

# Evaluation of growth, yield and nutrient composition of calyx and leave of roselle (*Hibiscus sabdariffa* L.) cultivars as affected by salt stress conditions in far north region of Cameroon

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## Abstract

**Contexte:** In Sahelian and coastal areas, salinity is a permanent threat to the growth and development of plants. The choice of tolerant salt species would be a solution to this constraint.

**Objective:** The purpose of this work is to study the effect of the different NaCl concentrations on the growth, the yield and the nutritional composition of leaves and the calyx in two cultivars of Roselle (*Hibiscus sabdariffa* L.): green bissap and red star.

**Methodology:** The plants were subjected to different doses of NaCl ranging from 0, 60, 120 to 240 mM for 60 days according to a completely random blocking device having four blocks.

**Results:** Increase in NaCl levels had no appreciable effect on growth, yield and chemical composition of leaf and calyx of roselle. Application of NaCl (from 0 to 240 mM) leads to a decrease of leaf yield (to 27.5% and 24.7% in Red star and Green bissap respectively), calyx yield (to 23.1% and 31.6% in Red star and Green bissap respectively), seed yield (to 29.8% and 32.8% in Red star and Green bissap respectively) and accumulation of osmolytes, fiber content, Na content of leaf and calyx and total anthocyanin content of calyx (to 25% and 66.5% in Red star and Green bissap respectively). The Na<sup>+</sup> content obtained in the aerial parts (leaf and calyx) is very lowest in the Red star cultivar and the highest value showed in Green bissap.

**Conclusion:** The Red star (red bissap) cultivar is more salt tolerant than Green bissap and we can suggest that it could be a promising crop for farmers in salinity-prone areas and the coastal belts in tropical and sub-tropical regions.

**Key words:** Calyx, growth, nutritional composition, roselle, salinity, tolerance.

## 1 Introduction

Roselle (*Hibiscus sabdariffa* L.) is mainly grown in Sahelian areas such as the region of the far north of Cameroon. The leaves and the calyx of the plant are used as vegetable or decoction or drink (Adam, 2013). In the northern areas of Cameroon, young leaves are also used as ingredients in sauces and serve as a nutrient complement to cereals such as rice, sorghum and millet. The plant is also used to treat many diseases such as cough, teeth and hypertension disorders (Hassane, 2005). From the nutritional point of view, the leaves are rich in Ca, K, Mg, N and P (Atta et al., 2010a). The calyx are rich in Ca, K, Mn, Na and Fe that the seeds (Atta et al., 2013). The roselle seeds contain about 60% of proteins, 20% of fat, 15% oil, 5% ash and carbohydrates (Halimatu et al., 2007; Mera et al., 2009). Roselle is also cultivated for its fiber in certain production areas (Maunde, 2010; Mohammed et al., 2013). Despite the benefits of this culture, in Cameroon the yield is usually low because of the limited genetic potential of the varieties used, a lack of supply of fertilizers and especially salt stress.

Salinity in soils is responsible for the reduced yield for several crops. Munns and Tester (2008) reported that 45 million hectares of land had been affected by salinity worldwide, and 1.5 million hectares are taken out of cultivation each year due to over-accumulation of salts in the soil. Salinity leads to reductions in several metabolic processes associated with growth, development, and crop productivity (Fayez and Bazaid, 2014). Seed germination, for example, is inhibited by salt stress

in several plant species (Ahammed et al., 2018; Gu et al., 2018; Wilayasinghe et al., 2019). Salt tolerance is very complex because multiple genes are involved. In this case, it seems that the most effective way to overcome the salinity problem may be the introduction of salt-tolerant crops. Indeed, according to degree of stress in the middle, plants are exposed to changes in their morphological, physiological, anatomical and biochemical behavior (Nasir- Khan, 2010). In these circumstances, plants are in a stressful situation and develop defense mechanisms (Denden *et al.*, 2005). These mechanisms, the osmotic adjustment plays a vital role in the resistance or tolerance of the plant to stress (Munns, 2002). The plant will have to synthesize organic solutes to adjust its water potential and on the other hand synthesizing osmoprotectors, mainly amino compounds and sugars and to accumulate them in cytoplasm and organelles (Chen and Jiang, 2010).

Therefore, this experiment was conducted to evaluate the effects of different levels of salt stress on the growth, yield, leaf and calyx nutritional quality of *Hibiscus sabdariffa* L. (Roselle) cultivars. Comparison of these parameters in these roselle cultivars may be helpful to provide additional information on the mechanisms of salt tolerance and develop salt tolerant cultivars for breeding program.

## 2 Materials and methods

### 2.1 Study site

The study was conducted from 16 April 2022 to 20 November 2022 at Palar harde, in the Maroua city, Department of Diamare, Region of Far north of Cameroon (latitude : 10°36'37,57''N, longitude : 14°17'34,41'' E). The climate is tropical of a hot sudano-sahelian type, average annual rainfall is estimated at 700 mm. The rainy season lasts about 3 to 4 months from June to September. The temperatures range from 25°C to 30°C in rainy season and culminate at 45°C in the dry season. The soil of the experimental site is mainly the sandy-clay type. In these periods of heat, it is a consequence of precipitation related to an important evaporation thus promoting the accumulation of salt in the soil (Munns et al., 2006).

### 2.2 Plant growth conditions and treatment

Two *Hibiscus sabdariffa* L. cultivars have been used as vegetable material: red star (large trilobed leaves, red calyx and stem) and green bissap (large trilobed leaves, green stem and white calyx). Seeds were provided by the breeding program of the TECHNISEM (SEMAGRI, Maroua). These ecotypes distinguished from the color of calyx and leaves, the shape of the leaves and type of production (calyx, leaves and seeds). Ten kilograms of the sieved soil was weighed into pots, each of seven litres capacity perforated at the bottom to allow unimpeded drainage. The experiments were arranged in a completely randomized block design with one plant per pot and four replicates per treatment with as factors two cultivars of roselle and four levels of salinity (0, 60, 120 and 240 mM NaCl).

The seeds were planted in cavity trays in the greenhouse on the 18th of May 2022 and transplanted into the prepared polythene bags containing 5 kg of soil on the 4th of June 2022. The plants were watered immediately after transplanting to avoid drought stress. Before initiating treatments plants were irrigated with normal tap water using a hand sprinkler to full saturation for two weeks in order to improve root development (Imana et al., 2010). After which 500 ml of water was applied to each pot and this was able to wet the soil to full saturation. All plants were fertilized daily with a modified nutrient solution (in g L<sup>-1</sup>): 150 g Ca(NO<sub>3</sub>)<sub>2</sub>, 70 g KNO<sub>3</sub>, 15 g Fe-EDTA, 0.14 g KH<sub>2</sub>PO<sub>4</sub>, 1.60 g K<sub>2</sub>SO<sub>4</sub>, 11 g MgSO<sub>4</sub>, 2.5 g CaSO<sub>4</sub>, 1.18 g MnSO<sub>4</sub>, 0.16 g ZnSO<sub>4</sub>, 3.10 g H<sub>3</sub>BO<sub>3</sub>, 0.17 g CuSO<sub>4</sub> and 0.08 g MoO<sub>3</sub> (Hoagland and Arnon, 1950). The pH of the nutrient solution was adjusted to

7.0 by adding  $\text{HNO}_3$  0.1 mM. The amendment in each case was applied 6 WAS with  $12 \text{ t ha}^{-1}$  of organic fertilizer.

### 2.3 Soil moisture content determination, irrigation water and analysis

Soil samples were collected from representative spots on the experimental site from where soil was collected for potting using soil auger to a depth of 20 cm, the samples were made into a sample. A sub-sample was taken, air-dried, crushed and sieved with 2-mm mesh sieve after which physical and chemical analyses were carried out (Table 1). The following chemical analyses were done on the soil and tap water (Tables 1 and 2). Organic carbon (C), was determined by the wet oxidation procedure (Walkley and Black, 1934) and total Nitrogen (N) by micro-Kjeldahl digestion method. Magnesium (Mg) was extracted using the Mehlich 3 method and determined by auto ANALYSER 5 (Technicon 2). The total and available soil phosphorus (P) were determined by the method of Okalebo et al. (1993). Soil was measured potentiometrically in 1:2.5 soil: water mixture. Calcium (Ca), potassium (K) and sodium (Na) were determined by a flame photometer (JENWAY) as described by Taffouo et al. (2008).  $\text{Ca}^{2+}$ ,  $\text{Mg}^{2+}$ ,  $\text{Na}^+$ ,  $\text{K}^+$ ,  $\text{HCO}_3^-$ ,  $\text{SO}_4^{2-}$ ,  $\text{NO}_3^-$ ,  $\text{Cl}^-$  content in water tap was determined by using colorimetric amperometric titration method (Taleisniket al., 1997) (Table 2). Electric conductivity and pH were determined by conductometer.

Table 1. Physical and chemical characteristics of soil used

Physio-chemical properties	Quantity
Clay %	$39.98 \pm 2.81$
Sand %	$68.04 \pm 2.87$
Total carbon %	$0.82 \pm 0.11$
Total nitrogen %	$0.35 \pm 0.27$
Ratio C/N	$3.77 \pm 1.08$
Phosphorus (%)	$0.29 \pm 0.12$
Potassium ( $\text{meq } 100\text{g}^{-1}$ )	$2.25 \pm 1.06$
Sodium ( $\text{meq } 100\text{g}^{-1}$ )	$1.14 \pm 0.66$
Calcium ( $\text{meq } 100\text{g}^{-1}$ )	$11.27 \pm 1.92$
Magnesium ( $\text{meq } 100\text{g}^{-1}$ )	$2.65 \pm 1.07$
pH	$6.23 \pm 1.11$
EC ( $\text{dS/m}$ )	$3.12 \pm 0.79$

Table 2. Chemical characteristics of irrigation water

Chemical characteristics									
Irrigation Water	$\text{Ca}^{2+}$ ( $\text{mg g}^{-1}$ )	$\text{Mg}^{2+}$ ( $\text{mg g}^{-1}$ )	$\text{K}^+$ ( $\text{mg g}^{-1}$ )	$\text{HCO}_3^-$ ( $\text{mg g}^{-1}$ )	$\text{Na}^+$ ( $\text{mg g}^{-1}$ )	$\text{SO}_4^{2-}$ ( $\text{mg g}^{-1}$ )	$\text{Cl}^-$ ( $\text{mg g}^{-1}$ )	pH	CE ( $\text{dS m}^{-1}$ )
Tap water	230.9	117.1	22.9	63.8	442.5	515.7	27.9	7.31	1.96

### 2.4 Plant growth and yield parameters

Seedlings were harvested 20 WAS by carefully removing and washing the soil particles from the roots, after which the plants parts were separated into shoots and roots (Metwally et al., 2013). Leaves and calyx of greenbissap and red star cultivars were analysed. The tissues (leave and calyx) were dried for 24 h at  $105^\circ\text{C}$  (Taffouo et al., 2008). The dry samples were weighted. Plant samples were harvested after 4 months culture and under 10 weeks of salt stress, plant were collected to determine agro morphological characters (plantheight, total leaf area, longer of calyx, diameter of calyx, leave, seed and calyx yield) roselle cultivars.

The leaf and calyx relative water content (RWC) was recorded according to the formula as follows:  $RWC = (FFW - FDW) / (TW - FDW) \times 100$ , where FFW is fresh weight, FDW is dry weight, and TW is turgid weight (Sánchez et al., 2004).

The TLA (length  $\times$  width  $\times$  0.80  $\times$  total number of leaves  $\times$  0.662) was calculated using the methodology described by Kumar et al. (2002).

### **2.5 Osmolytes and antioxidant content**

TSC content in leaves and calyx was measured by the phenolsulphuric method according to Dubois et al. (1956). For this purpose, leaf and calyx material (50 mg) was oven-dried until the constant dry mass was reached. Dried leaf material was powdered in a mortar and pestle and TSS was extracted by 70 % ethanol. After centrifugation of extract at 3,500 rpm for 20 min, a reaction mixture was prepared. This mixture consisted of 1,000  $\mu$ l supernatant, 300  $\mu$ l phenol, and 2,000  $\mu$ l concentrated sulphuric acid. Absorbances of these mixtures were read at 470 nm and the TSC content of the leaves and calyx was calculated by a standard curve using sucrose.

Fiber content (FC) analysis have been realised by the method of Van Soest, 1963.

Soluble protein content (SP) was determined by Bradford's method (1976). Briefly, appropriate volume (from 0 - 100  $\mu$ l) of sample was aliquoted into a tube and the total volume was adjusted to 100  $\mu$ l with distilled water. A 1 ml of Bradford working solution was added to each sample well. Then the mixture was thoroughly mixed by vortex mixer. After left for 2 min, the absorbance was read at 595 nm. The standard curve was established by replacing the sample portions in the tubes with proper serial dilutions of bovine serum albumin.

Total anthocyanin content (TAC) was analyzed by the procedure of Mancinelli et al. (1975). Leaf and calyx sample (0.1 g  $\times$  2) was soaked in 10 ml of a mixture of methanol and 1 N HCL (85/15; v/v) for 72 h at 4 °C. The crude extracts were filtered through a 0.45  $\mu$ m syringe filter before measurement of total anthocyanin content at 530 and 657 nm. The content was expressed as mg g<sup>-1</sup> fresh mass.

For estimation of vitamin C, 1 g of frozen leaf and calyx tissues was homogenised in 5 mL of ice-cold 6% m-phosphoric acid (pH 2.8) containing 1 mM EDTA (Gossett et al., 1994). The homogenate was centrifuged at 20,000  $\times$  g for 15 min at 4°C. The supernatant was filtered through a 30- $\mu$ m syringe filter, and 50  $\mu$ L of the filtrate was analyzed using an HPLC system (PerkinElmer series 200 LC and UV/VIS detector 200 LC, USA) equipped with a 5- $\mu$ m column (Spheri-5 RP-18; 220  $\times$  4.6 mm; Brownlee) and UV detection at 245 nm with 1.0 mL/min water (pH 2.2) as the mobile phase, run isocratically (Gahler et al., 2003).

### **2.6 Nutrient content**

Ca, K, Na, Mg and P contents in the leaf and calyx tissue of the plants were evaluated in dry, ground, and digested samples in a CEM microwave oven (Abreu et al., 1995). P was determined by colorimetry; potassium by flame photometry; calcium, sodium and magnesium by atomic absorption spectrometry (Malavolta et al., 1997). Iron content were determined by method reported in (Pauwels et al., 1992). Leaf and calyx of roselle was dry ashed at 450°C for 2 hours and digested on heat cave with 10 ml HNO<sub>3</sub> 1 M. The solution was filtrated and adjusted at 100 ml with HNO<sub>3</sub> at 1/100 and analyzed with an atomic absorption spectrophotometer (Rayleigh, WFX-100).

### **2.7 Statistical analysis**

The experiment was conducted as a factorial completely randomized design with four NaCl treatments (0, 60, 120 and 240 mM NaCl) and two cultivars (Red star and Green bissap) in four

replications. Data are presented in term of mean ( $\pm$ standard deviation). All data were statistically analysed using Statistica (version 9, Tulsa, OK, USA) and first subjected to analyses of variance (ANOVA). Statistical differences between treatment means were established using the Fisher LSD test at  $p < 0.05$ .

### 3 Results and discussion

#### *Effect of salinity on growth characteristics, relative water content, leaf, calyx and seed yield*

The results of the analysis of variations indicate significant differences ( $P < 0.05$ ) of PH, TLA, DC, LC, LRWC, CRWC, LY, CY and SY between the four doses of NaCl in both cultivars studied. From control to 240 mM NaCl, PH, TLA, DC, LC, LRWC and CRWC decreased to 24.7, 17.6, 32.2, 23.5, 9 and 9.1% for Green bissap and 26, 16.6, 19.8, 9.1, 9.2 and 6.2% for Red star (Figure 1A, 1B, 1D, 1E and 1F).

Prodjinoet al.(2018) reported that reducing the growth of the vegetative apparatus aerial under abiotic stress allows the plant to accumulate energy and resources to mitigate stress before the imbalance of the organism increases to a threshold where damage is irreversible. For Ben-Ahmed et al.(2008), the reduction of growth seems to be associated with strong  $\text{Na}^+$  accumulation in the plant.

The best leaf yield ( $481.85 \text{ kg ha}^{-1}$ ) and seed yield ( $456.29 \text{ kg ha}^{-1}$ ) has been obtained in Green bissap variety while calyx yield ( $452.85 \text{ kg ha}^{-1}$ ) was recorded in Red star cultivar (Table 3 and Figure 1D). These yields decrease significantly based on growing salts. Indeed, the 60 mM NaCl contribution reduced leaves, calyx and seeds yield to 9.9%, 10.1% and 10.1% in Green bissap and 8.2%, 6.6% and 7.4% in Red star respectively. This reduction is however to 24.7%, 31.6% and 32.8% in Green bissap and to 27.5%, 23.1% and 29.8% in Red star cultivar respectively at 240 mM NaCl. These results were similar to that of Apel and Hirt (2004) who reported that seedling growth, flowering production and fruit were affected by high salt concentration, leading to decreased in yield.

#### *Effect of salinity on osmolytes and antioxidant content*

Salt has a significant effect on the accumulation of TAC, TSC, FC, SP and reduction of ascorbic acid content ( $p < 0.05$ ). The results indicate that TAC, TSC, FC and SP, under saline constraints (60, 120 and 240 mM NaCl) are very important in both roselle cultivars (Figure 1C and Table 3). From control to 240 mM NaCl, TAC, TSC, FC and SP increased by (66.5% in calyx), (137.9% in leave and 122.5% in calyx), (195.5% in leave and 135.3% in calyx) and (167.9% in leave and 166.7% in calyx) respectively in Red star cultivar. And in Green bissap, this increase varies by (25% in calyx), (130% in leave and 126.9% in calyx), (146.7% in leave and 114.9% in calyx) and (178.2% in leave and 168.3% in calyx) respectively.

This results were similar with the finding of Petropoulos et al. (2017) who suggested that salt stress elevated sugar and protein in *Cichorium spinosum*. Soluble sugars play a dual role in stressed plants, since they participate in metabolic events and deshydration (Lepengue et al., 2012), role that is decisive in the osmotic adjustment and the level of stabilization of some proteins (Bouatrous, 2013). The increment of protein and fiber contents in roselle at 60, 120 and 240 mM NaCl could be contributed to human diet in the communities of saline prone area compared to non-saline area. According to the data obtained from the study, accumulation of anthocyanins with increasing of salinity allow the plant to develop resistance to environmental stresses (Steyn et al., 2002) and it either act as a hydroxyl radical scavenger (Cooper, 2001), or as a solute that inhibits lipid per oxidation and stimulates the activities of antioxidant enzymes which act as defense system in plants in response to a biotic events (Hassanein et al., 2005). In addition, anthocyanin is an important flavonoid as

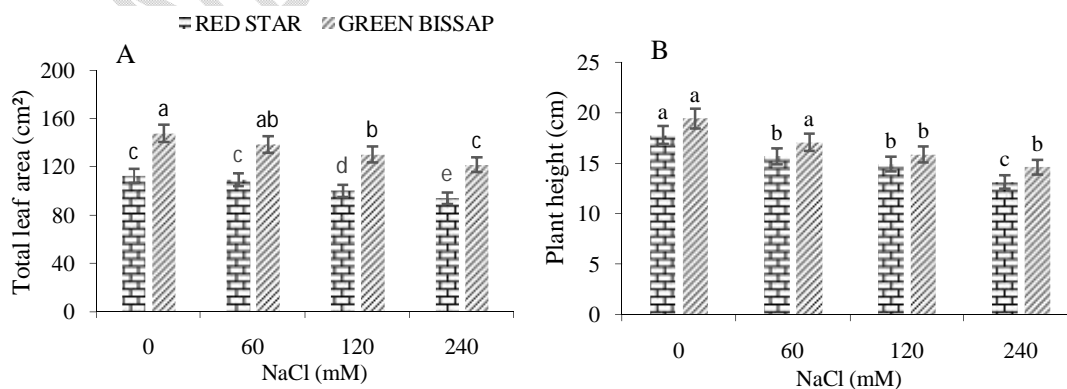
amodulator of salinity stress and plays an important role in the prevention of stress-induced oxidative damage in plants (Eryilmaz, 2006).

ASA decreased significantly from control to 240 mM NaCl in Red star by (191.87 to 90.91mg kg<sup>-1</sup> in leave and 503.25 to 398.53mg kg<sup>-1</sup> in calyx). In Green bissap cultivar, this reduction varie by(185.63 to 80.29 mg kg<sup>-1</sup> in leave and 483.55 to 340.57mg kg<sup>-1</sup> in calyx) (Table 3).Our results reveal that in leaf and calyx of all roselle cultivars, the ascorbic acid content decreased significantly under NaCl stress. This decrease was observed byRatnakar and Rai (2013) in the leaves of *Amaranthus polygamous* L. Navarro et al. (2006) found that salinity decreased the ASA content of pepper fruits, and this effect was dependent on the maturity stage.

### ***Effect of salinity on mineral composition of leaves and calyx***

The effect of salinity on the uptake of Na, P, Fe, Ca, Mg and K was investigated at the full blooming stage at the plant harvest and the data are shown in Table 4. The obtained results demonstrated that in the full blooming stage P, Fe, Ca, Mg and K concentrations decreased from control to 240 mM NaCl by 16.4, 19.1, 23.6, 11, 46.3%for the calyx and 33.7, 38.3, 34, 52.9, 58.3% for leaf of the Red starcultivar respectively; by 19, 22.85, 28.5, 16.8, 49.3% for the calyx and 37.5, 40.3, 34.3, 57.6, 60.1% for leaf of the Green bissap cultivar respectively. On the other hand, Na content increased for Red star (130.4% in leave and 109.4% in calyx) and for Green bissap(158.4% in leave and 127.6% in calyx) from control to to 240 mM NaCl.

The obtained results indicated that the contents of P, Fe, Ca, Mg and K in roselle leaves and calyx were significantly decreased in the plants treated with NaCl compared with the control plants(Hand et al., 2017; Nouck et al., 2021). The reduction in P, K, Mg content under saline conditions attribute to that Na saltsraised the pH of the soil, and in turn reduced the availability of P, Mg, K to the plant. The increase in Na concentration in plants with the salinity may be attributed to the ability of plants to use Na to maintain an osmotic potential gradient between the plant tissues and the external solution (Sivritepeet *al.*, 2003).During the experimentation, the lowest Na<sup>+</sup>content in the aerial partsare observed in the Red star cultivar and the highest in the variety Green bissap. Niu et al. (2010) andAktaset al.(2006) reported thatsalt tolerant pepper genotypes accumulateless Na<sup>+</sup>in the leavesthan sensitive genotypes. These studies indicate that genotypes with the ability to exclude Na<sup>+</sup> are generallymoretolerant to salinity.



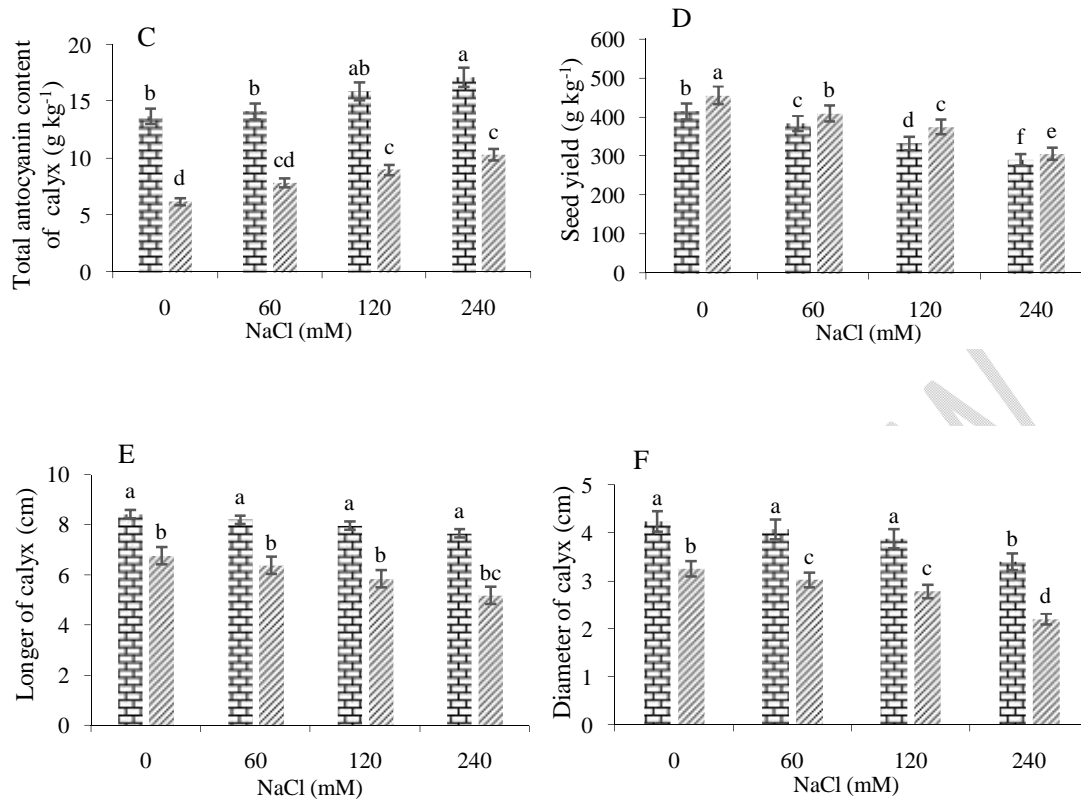


Fig. 1. Effects of salinity rates (60, 120 and 240 mM NaCl) on growth, seed yield and total anthocyanin content of calyx of two roselle cultivars (20 WAS). Total leaf area (A), Plant height (B), Total anthocyanin content of calyx (C) Seed yield (D), Longer of calyx (E) and Diameter of calyx (F). Bars are means (n=5)  $\pm$  SD. Means followed by different letter are significantly different (p < 0.05).

Table 3. Effects of salt stress rates on leaf and calyx nutritional components, leaf and calyx yield and relative water content of two roselle cultivars (20 WAS)

Cultivars	Organs	Salt stress (mM NaCl)	SP (g kg <sup>-1</sup> )	TSC (g kg <sup>-1</sup> )	RWC (%)	ASA (mg kg <sup>-1</sup> )	FC (g kg <sup>-1</sup> )	Yield (kg ha <sup>-1</sup> )
Red bissap (Red star)	Calyx	0	24.92 $\pm$ 1.13l	61.75 $\pm$ 1.77h	91.26 $\pm$ 2.11e	503.25 $\pm$ 4.21a	1.73 $\pm$ 0.95n	497.37 $\pm$ 4.16b
		60	28.34 $\pm$ 1.08l	65.12 $\pm$ 0.81h	89.44 $\pm$ 2.24f	473.31 $\pm$ 4.64b	1.89 $\pm$ 0.91	464.74 $\pm$ 2.19b
		120	34.63 $\pm$ 1.71k	70.73 $\pm$ 0.89g	88.53 $\pm$ 2.25f	422.24 $\pm$ 3.39b	2.01 $\pm$ 0.58n	411.82 $\pm$ 3.22b
		240	41.58 $\pm$ 0.89j	75.65 $\pm$ 1.03g	85.61 $\pm$ 2.37f	398.53 $\pm$ 4.42c	2.34 $\pm$ 0.66o	382.53 $\pm$ 3.75c
	Leaf	0	19.17 $\pm$ 0.86m	44.34 $\pm$ 2.65n	89.42 $\pm$ 2.55f	191.87 $\pm$ 3.19d	1.12 $\pm$ 1.12n	481.85 $\pm$ 3.55b
		60	23.41 $\pm$ 0.79l	49.25 $\pm$ 2.02j	86.34 $\pm$ 1.61f	149.19 $\pm$ 3.23d	1.43 $\pm$ 1.02n	442.53 $\pm$ 2.88b
		120	27.85 $\pm$ 1.02l	55.38 $\pm$ 1.33i	84.26 $\pm$ 1.82f	100.12 $\pm$ 3.44d	1.81 $\pm$ 0.83n	384.62 $\pm$ 3.11c
		240	32.19 $\pm$ 1.17k	61.16 $\pm$ 1.73h	81.19 $\pm$ 2.09f	90.91 $\pm$ 3.51e	2.19 $\pm$ 1.12n	349.38 $\pm$ 2.16c
	Calyx	0	21.14 $\pm$ 1.11l	56.37 $\pm$ 1.04i	93.41 $\pm$ 2.93e	483.55 $\pm$ 4.41b	0.94 $\pm$ 0.44o	452.85 $\pm$ 2.37b
		60	24.75 $\pm$ 1.05l	60.83 $\pm$ 1.09h	90.69 $\pm$ 1.92e	433.28 $\pm$ 3.92b	1.26 $\pm$ 0.77n	406.93 $\pm$ 2.47b
		120	28.35 $\pm$ 1.04j	65.51 $\pm$ 0.57h	88.17 $\pm$ 2.88f	392.34 $\pm$ 4.17c	1.63 $\pm$ 0.21n	375.65 $\pm$ 3.61c
		240	35.58 $\pm$ 1.09k	71.53 $\pm$ 4.57g	84.88 $\pm$ 2.37f	340.57 $\pm$ 4.85c	2.02 $\pm$ 1.07n	309.83 $\pm$ 3.85c

Green bissap	Leaf	0	20.73±0.91l	42.52±2.61j	90.76±2.54e	185.63±3.46d	0.75±1.08o	613.77±3.59a
		60	25.21±1.15l	46.87±2.02j	87.33±2.66f	138.49±3.55d	0.98±0.93o	552.74±2.15a
		120	31.37±1.14k	50.58±1.77i	85.51±1.74f	91.94±3.28e	1.47±0.74n	509.56±2.23a
		240	36.95±0.99k	55.29±1.11i	82.57±1.65f	80.29±3.27f	1.85±0.38n	462.18±2.91b

Two way ANOVA results

Cultivars (C)	*	*	NS	*	*	*
Organs (O)	NS	*	*	**	*	*
Salt stress (SS)	*	*	*	*	*	*
Interaction O x SS	*	*	*	*	*	*
Interaction C x SS	*	*	*	*	*	*

Values shown are means (n=5) ± SD; within columns, means followed by different letter are significantly different (p < 0.05). \*\*, \* significant at 1 and 5% probability levels, respectively, NS not significant

Table 4. Effects of salt stress rates on leaf and calyx mineral composition of two roselle cultivars (20 WAS).

Cultivars	Organs	Salt stress (mM NaCl)	Na (mg kg <sup>-1</sup> )	K (g kg <sup>-1</sup> )	Ca (mg kg <sup>-1</sup> )	Mg (mg kg <sup>-1</sup> )	P (mg kg <sup>-1</sup> )	Iron (mg kg <sup>-1</sup> )
Red bissap (Red star)	Calyx	0	887.93±3.11b	3.95±1.75o	486.25±2.79f	939.65±3.22a	702.55±3.93c	43.38±2.26k
		60	906.01±4.05a	3.24±0.82o	449.14±3.78f	901.12±3.18a	665.14±4.07d	40.14±3.08k
		120	938.47±3.09a	2.81±1.72o	408.04±2.83f	868.33±3.27b	629.73±4.11d	37.02±3.05l
		240	971.51±4.11a	2.12±0.90o	371.28±3.91g	835.35±3.45b	587.47±4.12e	35.09±3.07ll
	Leaf	0	36.86±3.24l	2.83±0.69o	342.02±2.65g	187.58±3.11i	398.62±3.71g	9.15±3.13n
		60	39.45±4.05l	2.25±0.85o	309.16±2.22g	146.34±2.28i	345.77±3.27g	7.88±3.04n
		120	43.08±3.18k	1.92±0.77o	269.47±2.66h	113.22±3.44i	307.56±3.11g	6.04±2.79n
		240	48.19±3.12k	1.18±0.61p	225.83±3.02h	88.35±3.57j	264.38±2.19h	5.65±2.91n
Green bissap	Calyx	0	962.22±3.33a	3.61±1.08o	421.38±2.14f	918.46±2.52a	695.52±3.12d	29.66±3.02m
		60	998.25±2.92a	3.01±0.805o	382.61±3.12g	855.48±2.23b	651.83±3.09d	27.09±2.28m
		120	1151.01±2.55a	2.59±1.05o	348.74±3.14g	809.56±2.71b	614.62±3.16d	25.41±2.72m
		240	1227.33±2.77a	1.83±7.57o	301.12±2.39g	763.71±3.75c	563.44±3.18e	22.85±2.55m
	Leaf	0	33.48±3.16ll	2.58±0.62o	363.56±2.44g	191.64±3.43i	413.96±3.18f	8.39±2.53n
		60	39.55±2.83l	2.04±1.21o	324.38±2.26g	149.27±3.23i	365.25±2.77g	6.62±3.67n
		120	45.48±2.84k	1.62±1.25p	280.72±2.39h	109.33±3.64i	319.41±3.66g	5.97±3.82n
		240	53.02±2.86k	1.03±1.09p	238.79±2.54f	81.17±3.14j	258.73±2.24h	5.01±3.08n

Two way ANOVA results

Cultivars (C)	*	NS	*	*	*	*
Organ (O)	**	NS	**	**	**	*
Salt stress (SS)	*	*	*	*	*	*
Interaction O x SS	*	*	*	**	*	*
Interaction C x SS	**	*	*	**	*	*

Values shown are means (n=5) ± SD; within columns, means followed by different letter are significantly different (p < 0.05). \*\*, \* significant at 1 and 5% probability levels, respectively, NS not significant

## Conclusion

The results of this experimentation reported genotypical differences in salinity. Salinity stress significantly decreased the growth and yield, enhanced the osmolytes, fiber content, total anthocyanin and Na content of the leaf and calyx of a roselle. Under the higher level of salinity conditions, the protein, soluble sugar, fiber content and total anthocyanin content of roselle leaves and calyx were very high in comparison to the control conditions, which allows for the assignment of roselle as a valuable food source for human diets and health benefits. Indeed, the variety Green bissap was more sensitive to NaCl than Red star. Red star has been more tolerant to salinity as it has been affected significantly than when the dose of salt is high (240 mM NaCl).

**Abbreviations:** Ascorbic acid-ASA; Calcium-Ca; calyx yield-CY; days after planting-DAP; days after sowing-DAS; diameter of calyx-DC; dry weight-DW; electric conductivity-EC; fiber content-FC; fresh weight-FW; leaf yield-LY; longer of calyx-LC; magnesium-Mg; nitrogen-N; organic carbon-C; phosphorus-P; plant height-PH; potassium-K; relative water content-RWC; seed yield-SY; shoot dry weight-SDW; shoot fresh weight-SFW;

sodium-Na; soluble proteins-SP; total anthocyanin-TAC;total leaf area-TLA;total solubles carbohydrates-TSC; week after sowing-WAS.

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### **Conflict of interests**

The authors declare that there is no conflict of interests.

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## ANNEX

Table Effects of salt stress rates on growth, seed yield and total anthocyanin content of calyx of two roselle cultivars (20 WAS)

Cultivars	Salt stress mM NaCl	TLA (cm <sup>2</sup> )	PH (cm)	TAC of calyx (g kg <sup>-1</sup> )	SY (kg ha <sup>-1</sup> )	LC (cm)	DC (cm)
Red bissap (Red star)	0	112.61±3.11f	17.82±1.44h	13.74±0.88i	415.24±4.54a	8.43±1.01j	4.25±1.01k
	60	109.44±4.11f	15.73±1.25h	14.15±0.86i	384.41±4.67b	8.21±1.21j	4.08±0.85k
	120	100.25±3.66g	14.94±1.33h	15.91±1.02i	333.53±3.92b	7.97±0.97j	3.89±0.72k
	240	93.97±3.14g	13.18±0.93i	17.17±1.09i	291.68±3.15c	7.66±1.88j	3.41±0.98k
Green bissap	0	147.88±2.92d	19.46±1.88h	6.21±0.74j	456.29±4.01a	6.78±1.25j	3.26±1.02k
	60	138.56±2.89d	17.11±1.66h	7.86±1.09k	410.34±3.07a	6.39±1.74j	3.03±1.22k
	120	130.42±3.99d	15.92±1.22h	9.01±1.12k	375.83±3.05b	5.86±1.09k	2.79±0.94l
	240	121.84±2.98e	14.66±1.31h	10.34±1.06k	306.72±2.98b	5.19±1.04k	2.21±0.88l
Two way ANOVA results							
Cultivars (C)		*	*	**	**	NS	NS
Salt stress (SS)		*	*	NS	*	*	*
Interaction C x SS		**	*	*	**	*	*

Values shown are means (n=5) ± SD; within columns, means followed by different letter are significantly different (p < 0.05). \*\* , \* significant at 1 and 5% probability levels, respectively, NS not significant