supply is one of the major responses of salt stress resistant genotypes in glycophyte species, and potassium ions are known to be a major component of osmotic adjustment during stress (Wu et al., 1996). Thus, in rice, Lutts et al. (1996) reported that a salt tolerant variety maintained high amounts of K<sup>+</sup> in the leaves compared to salt sensitive genotypes when both types were under salt stress. The same trend has been reported in durum wheat (Almansouri et al., 1999). In the present study, the reduction in potassium content observed is clearly less pronounced in the resistant cultivar *Yodana* compared to the sensitive cultivar *Keleya* in both leaves and roots. Thus, the relative resistance of cultivar *Yodana* to salinity appears to be primarily associated with maintaining a good K<sup>+</sup> supply in the presence of NaCl. Comparing the effect of foliar application of both pure glycine betaine and sugarbeet extract on okra response to salt stress, Habib et al. (2012) have revealed that this application significantly reduced the adverse effects of salt stress in terms of plant growth, yield and leaf K<sup>+</sup> content indicating that leaf K<sup>+</sup> accumulation is part of the strategy which mediated the salt tolerance acquired following the foliar application of both pure glycine betaine and sugarbeet extract. This result confirmed the importance of leaf K<sup>+</sup> accumulation as part of okra plants salt tolerance strategy.

The salt-resistant cultivar *Yodana* accumulated less Na<sup>+</sup> in both leaves and roots and maintained higher K<sup>+</sup> content than the salt-sensitive cultivar *Keleya*. Consequently, the salt-resistant *Yodana* maintained a significantly higher K<sup>+</sup>/Na<sup>+</sup> selectivity ratio in both leaves and roots than the sensitive *Keleya*.

### **Implication of organic solutes accumulation in the salinity resistance of okra cultivars**

Biosynthesis of osmoprotectants and compatible solutes are among the physiological principle and biochemical mechanisms developed by plants in order to survive in soils with high salt concentration (Gupta and Huang, 2014). Proline and soluble sugars are the main parts of these osmoprotectants and compatible solutes. Salinity caused an increase in proline content in both cultivars either in leaves or in roots. Accumulation of proline is frequently reported in plants subjected to salt stress (Mishra and Saxena, 2009; Bouassaba and Chougui, 2018). It has often been considered as a compatible osmoregulator which may be involved in the mechanisms of resistance to salt stress (Ehsanpour and Fatahian, 2003; Bouassaba

and Chougui, 2018). Other functions have been suggested regarding the accumulation of proline in stressed tissues ; it could be: (1) a protective agent for enzymes and membranes (Van Rensburg et al., 1993; Solomon et al., 1994), (2) a free radical scavenger (Smirnov and Cumbes, 1989), (3) a carbon and nitrogen storage compound (Jäger and Meyer, 1977) or (4) it could be involved in the regulation of cytosolic pH (Venekamp, 1989). However, results of the present study on stressed okra plants showed that the accumulation of proline is much more marked in resistant cultivar *Yodana* compared to the sensitive cultivar *Keleya* in both leaves and roots. We can therefore suggest that the overproduction of proline is okra plants response to salt stress and that the salinity resistance of the cultivar *Yodana* is associated with high proline accumulation. These results indicated that proline plays an important role in salinity tolerance as previously reported in several species (Watanabe et al., 2000; Mishra and Gupta, 2005). However, other authors have reported an opposite tendency in several species including sugar cane (Wahid, 2004; Gandonou et al., 2005, 2011), rice (Lutts and Guerrier, 1995; Lutts et al., 1996), tomato (Pérez-Alfocea et al., 1994) and amaranth (Wouyou et al., 2019). In other okra genotypes, Habib et al. (2012) have reported that foliar application of both pure glycine betaine and sugarbeet extract significantly reduced the adverse effects of salt stress in terms of plant growth and yield but reduced leaf proline in comparison to salt stress indicating that the salt tolerance acquired following the foliar application of both pure glycine betaine and sugarbeet extract was accompanied by a low leaf proline accumulation. This result suggested that proline hyper accumulation in leaves is not part of the strategy developed by okra treated plants to tolerate salt stress. Thus, the implication of proline in salinity tolerance in okra depends on the genotype and the type of salt-tolerance (genetic or artificial) taking into account.

In general, salinity caused an increase in soluble sugars content in both cultivars either in leaves or in roots. The results are consistent with the general trend. Indeed, the effects of salt stress generally result in an increase in the content of soluble sugars in both the leaves and the roots in several plant species (Bouassaba and Chougui, 2018; Wouyou et al., 2019) including okra (Abbas et al., 2014). Generally, the more tolerant cultivars accumulate more soluble sugars than the sensitive ones. Thus, Gandonou et al. (2011) reported that in sugarcane, the salinity resistant cultivar CP66-346 accumulates more soluble sugars in the leaves than the sensitive CP65-357 in the presence of salt stress. The same trend has been reported in calli, selected or not in this same species (Gandonou et al., 2005, 2006). On the other hand, in *Carthamus tinctorius*, Ashraf and Fatima (1995) reported that two salt-resistant accessions showed different responses: one accumulates more soluble sugars than sensitive accessions, while the other

resistant accession accumulates similar amounts of sugars as sensitive accessions, although it is more tolerant. Soluble sugars are known for their role in osmoregulation in plants exposed to osmotic stress. According to Cram (1976), among the osmotically active organic compounds, sugars contribute more than 50% of the total osmotic potential in glycophytes subjected to salt stress. The fact that the salt sensitive cultivar *Keleya* accumulated more soluble sugars in both leaves and roots than the salt resistant *Yodana*, seems to indicate that soluble sugars did not play an important role in cultivar *Yodana* salt resistance.

## Conclusion

Salt stress caused an increase in sodium ions, free proline and soluble sugars content and a decrease in potassium ion content in both leaves and roots in both okra cultivars with a significant difference in the reaction of okra cultivars. The overall reaction of the cultivars indicates that the relative salt resistance of cultivar *Yodana* is reliable to a good sodium ion exclusion and a good potassium ion accumulation mainly in leaves associated with maintaining of a good  $K^+/Na^+$  ratio. Free proline appears also as a good salt resistance indicator in this cultivar.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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