# Evaluating the effect of salt stress conditions on the growth, yield and nutrient composition of calyces and leavesof roselle (*Hibiscus sabdariffa*L.)cultivarsin far north region of Cameroon


## Abstract

***Context*:**In sahelian and coastal areas, salinity is a permanent treat to the growth and devolpment of plants. The choice of salt-tolerant species would be a solution to this constraint.

***Objective:***The purpose of this work was to study the effect of the different NaClconcentrations on the growth, the yield and the nutritional composition of leaves and the calyx in two cultivarsof Roselle (*Hibiscus sabdariffa* L.)i.e: green bissap and red star.

***Methodology*:**The plants were subjected to different doses of NaCl ranging from 0, 60, 120 to 240 mMfor 60 daysin a completely random blocking design having four block.

***Results*:**Increase in NaCl levels had no appreciable effect on growth, yield and chemical composition of leave and calyce of roselle.Application of NaCl (from 0 to 240 mM)led to a decrease ofleaf yield (27.5% and 24.7% in Red star and Green bissap respectively), calyce yield (23.1% and 31.6% in Red star and Green bissap respectively), seed yield (29.8% and 32.8% in Red star and and Green bissap respectively)and accumulation of osmolytes, fiber content, Na content of leave and calyce and total anthocyanin content of calyce(25% and 66.5% in Red star and Green bissap respectively). The $Na^{+}$content obtained in the aerial parts (leave and calyce) was very low in the Red star cultivar and high in Green bissap.

***Conclusion*:**The Red star (red bissap) cultivar is moresalt tolerantthan the Green bissap and therefore suggests that it could be a promising crop for farmers in salinity-prone areas and the coastal belts in tropical and sub-tropical regions.



**Keys words**: Calyce, 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 calyce of the plant are used as vegetable or decoction or drink (Adamou, 2013). In the northen areas of Cameroon, young leaves are also used as ingredients in sauces and serve 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). Thecalyces are rich inCa, K, Mn, Na and Fe (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 crop, 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 synthetisizing osmoprotectors, mainly amino compounds and sugars and to accumulate them in cytoplasm and organists (Chen and Jiang, 2010).

Therefore, this experiment was conducted to evaluate theeffects of differents levels of salt stresson the growth, yield, leave 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 20November 2022 at Palar harde, in the Maroua city, Department of Diamare, Region ofFar northof Cameroun (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 to 45°C in the dry season. The soil of the experimental site is mainly the sandy-clay type. In these periods of heaplers, it is a consequitus 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. cultivarshas been used as vegetable material: red star (large trilobed leaves, red calyx and stem) and green bissap (large trilobed leaves, green stemandwhite 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, leavesand 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 treatmentmaking it a two factor experiment, i.e.twocultivars of roselle and four level of salinity (0, 60, 120 et 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 containing5 kg of soil on the 4th of June 2022. Theplants 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 modifiednutrient solution (in g $L^{-1}$): 150 g $Ca(NO_3)_2$, 70 g $KNO_3$, 15 gFe-EDTA, 0.14 g $KH_2PO_4$, 1.60 g $K_2SO_4$, 11 g $MgSO_4$, 2.5 g$CaSO_4$, 1.18 g $MnSO_4$, 0.16 g $ZnSO_4$, 3.10 g $H_3BO_4$, 0.17 g$CuSO_4$ and 0.08 g $MoO_3$ (Hoagland and Arnon, 1950). ThepH of the nutrient solution was adjusted to

7.0 by adding$HNO_3$ 0.1 mM.The amendment in each case was applied 6 WAS with 12 t $ha^{-1}$ of organic fertilizer.

### *2.3Soilmoisture 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 at a depth of 20 cm, the samples were made into a composite 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 5Technicon 2). The total and available soil phosphorus (P) were determined by the method of Okalebo et al. (1993). Soil was measured potentionmetrically in 1:2.5 soil: water mixture. Calcium (Ca), potassium (K) and sodium (Na) were determined by aflame photometer (JENWAY) as described by Taffouo et al. (2008). $Ca^{2+}$, $Mg^{2+}$, $Na^{+}$, $K^{+}$, $HCO_3^{-}$, $SO_4^{2-}$, $NO_3$, $Cl^{-}$content in water tap weredetermined by using colorimetric amperometric titration method (Taleisnik et 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 ± 2.81 |
| Sand% | 68.04 ± 2.87 |
| Total carbon % | 0.82 ± 0.11 |
| Total nitrogen % | 0.35 ± 0.27 |
| Ratio C/N | 3.77 ± 1.08 |
| Phosphorus (%) | 0,29 ± 0.12 |
| Potassium (meq $100g^{-1}$) | 2.25 ± 1.06 |
| Sodium (meq $100g^{-1}$) | 1.14 ± 0.66 |
| Calcium (meq $100g^{-1}$) | 11.27 ± 1.92 |
| Magnésium (meq $100g^{-1}$) | 2.65 ± 1.07 |
| pH | 6.23 ± 1.11 |
| EC(dS/m) | 3.12± 0.79 |

Table 2.Chemical characteristics of irrigation water

| *Chemical characteristics* | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Irrigation Water | $Ca^{2+}$(mg $g^{-1}$) | $Mg^{2+}$(mg $g^{-1}$) | $K^{+}$(mg $g^{-1}$) | $HCO_3^{-}$ (mg $g^{-1}$) | $Na^{+}$ (mg $g^{-1}$) | $SO_4^{2-}$ (mg $g^{-1}$) | $Cl^{-}$(mg $g^{-1}$) | pH | CE (dS $m^{-1}$) |
| Tap water | 230.9 | 117.1 | 22.9 | 63.8 | 442.5 | 515.7 | 27.9 | 7.31 | 1.96 |

### *2.4Plant 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 calycesof greenbissap and red starcultivars were analysed.The tissues (leave and calyx) were dried for 24 h at 105˚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, length of calyce, diameter of calyce, leave, seed and calyce yield) rosellecutivars.

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

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

***2.5Osmolytes and antioxidant content***

TSC content in leaves and calyce were 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 µl supernatant, 300 µl phenol, and 2,000 µl concentrated sulphuric acid. Absorbances of these mixtures were read at 470 nm and the TSC content of the leavesand calyces were calculated by a standard curve using sucrose.

Fiber content (FC) analysis was done by the method of Van Soest, 1963.

Soluble protein content (SP) was determined by Bradford's method (1976). Briefly, appropriate volume (from 0 - 100 µl) of sample was aliquoted into a tube and the total volume was adjusted to 100 µl with distilled water. A 1 ml of Bradford working solution was added to each sample. Then the mixture was thoroughly mixed by vortex mixer. After 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 anthocyanincontent (TAC) was analyzed by the procedure of Mancinelli et al. (1975). Leaf and calyx sample (0.1 g × 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 µm syringe filter before measurement of total anthocyanin content at 530 and 657 nm.The content was expressed as mg $g^{-1}$ 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 × g for 15 min at 4˚C. The supernatant was filtered through a 30-µm syringe filter, and 50 µL of the filtrate was analyzed using an HPLC system (PerkinElmer series 200 LC and UV/VIS detector 200 LC, USA) equipped with a 5-µm column (Spheri-5 RP-18; 220 × 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)**.**

***2.6Nutrient 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 wasdetermined by method reported in (Pauwels et al., 1992). Leaf and calyceof roselle were dry ashed at 450˚C for 2 hours and digested on heat cave with 10 ml $HNO_3$1 M. The solution was filtrated and adjusted at 100 ml with $HNO_3$ at 1/100 and analyzed with an atomic absorption spectrophotometer (Rayleigh, WFX-100).

***2.7 Statistical analysis***

The experiment was conducted as a factorial in a completely randomized design in four replications. Factor one was NaCl treatments (0, 60, 120 and 240 mMNaCl) and factor twowas rosellecultivars (Red star and Green bissap). Data are presented in term of mean (±standard deviation). All data were statistically analysed using Statistica (version 9, Tulsa, OK, USA) and subjected to analysies 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, calyce and seed yield*

The results of the analysis of variace indicate significant differences ($P<0.05$) of PH, TLA, DC, LC, LRWC, CRWC, LY, CY and SYbetwen 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).

Prodjinoto et al.(2018) reported thatreducing the growth of the vegetative apparatusaerial 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 $Na^+$ accumulation in the plant.

The highest leaf yield (481.85 kg $ha^{-1}$) and seed yield (456.29 kg $ha^{-1}$)was obtained in Green bissapvariety while calyce yield (452.85 kg $ha^{-1}$) was recorded in Red starcultivar (Table3 and Figure 1D). These yields decrease significantly base on growing salts. Indeed, the 60 mMNaClcontribution reduced leaf, calyce and seed yieldto9.9%, 10.1% and 10.1%in Green bissap and8.2%, 6.6% et 7.4% in Red starrespectively. This reduction is however to24.7%, 31.6% and 32.8% in Green bissap and to 27.5%, 23.1% and 29.8% inRed starcultivar respectively at 240 mM NaCl. These results were similar to that ofApel and Hirt (2004) who reported that seedling growth, flowering production and fruit were affected by high saltconcentration, leading to decreased yield.

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

Salt has a significant effect on the accumulation of TAC, TSC, FC, SPand reduction of ascorbic acid content ($p<0.05$). Theresults 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 calyce), (137.9% in leaf and 122.5% in calyce), (195.5% in leaf and 135.3% in calyce) and (167.9% in leaf and 166.7% in calyce) respectively in Red star cultivar. And in Green bissap, this increase varies by (25% in calyce), (130% in leaf and 126.9% in calyce),(146.7% in leaf and 114.9% in calyce) and (178.2% in leaf and 168.3% in calyce) 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 dehydration (Lepengueet al., 2012), role that is decisive in the osmotic adjustment and the level of stabilization of same 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, accumulaion of anthocyanins with increasing 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 actas 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 (Eryılmaz, 2006).

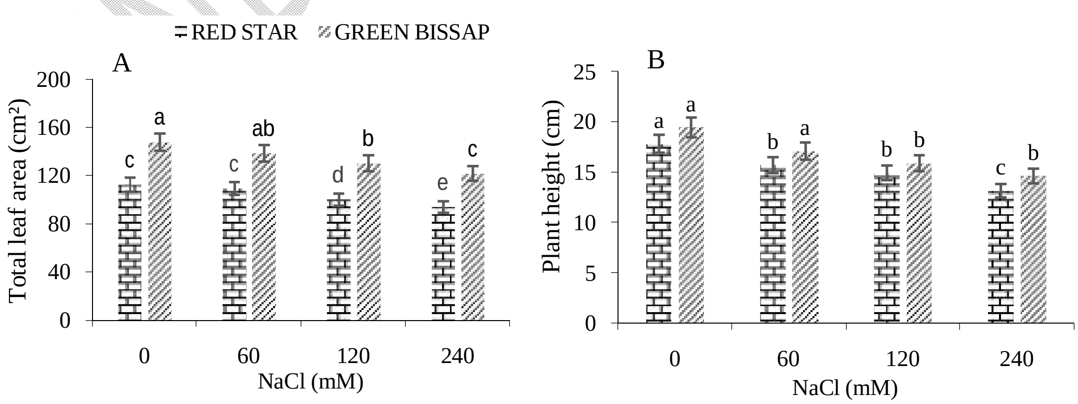
ASA decreased significatively from control to 240 mM NaCl in Red star by (191.87 to 90.91mg $kg^{-1}$in leaf and 503.25 to 398.53mg $kg^{-1}$in calyce). In Green bissap cultivar, this reduction varie by(185.63 to 80.29 mg $kg^{-1}$in leaf and 483.55 to 340.57mg $kg^{-1}$ in calyce) (Table 3).The results revealed that in leaf and calyce 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 leaf and calyce***

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 calyceand 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 calyceand 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 leaf and 109.4% in calyce) and for Green bissap(158.4% in leaf and 127.6% in calyce) 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 calyce 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 (Sivritepe *et al.,* 2003).During the experimentation, the lowest $Na^{+}$content in the aerial parts were observed in the Red star cultivar and the highest in the Green bissapvariety. Niu et al. (2010) and Aktas et al.(2006) reported thatsalt tolerant pepper genotypes accumulateless $Na^{+}$in the leavesthan sensitive genotypes. These studies indicate that genotypes with the ability to exclude $Na^{+}$ are generallymoretolerant to salinity.





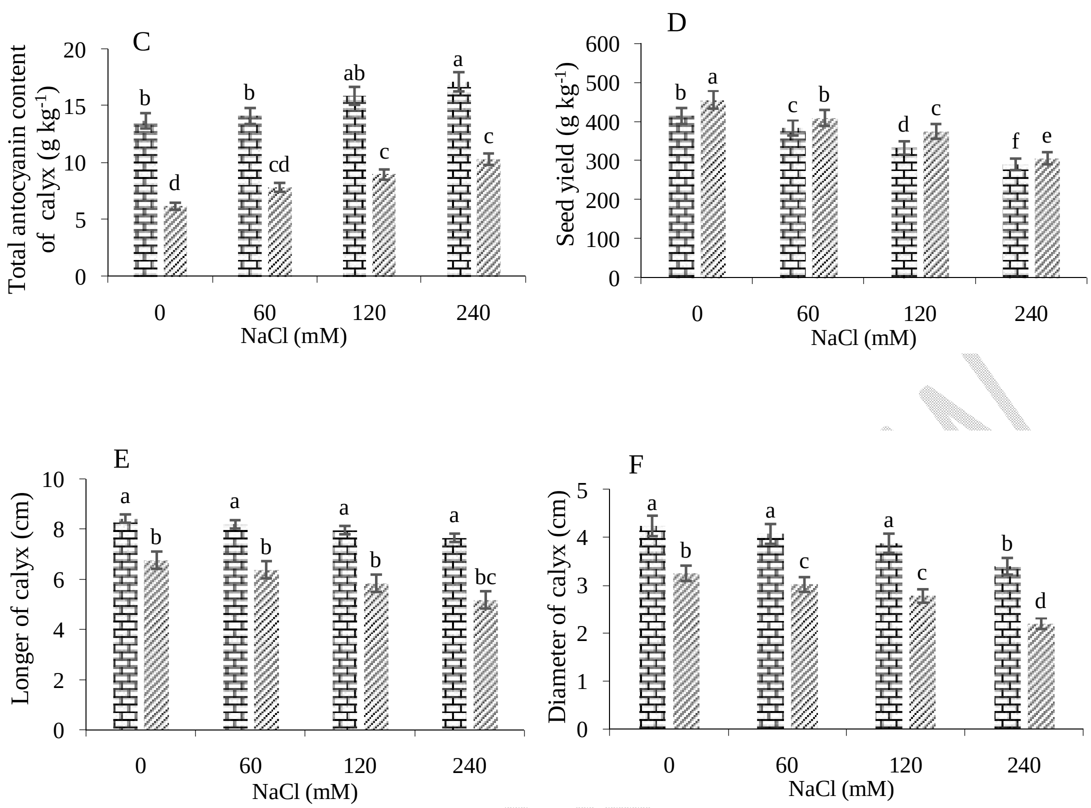



Fig. 1. Effects of salinityrates (60, 120 and 240 mM NaCl)on growth, seed yield and total anthocyanin content of calyce of two roselle cultivars (20 WAS).Total leaf area(A), Plant height (B),Total antocyanin content ofcalyce(C) Seed yield (D), Length of calyce(E) and Diameter of calyce(F). Bars are means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

Table 3. Effects of salt stress rates on leaf and calyxnutritional components, leaf and calyce yieldand relative water content of two roselle cultivars (20 WAS)

| Cultivars | Organs | Salt stress (mM NaCl) | SP (g kg$^{-1}$) | TSC (g kg$^{-1}$) | RWC (%) | ASA (mg kg$^{-1}$) | FC (g kg$^{-1}$) | Yield (kg ha$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Red bissap (Red star) | Calyce | 0 | 24.92±1.13l | 61.75±1.77h | 91.26±2.11e | 503.25±4.21a | 1.73±0.95n | 497.37±4.16b |
| | | 60 | 28.34±1.08l | 65.12±0.81h | 89.44±2.24f | 473.31±4.64b | 1.89±0.91 | 464.74±2.19b |
| | | 120 | 34.63±1.71k | 70.73±0.89g | 88.53±2.25f | 422.24±3.39b | 2.01±0.58n | 411.82±3.22b |
| | | 240 | 41.58±0.89j | 75.65±1.03g | 85.61±2.37f | 398.53±4.42c | 2.34±0.66o | 382.53±3.75c |
| | Leaf | 0 | 19.17±0.86m | 44.34±2.65n | 89.42±2.55f | 191.87±3.19d | 1.12±1.12n | 481.85±3.55b |
| | | 60 | 23.41±0.79l | 49.25±2.02j | 86.34±1.61f | 149.19±3.23d | 1.43±1.02n | 442.53±2.88b |
| | | 120 | 27.85±1.02l | 55.38±1.33i | 84.26±1.82f | 100.12±3.44d | 1.81±0.83n | 384.62±3.11c |
| | | 240 | 32.19±1.17k | 61.16±1.73h | 81.19±2.09f | 90.91±3.51e | 2.19±1.12n | 349.38±2.16c |
| | Calyce | 0 | 21.14±1.11l | 56.37±1.04i | 93.41±2.93e | 483.55±4.41b | 0.94±0.44o | 452.85±2.37b |
| | | 60 | 24.75±1.05l | 60.83±1.09h | 90.69±1.92e | 433.28±3.92b | 1.26±0.77n | 406.93±2.47b |
| | | 120 | 28.35±1.04j | 65.51±0.57h | 88.17±2.88f | 392.34±4.17c | 1.63±0.21n | 375.65±3.61c |
| | | 240 | 35.58±1.09k | 71.53±4.57g | 84.88±2.37f | 340.57±4.85c | 2.02±1.07n | 309.83±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 calyce mineral composition of two roselle cultivars (20 WAS).

| Cultivars | Organs | Salt stress (mM NaCl) | Na (mg kg$^{-1}$) | K (g kg$^{-1}$) | Ca (mg kg$^{-1}$) | Mg (mg kg$^{-1}$) | P (mg kg$^{-1}$) | Iron (mg kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Red bissap (Red star) | Calyce | 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 | Calyce | 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±3.161l | 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 reportedgenotypical differences of tolerance to salinity. Salt stress significantly decreased the growth and yield, enhanced the osmolytes, fiber content, total anthocyanin and Na content of the leaf and calyce of a roselle. Under the higher level of salinity conditions,the protein, soluble sugar, fiber content and total anthocyanin content of roselleleaves and calyce were very high in comparison tothe control conditions, which allows for the assignment of roselleas a valuable foodsource for human diets and health benefits. Indeed, the Green bissapvarietywas 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; calyce yield-CY; days after planting-DAP; days after sowing-DAS;diameterofcalyce-DC; dry weigth-DW; electric conductivity-EC; fiber content-FC; fresh weigth-FW; leaf yield-LY; length of calyce-LC; magnesium-Mg; nitrogen-N;organic carbon-C; phosphorus-P; plant height-PH;

potassium-K; relative water content-RWC; seed yield-SY; shoot dry weigth-SDW;shoot fresh weigth-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.

### Artificial intelligence

No generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.



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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 ($cm^2$) | PH (cm) | TAC of calyx ($g\ kg^{-1}$) | SY ($kg\ ha^{-1}$) | 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