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(RESEARCH ARTICLE)

Growth parameters, mineral distribution, chlorophyll content, biochemical constituents and non-enzymatic antioxidant compounds of white yam (*Dioscorea rotundata* (L) var. gana) grown under salinity stress.

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Abstract

Soil salinity has a negative impact on crop production over the world. The effect of salt stress on growth, chlorophyll content, mineral distribution, biochemical constituents and non-enzymatic antioxidant compounds of white yam (*Dioscorea rotundata* (L) var. gana) cultivar regularly consumed in Cameroon were investigated. Plants were subjected to four different levels concentrations of NaCl (0, 50, 100 and 200 mM), with 0 mM NaCl as a control. The supply of intake doses of NaCl in the culture medium significantly ($P < 0.001$) decreased the dry biomass (roots and shoots), growth parameters (number of leaves, noose diameter, leaf area and stem height) and chlorophyll contents from 100 mM NaCl. Mineral elements (K, Ca and Mg) and K/Na ratio significantly ($P < 0.001$) decreased in roots and shoots with increasing salinity. The higher sodium (Na) concentrations were recorded in shoots than in roots. The different biochemical constituents (proline (PRO), total soluble carbohydrates (CH), soluble proteins (PR) and total free amino acids (FAA)) and non-enzymatic antioxidants compounds (total phenolic (TP) and flavonoids (FLA) contents) significantly ($P < 0.001$) increased from 50 mM NaCl. The main strategy in cv. gana seems to increase osmotic adjustment through high accumulation of CH, PR, FAA and PRO in the leaves and they could eventually be considered as biochemical indicators of early selection and osmotic adjustment ability for salt tolerant plants. The gana variety could be encouraged to be planted on salt affected soils for the better development in salty areas.

Keywords: *Dioscorea rotundata*; growth parameters; salinity; biochemical constituents; mineral distribution

1. Introduction

Salinity is the main abiotic stress, which reduces plant yield in the world [1, 2, 3]. About 7% of the world's land surface, 5% of the cultivated land and 20% of irrigated land has suffered from salt problem [4]. The causes of salinization are the saline parent bed rocks, mineral degradation, invasion of sea water in coastal regions and the over use of saline water irrigation [3,5]. Soil salinity supply modifies ion transport and plant organ content [6]. NaCl is the main cause of soil salinization and sodium is toxic to cell metabolism. It adversely affects the functioning of some cellular enzymes, plant germination, growth and yield, in higher concentration. It also causes osmotic imbalance, membrane disorganization, reduction in growth, water up take, inhibition of cell division and expansion [2, 5, 7]. The effects of NaCl on growth, mineral uptake, photosynthesis, biochemical constituent and productivity were published by many authors [2, 3, 8, 9]. They showed that sodium (Na) inhibits potassium (K), calcium (Ca) and magnesium (Mg) in plant organs

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which results in K^+/Na^+ antagonisms. They also reported that photosynthetic activity is strongly affected by salinity stress which is directly related to the closure of stomata due to low intercellular CO_2 levels [10]. The results from these salinity treatments have shown increased the concentrations of carbohydrates, proteins, amino acids and proline. Such salt tolerance is determined by osmotic adjustment, the maintenance of ion homeostasis, the control of ion and water flux, the specific protein and free radical enzymes involved in the protection of protoplast functions [11]. Proline (PRO) is the main amino acid in the cytoplasm that contributes to the stability of the osmotic pressure of ions in the vacuoles. Under saline conditions, PRO is the main compatible solute which accumulates and performs well in the process of adapting cells to cold, salt and water stress [12]. Proteins (PR) are involved in osmotic adjustment; they are stored as nitrogen under salt-stress and re-used when the stress is removed [13]. Previous authors showed that salt-tolerant plants store more soluble carbohydrates (CH) under salt stress conditions [14]. The production and accumulation of free amino acids (FAA) by plant tissue during drought, salt and water stress is an adaptive response according to [12]. Total phenolic (TP) and flavonoids (FLA) compounds are a group of secondary metabolites which play a key role between plant, surrounding environment and they are implicated to stress resistance against biotic and abiotic factors [15]. Their accumulation during stress could be cellular adaptive mechanisms for scavenging oxygen free radicals [16].

White yam (*Dioscorea rotundata* (L) var. gana) is an important cash crop ranked as third tubers produced after cassava and cocoyam in Cameroon. They can be consumed according to the varied ways by cooking, fried or roasted [17]. Tubers improve human health by supplying the much needed proteins and micronutrients vitamin C and D as well as a lot of potassium. Investigating on crop plant species or with genetic potential for salinity tolerance are better approaches for developing salt tolerant cash crop cultivars [18]. Therefore the aim of this work is to study the effects of NaCl on growth, mineral uptake, chlorophyll contents, biochemical constituents and non-enzymatic antioxidant compounds of white yam (*Dioscorea rotundata* (L) var. gana). Comparison of these parameters can be helpful to provide additional information on the mechanism of salt tolerance and improve salt tolerant plants for research and breeding program.

2. Material and methods

2.1. Study area

The study was performed in a greenhouse of the Faculty of Science of the University of Douala in Cameroon, Campus II, from September 2019 to June 2020. Douala (3° 40' - 4° 01 N and 9° 16 - 9° 52' E, elevation 13 m) is the economic capital of Cameroon, located at the Wouri estuary and belongs to the Northern equatorial climate zone with a particular climate call Cameroonian equatorial climate type characterized with two seasons: (i) a dry season from December to March and (ii) rainy season that runs from March to December. The maximum rain fall is from July to September. Average rain fall and temperature are 400 mm/year and 26.7 °C and relative humidity is nearest to 100% [19]. Prevailing wind carries the Monsoon.

2.2. Plant material

White yam (*Dioscorea rotundata* (L) var. gana) grows well in the limit of the tropical forest and the savannah [17]. The roots can reach 2.5 m in length in the soil and is used as medicine and for food consumption. The samples were provided by the Institute of Agronomic Research and Development (IRAD) breeding program of Cameroon.

2.3. Plant growth and treatment

After breaking dormancy, the samples of white yam variety "gana" were sterilized with 3% of sodium hypochlorite during 15 minutes, washed five times with demineralized water and transplanted into 5 L polythene bags filled with 5 kg of sterilized sand. The plants were arranged in a complete randomized block design, with one plant each and five replications per treatment. They were enriched on daily basis with a modified nutrient solution. (in g/L) made of 150 g $Ca(NO3)_2$, 70 g KNO_3, 15 g Fe–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 [20]. The pH of the nutrient solution was adjusted to 7.0 by adding HNO_3 0.1 mM. White yams plants were subjected to different salt concentrations (0 (control), 50, 100 and 200 mM NaCl) in culture medium for a period of six weeks for the determination of physiological and biochemical responses of cultivars to salt stress. The average day and night temperatures in the greenhouse were between 26 and 20 °C respectively during the growth period with an average relative air humidity of 69.5%. Parameters were evaluated under greenhouse condition: growth parameters (dry biomass of roots and shoots, biochemical constituents (soluble proteins, carbohydrates, total free amino acids and proline content) as well as mineral (Na, K, Ca and Mg contents of roots and shoots).

2.4. Growth parameters

After the plants were harvested, the number of leaves, stem height, leaf area, noose diameter and dry biomass were recorded. Roots and shoots were separately dried at 60 °C during 72 Hours and their dry biomasses were determined. Leaf area was calculated using the Taillièz and Ballo formula [21]. Surface area (cm^2) = 1/3 (Length × Width). The stem height and noose diameter were determined by measuring with a ruler and vernier caliper respectively, and the number of leaves were determined by counting.

2.5. Mineral content

In order to determine the mineral distribution of Na, K, Ca and Mg in the shoots and roots, 1 g of dry biomass were separately crushed and thoroughly mixed with 20 mL of HCl 1/10 for 24 hours. The sodium, potassium, calcium and magnesium concentrations in plants partitioning were determined through atomic absorption spectrophotometer (Rayleigh WFX-100) [22] method.

2.6. Chlorophyll content

The chlorophyll content was determined using the Arnon [23] method. 0.80 g sample of fresh leaves were crushed and their contents extracted with 80% of alkaline acetone (v/v) and the filtrate was analyzed with spectrophotometer (Pharmaspec model UV-1700) at 645 and 663 nm wavelengths.

2.7. Biochemical constituents

2.7.1. Proline content

Proline content (PRO) was estimated using Bates et al. [24] method. 0.5 g of fresh leaves were weighed and put inside a flask. 10 mL of 3% aqueous sulphosalicylic acid was poured in the same flask. The mixture was homogenized, and then filtered with a Whatman N°2 filter paper. 2 mL of filtered solution was poured into a test tube, and then 2 mL of glacial acetic acid and ninhydrin acid were respectively added into the same tube. The test tube was heated in a warm bath for 1 h. The reaction was stopped quenched by placing the test tube in an ice bath. 4 mL of toluene was added to the test tube and stirred. The toluene layer was separated at room temperature, and the mixture purple color was read at 520 nm by spectrophotometer UV (Pharmaspec model UV-1700). At 520 nm, the absorbance was recorded and the concentration of PRO was determined using a standard curve as µg/g FW.

2.7.2. Total free amino acids content

Total free amino acids content (FAA) was determined by the ninhydrin method [25]. Fresh leaves (1 g) were grounded in 5 mL of ethanol 80%, amino acids were then extracted using reflux technique in boiling ethanol for 30 min. After decanting, the supernatant was filtered using Whatman N°3 filter paper. The filtrate was collected and the residue used to repeat the extraction. The two mixed filtrates constituted the raw extract of amino acids that were measured using ninhydrin method. The absorbance of purplish bruise complex was read at 570 nm wavelength. The standard curve was established using 0.1 mg/ mL of glycine.

2.7.3. Soluble proteins content

Soluble proteins content (PR) was evaluated using Bradford [26] method. The protein standard protein used was the bovine serum albumin (BSA). 0.1 g of fresh leaves were homogenized with 4 mL of an already prepared sodium-phosphate buffer, pH 7.2. The mixture was then centrifuged at 13 000 rpm for 4.5 min at 4°C. 1 mL of the supernatant was poured into a tube containing 5 mL of the Bradford reagent. The mixture was shook and incubated in the dark for 15 min. The absorbance of the resulting blue complex was read at 595 nm with a spectrophotometer UV (PG instruments T60). The standard curve was obtained using BSA 1 mg/mL.

2.7.4. Soluble carbohydrate content

Soluble carbohydrate **(**CH) content was obtained using phenol-sulphuric acid [27]. The fresh leaves (1 g) were grounded in 5 mL of 80% ethanol twice and filtered with the whatman No 2 filter paper. The collected extracts were diluted by deionized water to 50 mL. 1 mL of each sample was poured in test tube, then 1 mL of phenol solution and 5 mL of sulphuric acid were added. The mixture was then swirled. The wavelength was read at 490 nm by a spectrophotometer (Pharmaspec UV-1700 model). The quantity of CH was deduced from the glucose standard curve.

2.8. Non enzymatic antioxidants

2.8.1. Total phenolic content

Total phenolic content (TP) was determined using the folin-ciocalteu method [28]. 1 g of fresh leaves were grounded at 4 °C during 20 minutes in 3 mL of 0.1 NHCl, the homogenate was centrifuged at 6000 g during 40 minutes. The pellet re-suspended in 3 mL of 0.1 NHCl and centrifuges previously. The two supernatant are mixed and constitute the crude extract of soluble phenol. The reaction mixture containing 15 µL of extract 100 µL folin-ciocalteu reagents, 0.5 mL of 20% Na_2CO_3 was incubated at 40 °C for 20 minutes and absorbance read at 720 nm wavelength with a spectrophotometer (Pharmaspec UV-1700 model). The TP (mg/g FW) content was determined through a standard curve established by using chlorogenic acid.

2.8.2. Flavonoids content

Flavonoids content (FLA) of crude extract was determined by the aluminum chloride colorimetric method [29]. 50 µL of crude extract (1 mg/mL ethanol) were made up to 1 mL with methanol, mixed with 4 mL of distilled water and then 0.3 mL of 5% $NaNO_2$ solution; 0.3 mL of 10% $AlCl_3$ solution was added after 5 minutes of incubation, and the mixture was allowed to stand for 6 minutes. Then, 2 mL of 1 mol/L NaOH solution were added, and the final volume of the mixture was brought to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and absorbance was recorded on spectrophotometer (Pharmaspec UV-1700 model) at 510 nm wavelength. FLA content was calculated from a grutin calibration curve, and the result was expressed as grutin equivalent per g dry weight.

2.9. Statistical analysis

The experiment was performed in a complete randomized design. All data were presented in terms of mean (± standard deviation), statistically analysed using Graph pad Prism version 5.01 and subjected to analysis of variance (ANOVA). Statistical differences between treatment means were established using the Fisher Least Significant Difference (LSD) at $P < 0.05$.

3. Results and discussion

3.1. Plant growth

The growth parameters (stem height, number of leaves, noose diameter, leaf area and dry biomass) were generally influence by the intake doses of NaCl and decreased significantly in the culture medium during six weeks from 100 to 200 mM of NaCl (Table 1). Noose diameter and number of leaves significantly decrease ($P < 0.05$) from 100 mM of NaCl, leaf area significantly decreased ($P < 0.01$) while dry biomass and stem height significantly decreased ($P < 0.001$) from 100 mM of NaCl (Table 1). These results are in consonance with those of Amira and Abdul Qados [30] on *Vicia faba*; Assimakopoulou et al. [31] on *Phaseolus vulgaris* and Hand et al. [3] on *Capsicum annuum* L. They showed that, the number of leaves decreased with the intake doses of NaCl due to the accumulation of Na^+ in the cytoplasm of leaves. At the same time their fluid vacuoles cannot store salt and consequently decrease the salt concentrations in the cell and finally the leaves fell and died. According to Dadkhah [32] on *Beta vulgaris* L and Hand et al. [3] on *Capsicum annuum* L, the decrease of noose diameter with intake doses of NaCl is a consequence of physiological responses like the modification of ionic equilibrium, mineral nutrition disruption which inhibited the accumulation of mineral ions in the tissues. Mudgal et al. [33] and Hand et al. [3] adhere to the fact that the decrease in leaf area with the intake doses of NaCl in the culture medium is the consequence of several physiological responses including modification of ion balance, stomatal behavior, mineral nutrition and photosynthetic efficiency. According to Erum Mukhtar et al. [34] on *Brassica napus* L; Hand et al. [3] on *Capsicum annuum* L, the decrease of stem height under salinity stress can be due to the negative effects of salt on photosynthetic activities (Table 1). In additional, the disturbance of enzymatic activity affects the synthesis of proteins consequently induces the reduction of carbohydrates and growth hormones, the reduction of the plant turgidity and the competition of ions ensure the osmotic adjustment [35]. The results obtained showed a significant ($p < 0.001$) decrease in dry biomass of white yam in the culture medium from 100 mM NaCl in shoots and roots (Table 1). These results are similar with those obtained by Taffouo et al. [8]; Meguekam et al. [9]; Nouck et al. [2] and Hand et al. [3] respectively on *Vigna unguiculata* L. Walp, *Arachis hypogaea* L., *Lycopersicum esculentum* L. and *Capsicum annuum* L. They showed that the reduction of photosynthetic capacity can reduce the shoots and roots dry biomass. According to Hamrouni et al. [36] plant can tolerate the salinity stress by first reducing their root system in the aim to maintain the leaves and ensure their photosynthetic activity.

Table 1 Effects of NaCl on plant growth in white yam after six weeks of treatment.

	Treatment	Dry Biomass (g)					
Cultivar	**mM NaCl**	**RDB**	**SDB**	**ND (mm)**	**SH (cm)**	**NL**	**LA (cm^2)**
	0	19.50±1.45	79.81±5.63	2.95±0.14	53.28±3.18	9.22±0.49	25.53±2.89
	50	17.86±1.95^{a}ns	78.88±4.19^{a}ns	2.83±0.1^{a}ns	39.15±2.55^{a}***	9.02±0.33^{a}ns	23.58±2.04^{a}ns
gana	100	12.86±1.50^{b}***	62.39±5.46^{b}***	2.73±0.09^{b}*	33.69±1.99^{b}***	8.69±0.41^{b}*	19.36±2.51^{b}**
	200	10.44±0.89^{b}***	48.31±3.19^{c}***	2.61±0.09^{c}*	28.75±1.76^{c}***	5.52±0.59^{c}***	14.79±2.74^{c}***

Mean results of five replications ± SD. Based on the ANOVA method followed by all pairwise analysis using the student-Newman-keuls. nsP > 0.05; *P < 0.05; **P < 0.01 and ***P < 0.001 as compared to 0 mM NaCl. Letter showed the difference between the different concentrations (P < 0.05).

Table 2 Effects of NaCl on mineral distribution (µg/g MS) in white yam after six weeks of treatment.

	Treatment	Mineral distribution (µg/g MS)				
Cultivar	mM NaCl	Na^{+}	K^{+}	Ca^{2+}	Mg^{2+}	K^{+}/Na^{+}
	0	71.60±4.68	37.74±2.40	33.47±3.25	26.37±3.18	0.524
	50	89.±5.18^{a}*	31.25±2.56^{a}**	29.68±3.39^{a}ns	22.49±2.81^{a}ns	0.344
Roots	100	110.81±9.25^{b}***	26.46±2.10^{b}***	26.26±2.75^{ab}*	19.44±1.73^{a}**	0.234
	200	136.33±13.03^{c}***	19.30±2.44^{c}***	20.78±3.77^{b}***	18.22±2.20^{a}**	0.138
	0	182.51±12.79	102.39±9.40	88.62±7.82	73.42±4.61	0.556
	50	211.82± 18.71^{a}*	91.22±7.72^{a}ns	81.31±3.54^{a}ns	65.68±4.98^{a}ns	0.428
Shoots	100	247.84±16.51^{b}***	76.48±6.72^{b}***	75.37±4.18^{a}**	55.66±5.95^{b}***	0.302
	200	310.31±15.66^{b}***	70.68±4.45^{b}***	64.75±4.14^{b}***	44.30±3.69^{c}***	0.222

Mean results of five replications ± SD. Based on the ANOVA method followed by all pairwise analysis using the student-Newman-keuls. nsP > 0.05; *P < 0.05; **P < 0.01 and ***P < 0.001 as compared to 0 mM NaCl. Letter showed the difference between the different concentrations (P < 0.05).

3.2. Nutrient uptake

Salt treatments significantly ($p < 0.001$) increased Na^+ with the increase doses of NaCl (Table 2). These results are the same with those observed by Taffouo et al. [8]; Mehede et al. [37] and Hand et al. [3]. They showed that the predominance of salts enhances Na^+ uptake and this inhibits the uptake of other mineral elements like K^+. Additional white yam "gana" transfer Na^+ from the root to the aerial parts. These results corroborate those obtained by Wamba et al. [38] and Rais et al. [5] who showed that the competition between Na^+ and K^+ for aerial parts of plants can be caused by the loss of osmotic potential of root medium, specific ion toxicity and the lack of nutritional ions. Increase in the mineral content, increases with the increasing concentrations in NaCl with a greater accumulation in the shoots related to the roots. From the results obtained, Ca^{2+} significantly decreased in the culture medium with the intake doses of NaCl. According to Amuthavalli et al. [39] and Rahman et al. [40], calcium plays an important role in the osmotic adjustment process. The calcium also plays an important role by reducing the negative effect of NaCl and increasing the selectivity K^+/Na^+ [41, 42]. The decrease of magnesium with the intake doses of NaCl is according to Assimakopoulou et al. [31], higher accumulation of Na^+ in the soil could be the cause of toxicity, ionic disorder and the competition between ions which inhibited the translocation of other minerals (K^+, Ca^{2+} and Mg^{2+} which play a major role in the membrane permeability) to the aerial part of the plant.

3.3. Chlorophyll content

The different chlorophyll, a, b and (a+b) are highest at 0 mM NaCl and slopes down significantly ($p < 0.001$) with increase intake doses of NaCl (Fig. 1). This decrease in chlorophyll content under salt stress is a commonly reported phenomenon in diverse studies, because of its adverse effects on membrane stability. This was observed by [3, 8, 43]. These authors reported that chlorophyll content a, b and total decreases with increasing salt concentrations. The decrease in chlorophyll content was observed because of salt-induced weakening of protein pigment-complex and increase chlorophyllase (EC: 3:.1.1.14) [35].

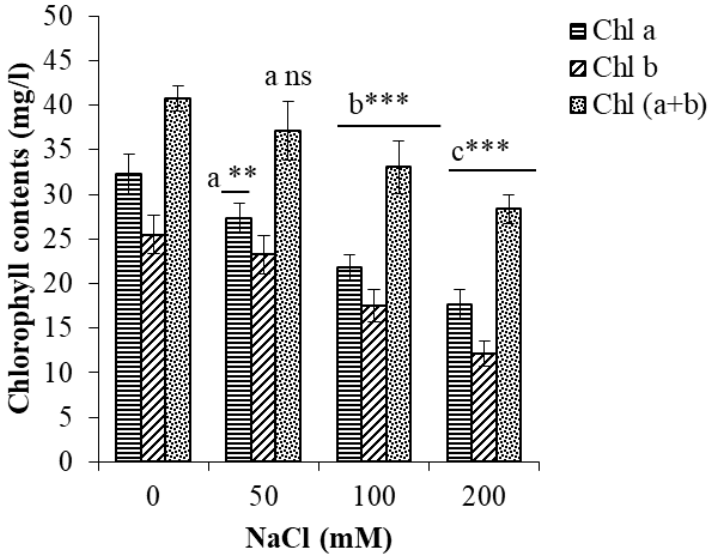

Figure 1 Effects of NaCl concentrations on the chlorophyll content a, b, (a+b) of white yam. Mean results of five replications ± SD. Based on the ANOVA method followed by all pairwise analysis using the student-Newman-keuls. nsP > 0.05; **P < 0.01 and ***P < 0.001 as compared to 0 mM NaCl. Letter showed the difference between the different concentrations (P < 0.05).

3.4. Non-enzymatic antioxidant

Results obtained on the effect of salinity on the accumulation of flavonoids agree with those of Salama et al. [44] who studied genotypic variations in phenolic, flavonoids and their antioxidising activities in maize plant grown in salinized media (Fig. 2). This study showed that an increase in salt concentrations led to a significant increase in total phenol and flavonoids contents. According to Hichem et al. [45] High accumulation of phenolics in plants is physiologically important in overcoming the salinity-induced oxidative stress. These results agree with those of Radi et al. [46] who reported that salinity stress affects the phenolic compounds content by the induced disturbance of the metabolic processes leading to an increase in phenolic compounds.

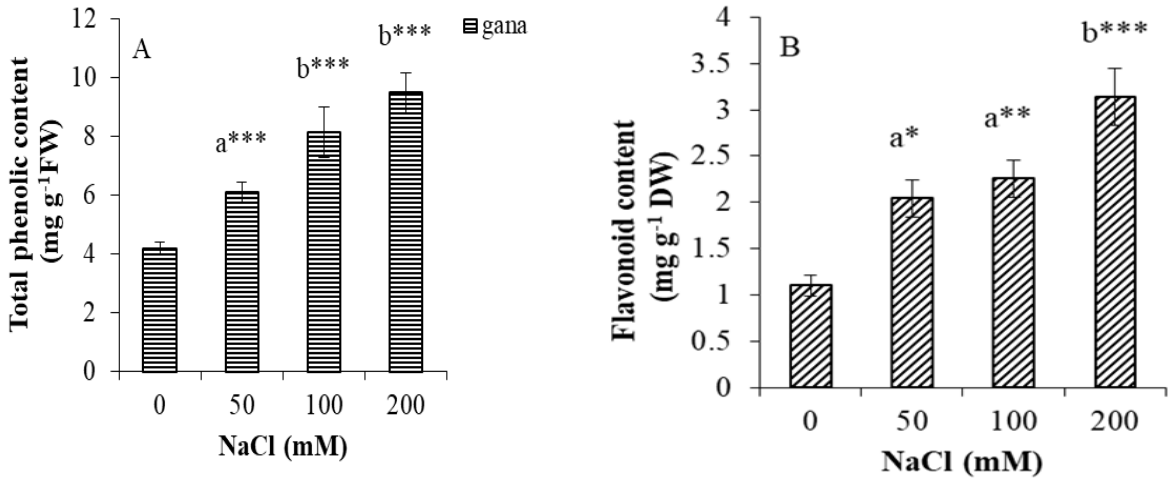

Figure 2 Effects of NaCl on accumulation of non-enzymatic antioxidants in white yam. A: Total phenolic content; B: Flavonoid content**.**

3.5. Biochemical constituents

The total soluble proteins significantly increased with intake doses of NaCl (Fig. 3). The same results were obtained by Kapoor and Srivastava [47] ; Amira and Abdul [30]; Nouck et al. [2] and Hand et al. [3] they showed that the accumulation of soluble proteins in plant tissues under conditions of environmental stress was due to regulatory osmotic adjustment in current stress.

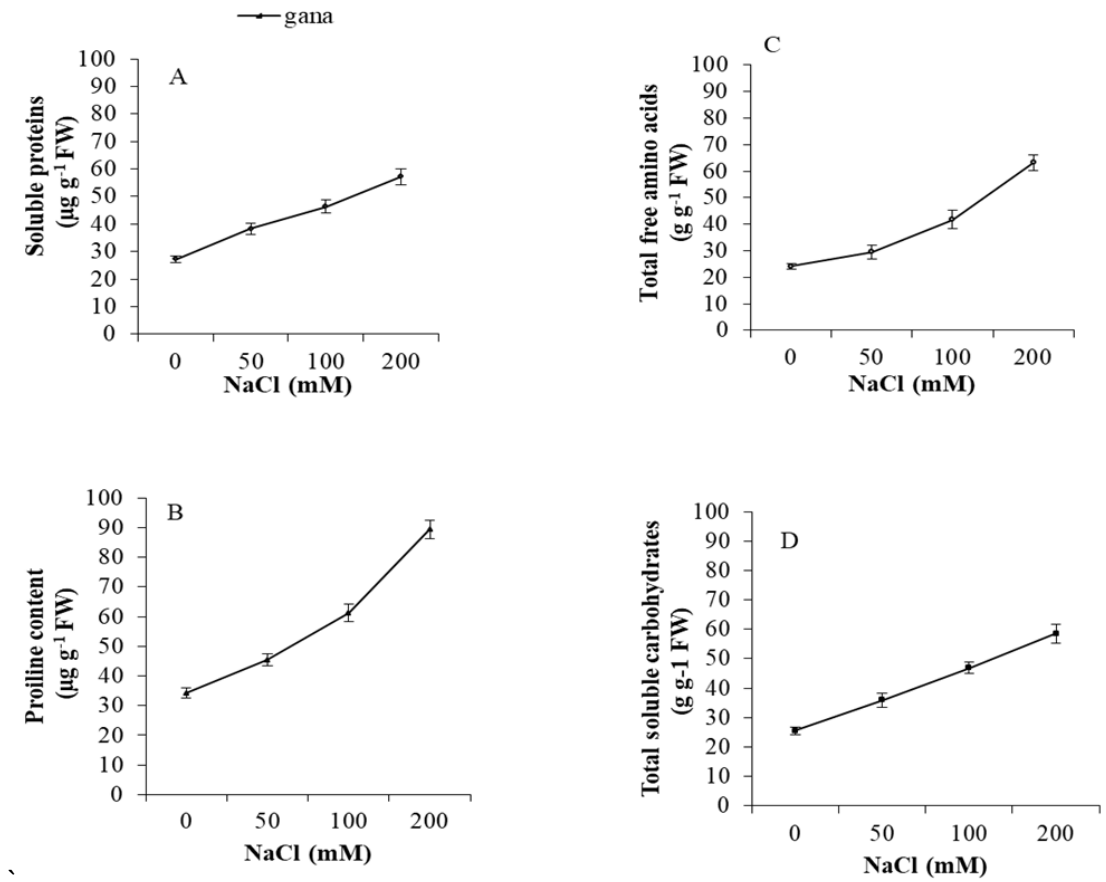

Figure 3 Effects of NaCl on accumulation of biochemical constituents in white yam. A: Total soluble proteins; B: Proline content; C: Total free amino acids and D: Soluble carbohydrates. Bars are means (n = 5) ± SD.

The osmotic balance of the cytoplasm relies on an active synthesis of organic compounds as total soluble proteins which enhance the plant salt tolerance. The total free amino acids in leaves increase with increase NaCl concentrations (Fig. 3). Similarly, Cusido et al. [48] and Nouck et al. [2] reported that salinity increased the levels of total free amino-acids and may be a good indicator for screening salt-tolerant genotypes. In addition this is because the increase of free amino-acids is due to the reduction of osmotic potential to maintain the turgid potential. From the results obtained the total soluble carbohydrates increase significantly with intake doses of NaCl (Fig. 3). According to Dhanapackiam and Llyas [49] and Movafegh et al. [43], the accumulation of total soluble carbohydrates in plant tissues under conditions of environmental stress was due to regulatory osmotic adjustment in current stress. The osmotic balance of the cytoplasm relies on an active synthesis of organic compounds such as soluble carbohydrates which enhances the plant salt tolerance. The results showed that the proline increased significantly with intake doses of NaCl (Fig. 3). According to Meguekam et al. [9]; Shah et al. [50] and Nouck et al. 2, the build-up of Proline (PRO) is a method of stress tolerance because its accumulation contributes to the acquisition of tolerance by maintaining turgor in cells of many species responsible for osmotic adjustment in tolerant plants grown under saline conditions

4. Conclusion

The results of this study showed that white yam (*Dioscorea rotundata* (L) var. gana) was affected by the supply of intake doses of NaCl for a period of six weeks in the culture medium. The dry biomass (roots and shoots), the growth parameters (number of leaves, stem height, leaf area and noose diameter) and chlorophyll contents decreased from 100 mM of NaCl. The biochemical constituents (Proline, total soluble carbohydrates, soluble proteins and total free amino acids) and non-enzymatic antioxidant compounds (Total phenolic and flavonoids compounds) increased from 50 mM of NaCl. The main strategy of salt-tolerance in gana seems to increase osmotic adjustment through the strongly accumulation of PR, CH, PRO and FAA in leaves. Thus, the higher accumulation of these compatible solutes, TP and FLA in leaves could be considered as potential biochemical indicators of early identification and osmotic adjustment ability for salt-tolerant plants in salt stress conditions. The mineral ions (Na, K, Ca, Mg and K/Na) in the plant partitioning (roots and shoots) were significantly decreased with increasing NaCl, and the highest concentrations of Na were observed in the shoot than roots.

Compliance with ethical standards

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Disclosure of conflict of interest

There is no conflict of interest.

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