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Modifications of growth, mineral uptake, chlorophyll content, osmolyte contents, antioxidant compounds and yield of three varieties of yam (*Dioscorea rotundata* L.) in saline conditions

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A vast area of the world is negatively impacted by salinity which causes loss of crop yields. Modifications in the growth, mineral uptake, chlorophyll content, osmolyte contents, antioxidant compounds and yield of three varieties of yam (*Dioscorea rotundata* L.) were evaluated in saline conditions, in a greenhouse (0, 50, 100 and 200 mM) and farm (0 and 50 mM). The leaf area, stem height, noose diameter, chlorophyll (a+b), dry weight of roots, shoots and ratio decreased (p < 0.05) with increasing salinity from 100 mM NaCl. K, Ca, Fe, Mn, Zn, Cu and K/Na decreased (p < 0.05) in plant organs in all varieties. Osmolytes (total soluble carbohydrates, total free amino acids, soluble proteins and proline) and secondary metabolites (total phenol, flavonoids, superoxide dismutase and peroxidase) increased (P < 0.001) with salinity in Gana and Kwete in contrast to Bagaa variety. The accumulation of osmolytes is suggested to be biochemical pointers to the initial identification of plant tolerance to salinity and the associated osmotic modifications. The tuber yield exhibited tolerance at 50 mM NaCl of Gana and Kwete variety implying an increase of white yam production saline areas.

Key words: Osmolytes, *Dioscorea rotundata,* salinity, mineral uptake, growth parameters, agronomic parameters.

INTRODUCTION

Saline soils are found in coastal and drought lands globally and are harmful to non-tolerant plants (Taibi et al., 2016; Singh et al., 2018; Hand et al., 2022). It naturally occurs with recurrent overflooding from ocean

and rain; salts formed from weathered soil minerals that were not fully leached and high evaporation rates resulting in the accumulation of salts (Singh, 2015; Hand et al., 2021). The global rise in salinity levels has not only

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Author(s) agree that this article remain permanently open access under the terms of the <u>Creative Commons Attribution</u> <u>License 4.0 International License</u> affected irrigated lands (20%) but also arable lands (40%) and the prevailing trends suggested that approximately 50% of farmlands will be subject to saline pressure by 2050 (Hussain et al., 2019; Kusvuran et al., 2021; Hand et al., 2021). Salinity stress has a dangerous effect on plant growth, metabolites, physiological parameters, and morphological responses, which reflects on the plants as a reduction in growth, productivity, weight and quality of the crops. This is caused by osmotic effects, harmful ions, and nutritional insufficiency which negatively impact the photosynthetic efficacy and ions homeostasis of plants (Abbas et al., 2018; Kusvuran et al., 2021).

Investigations on the mineral uptake, growth and agronomic parameters of glycophytes under salinity stress have been widely studied in agriculture (Orabi and Abdelhamid, 2016; Rahneshana et al., 2018; Kusvuran et al., 2021; Hand et al., 2022). Previous authors established that elevated concentrations of sodium (Na) hamper the uptake of some macroelements such as calcium (Ca) and potassium (K) in the plants in correlation with the K/Na antagonism. Also, trace elements like Cu (copper), zinc (Zn), iron (Fe) and manganese (Mn) showed deficiency symptoms under salinity stress, due to low solubility which alters their solubility and concentration in the soil (Borlu et al., 2018; Hand et al., 2021). Previous works explain that, excess salt affects photosynthesis and their activities are directly related to stomatal or non-stomatal limitations (Jaleel et al., 2008; Abdallah et al., 2020).

Previous works point to some osmolytes as mediators in the adjustment of osmotic imbalance, modulating the sequels of excess NaCl in the vacuole. Salt tolerance mechanisms developed by plants under salinity include ion homeostasis system via salt glands/salt bladders, primary metabolites like proline (PRO), total soluble sugars (CH), total free amino-acids (FAA), soluble proteins (PR), hormonal status, and secondary metabolites (Shabala et al., 2014; Theerawitaya et al., 2015).

Salinity induces excess accumulation of ROS enzymes like glutathione reductase (GR), superoxide dismutase (SOD), catalase (CAT) and peroxidase (POD) whose accumulation instigates harm to DNA, renders enzyme inactive, disables communication with other necessaries plant cell elements and protein oxidation (Rady et al., 2019; Kusvuran et al., 2021). Secondary metabolites reduce oxidative stress by scavenging free radicals in plant cells (Salama et al., 2015; Orabi and Abdelhamid, 2016). Previous works have reported that, salinity stress increases SOD and POD activities in plants (Chookhampaeng, 2011; Meguekam et al., 2014). Total phenol (TP) and flavonoids (FLA) are two non-enzymatic antioxidants which recreate a function in any interaction possessed with their environment. plants Their accumulation in plants under stress is pointed as cellular adaptive mechanism for scavenging oxygen free radicals (Mohamed and Aly, 2008; Meguekam et al., 2014; Hand et al., 2017).

White yam tubers contain essential proteins, micronutrients, K, vitamin C and D, improve human health and is ranked third tuber crop produced in Cameroon. Its preparation methods vary from boiling to frying or roasting. Studying the genetic potential of cash crops to generate tolerance is a better approach for improving salt tolerant and commercial crops (Munns and Tester, 2008). This study aimed to investigate modifications of growth, mineral uptake, chlorophyll content, osmolyte contents, antioxidant compounds and yield of three varieties of yam in saline conditions. Comparison of the study parameters will help to provide additional details on the tolerance process of NaCl and contribute to the development of salt tolerant crops for a breeding program.

MATERIALS AND METHODS

Experimental site and white yam samples

The study was conducted in two phases: in a greenhouse and in the field, at the Faculty of Science, University of Douala in Cameroon (3° 40' - 4° 01 N and 9° 16 - 9° 52' E, elevation 13 m). Douala is located in the coastal region at the Wouri estuary. The main climate type is the Cameroonian equatorial climate characterized by two seasons: (i) a dry season (3 months) and (ii) a rainy season (9 months). The prevailing wind is the Monsoon. There is abundant rain fall (3597 mm per year), with a relative temperature of 26.7°C and humidity closer to 100% (Hand et al., 2022). The work ran from October 2020 to December 2022. Samples of the three varieties of white yam (Gana, Kwete and Bagaa) used for the experiment were obtained from the Agronomic Institute Research and Development (IRAD) Nkolbisson, Yaounde-Cameroon.

Growth conditions and treatments

Samples of three white yam varieties (Gana, Kwete and Bagaa) were sterilized and planted into 5 L polythene bags filled with 5 kg of sterilized sand, arranged in a complete randomized block design (1 plant each and 5 replications/treatment). The experiment was supplied every day with Hoagland and Arnon (1950) modified nutrient solution (in g L⁻¹): of 150 g Ca(NO₃)₂, 70 g KNO₃, 15 g Fe-EDTA, 0.14 g KH₂PO₄, 1.60 g K₂SO₄, 11 g MgSO₄, 2.5 g CaSO₄, 1.18 g MnSO₄, 0.16 g ZnSO₄, 3.10 g H₃BO₄, 0.17 g CuSO₄, and 0.08 g MOO₃. The pH was adjusted to 7.0 by adding HNO₃ 0.1 mM. Some growth measurements (noose diameter, stem height, and leaf surface area), dry weight of roots, shoots, total and roots/shoots, chlorophyll (a+b), mineral uptake (K/Na, Na, K, Ca, Fe, Mn, Zn, and Cu contents) of plant organs and metabolites (Osmolyte contents (Soluble proteins, total free amino acids, proline and total soluble carbohydrates) and antioxidant compounds (total phenol, flavonoids, superoxide dismutase and peroxidase content) were evaluated. The temperature in the greenhouse ranged from 27 and 19°C, average relative air humidity of 75% and different salt concentrations (0 (control), 50, 100 and 200 mM NaCl).

Evaluation of growth measurements

After six weeks in the culture media, some growth measurements like dry weights partitioning (roots and shoots), noose diameter (measured with vernier caliper), leaf surface area calculated, LA $(cm^2) = 1/3$ (length x width) and stem height (measured with a ruler) were evaluated. Plant organs dry weights were obtained after being dried in an oven at 65°C during 72 h (Nouck et al., 2022).

Mineral uptake

Some mineral like Na, K, Ca, Fe, Mn, Zn, and Cu were determined by Pauwels et al. (1992). Dry weights (1 g) of roots and shoots were respectively added to 20 mL of HCl for 24 h. The filtrate was analysed with an atomic absorption spectrophotometer (Rayleigh WFX-100).

Chlorophyll (a+b)

Chlorophyll (a+b) was measured according to the method of Arnon (1949). 0.5 g of leaves of each variety of white yam (Gana, Kwete and Bagaa) were crushed, and extracted with 80% of alkaline acetone (v/v). The absorbance of extracts was measured at 645 and 663 nm with a spectrophotometer (Pharmaspec model UV-1700).

Osmolyte contents

Soluble protein content

PR content was evaluated using the Bradford (1976) method. A given volume.ranging from 0 to 100 μ l of sample was transferred in to a graduated test tube and the volume made up to 100 μ l with distilled water. 1 mL of Bradford solution was added. The mixture was homogenized using a vortex mixer. After 2 min, the absorbance was read at 595 nm using a UV spectrophotometer (PG instruments T60) against a concentration blank.

Proline content

Proline content was evaluated according to the method of Bates et al. (1973). 0.5 g of fresh leaves was crushed and 10 mL of 3% aqueous sulphosalicylic acid was added. The mixture was filtered with a Whatman paper No. 1. 2 mL and the filtrate were transferred into a test tube, and 2 mL of glacial acetic acid and ninhydrin reagent were added into the tube. The test tube was heated in a water bath at a temperature of 90°C for 20 min. The tube was removed and place in an ice water bath to stop the reaction. 4 mL of toluene reagent was added and purple-colour was obtained and the absorbance read at 520 nm using a UV spectrophotometer (Pharmaspec model UV-1700) against a concentration blank (μ g/g FW).

Soluble carbohydrate content

CH content was measured using the phenol-sulphuric acid method (Dubois et al., 1956). 1 g of fresh leaves was crushed in 5 mL 80% ethanol and filtered with the Whatman paper No. 1. The filtrate was made up to 50 mL with deionized water. 1 mL of the obtained solution was transferred into a test tube, followed by the addition of 1 mL of phenol solution and 5 mL of sulphuric acid and kept at 40°C in a water bath for 15 min. The absorbance of the obtained mixture was read at 490 nm using a UV spectrophotometer (Pharmaspec UV-1700 model) (μ g/g FW) of glucose.

Total free amino acids content

FAA content was assessed using the ninhydrin method (Yemm and

Cocking, 1955). 1 g of fresh leaves was crushed in 5 mL of 80% ethanol. Amino acids were then extracted using reflux technique by boiling in ethanol for 30 min. After centrifuging, the supernatant was collected and the pellets were used to repeat the extraction. The two filtrates were combined, and the raw extract of amino acid content was measured using ninhydrin method. The absorbance of purple-blue complex was read at 570 nm. The standard curve was established using 0.1 mg/mL of glycine.

Antioxidant compounds

Flavonoids content

Flavonoids content was quantified using the aluminium chloride colorimetric method (Chang et al., 2002). 50 μ L of crude extract (1 mg/mL ethanol) was completed to 1 mL with 95% methanol, 4 mL of distilled water and 0.3 mL of 5% NaNO₂ solution and 0.3 mL of 10% AlCl₃ solution was added successively. The mixture was incubated at room temperature for 5 min. 2 mL of 1 M NaOH solution was added, and the final volume made up to 10 mL with double-distilled water. The mixture was allowed to stand for 15 min, and the absorbance recorded at 510 nm using a spectrophotometer (Pharmaspec UV-1700 model). FLA content was calculated from a quecertin calibration curve expressed in mg ^{g-1} FW.

Total phenolic content

Total phenol content was quantified using the Folin Ciocalteu method (Marigo, 1973). 1 g of fresh leaves was crushed in 3 mL of 0.1 N HCl. After incubation at 4°C for 20 min, the homogenate was centrifuged at 6000 rpm. The supernatant was collected and the pellet were re-suspended in 3 mL of 0.1 N HCl and centrifuged as earlier stated. The two supernatants were combined. To 15 μ L of the extract, 100 μ L Folin-Ciocalteu reagent and 0.5 mL of 20% Na₂CO₃ was added. The mixture was incubated at 40°C for 20 min and absorbance read at 720 nm using a spectrophotometer (Pharmaspec UV-1700 model). A standard curve was established using Gallic acid and the results expressed as mg g⁻¹ fresh weight.

Peroxidase

This method was described by Jebara et al. (2005). Peroxidase (POD) activity was determined and expressed as µmol guaiacol min⁻¹g⁻¹ oxidized protein. The assay mixture of 3 ml was made up of 1.5 ml of 0.1 M phosphate buffer (pH 7.0), 1 ml freshly prepared 10 mM guaiacol, 0.1 ml enzyme extract and 0.1 ml of 12.3 mM H₂O₂. Initial absorbance was read at 436 nm and subsequent changes in absorbance were recorded at interval of 30 s on spectrophotometer (Pharmaspec UV-1700 model). The extinction coefficient 26.6 mM⁻¹ cm⁻¹ for the oxidized tetraguaiacol polymer was used to calculate the activity.

Superoxide dismutase

The SOD was measured according to the method described by Dhindsa et al. (1981). 3 mL of reaction mixture with 0.1 mL of 1.5 M Na_2CO_3 , 0.2 mL of 200 mM methionine, 0.1 mL of 3 mM EDTA, 0.1 mL of 2.25 mM p-nitroblue tetrazolium chloride (NBT), 1.5 mL of 100 mM potassium phosphate buffer (pH 7.5), 1 mL of distilled water and 0.05 mL of enzyme samples was used. Control experiment was the tube containing no enzyme. At the start of the reaction 0.1 ml 60 μ M riboflavin was added in each tube below a light source of two 15 W fluorescent lamps for 15 min. The reaction was quenched by switching off the light source and covering the

Physical and chemical property	Quantity	Physical and chemical property	Quantity
pH-water	6.45±0.10	Phosphorus (ppm)	4.60±0.10
Clay (%)	14.20±1.20	Potassium (g kg ⁻¹)	0.25±0.02
Coarse sand (%)	27.91±2.20	Sodium (g kg ⁻¹)	0.07±0.01
Fine sand (%)	25.60±1.80	Calcium (g kg ⁻¹)	0.23±0.01
Coarse silt (%)	26.00±1.60	Manesium (g kg ⁻¹)	0.17±0.01
Fine silt (%)	6.30±0.50	Zinc (mg kg ⁻¹)	0.29±0.02
Nitrogen (%)	0.32±0.02	Cu (mg kg ⁻¹)	1.42±0.01
Organic C (%)	0.75±0.04	Fe (mg kg ⁻¹)	3.26±0.10
Ratio C/N	2.34±0.02		

Table 1. Soil analysis of the Faculty of Science experimental site in Douala, Cameroon.

Source: Experimental data analysis.

tubes with black cloth. Absorbance was read at 560 nm. An illuminated blank without protein gave the maximum reduction of NBT and was considered to be the maximum absorbance at 560 nm. SOD activity was presented as absorbance of blank minus absorbance of sample, giving the total inhibition, calculated per microgram of protein and expressed as U mg⁻¹.

Agronomic parameters

Tuber yields and harvest index

The soil samples were collected from 0 to 20 cm depth in the study site (Table 1). Plots were set out in a randomized complete block in a split-plot arrangement with the dimension of 5 m in length and 2 m in width, space between plots 1.5 m and between plants 1 m. Two treatments in the farm 0 (control), 50 mM NaCl and three replications per treatment and per varieties. The following agronomic parameters were evaluated: (i) yield (t/h) = Total production/surface; (ii) harvest index (HI) = Wt/(Wt+ Biomass (shoot and tuber)) × 100 (Bijalwan and Manmohan, 2014), fifteen plants per replication for each variant of the experiment. Wt: Weight of tubers.

Statistical analysis

Data collected from the experiment was conducted as completely randomized design, inputted into Graph pad Prism version 5.01, expressed in terms of mean \pm standard deviation (SD) and submitted to analysis of variance (ANOVA). Statistical differences between treatment means were established using the Fisher Least Significant Difference (LSD) at (P < 0.05) concerning the difference between control and the different concentrations for the same variety, Duncan's Multiple Range Test (DMRT) was used for means separation of the different concentrations for the different varieties.

RESULTS AND DISCUSSION

Influence of salinity stress on plant growth of three varieties of white yam

The detrimental effects of NaCl in the culture medium observed in all growth parameters varied in relation with concentrations and varieties (Table 2). The reduction was significantly (P<0.01) accentuated in variety Bagaa

followed by Kwete compared to Gana from 100 mM NaCl. The relationships between variety \times salinity stress was significantly (P<0.05) reduced for cultivar height, leaf surface area and total biomass. These findings are substantiated by those of Gouveitcha et al. (2021) and Ors et al. (2021). They attributed the decrease in plant growth under salinity stress as a direct inhibition of cell division and expansion. In addition, they explained that the reduction observed was due to dehydration caused by the low water potential of cell, ions imbalance and toxicity caused by high presence of Na⁺ and Cl⁻ in the protoplasm.

A significant (p < 0.05) decrease of leaf area and stem height was observed with increasing soil salinity starting at 100 mM NaCl in all varieties (Table 2). The results of the work done by Kamran et al. (2020) proved that, leaf surface area development, photosynthesis activities, plant growth regulators, water absorption and plant height decrease in the presence of salt. The reduction of noose diameter with increasing intake doses of NaCl in the culture medium was explained by Gouveitcha et al. (2021) on two varieties of okra (Abelmoschus esculentus L. Moench); Santhi et al. (2015) on Solanum nigrum L and Nouck et al. (2022) on two varieties of Vigna unguiculata (L. Walp). According to these authors, the decrease in noose diameter was caused by modifications of nutrient elements and photosynthetic activity affecting the development of basts and cells. In our work, the prominent reduction in growth parameters of variety Bagaa followed by Kwete and Gana is proof that toxicity from the presence of salt affected the growth and development of variety Bagaa more, followed by Kwete and then Gana.

Dry weight biomass

The dry biomass of plant organs (roots and shoots) was decreased with increased levels of salt starting at 100 mM NaCl during the experiment in all varieties (Table 3). The relationships between variety \times salinity was significantly (P<0.05) reduced for roots + shoots dry

Veriety	Concentration	Growth parameters				
variety	(mM NaCl)	LA (cm²)	SH (cm)	ND (mm)		
	0	26.53±1.85 ^a	53.28±2.18 ^a	2.95±0.11 ^a		
Gana	50	23.58±2.04 ^b	39.15±1.55 ^b	2.83±0.10 ^a		
Gana	100	19.36±1.11°	33.89±1.01°	2.71±0.09 ^c		
	200	14.09±0.74 ^d	28.75±0.76 ^d	2.55±0.09 ^d		
	0	20.13±1.12 ^c	47.33±2.24 ^e	2.71±0.12 ^c		
Kwete	50	18.45±1.31°	36.19±1.26 ^f	2.41±0.09 ^f		
	100	16.17±1.02 ^g	31.11±0.92 [°]	2.33±0.07 ^f		
	200	11.61±0.61 ^h	26.07±1.03 ^d	2.24±0.07 ^h		
	0	18.05±0.64 ^c	41.18±2.71 ^b	2.52±0.09 ^d		
Davia	50	15.27±1.13 ^d	33.48±1.19°	2.33±0.08 ^f		
Вадаа	100	13.19±0.73 ^d	29.05±0.83 ^d	2.17±0.06 ^h		
	200	10.14±0.58 ^h	23.17±1.01 ¹	2.06±0.06 ¹		
	Two way ANOVA result					
Cultivar (C)		*	*	ns		
Salinity treatments (S)		*	*	*		
Interaction C×S		* * ns				

Table 2. Variation of growth parameters of three varieties of white yam cultivars at different salt concentrations.

Data are means (n=5) \pm SD; Data of each column followed by the same letter designate no significant difference (p < 0.05), Duncan's test. The punctuation mark designates the interactions among varieties and salt treatment. *Significant (p < 0.05), ns= Not significant. Source: Experimental data analysis.

weight. Our findings are consistent with those of Ors et al. (2021), who explained that the reduction in plant dry biomass in salty stress area was due to Na⁺ toxicity from plant tissue which reduced photosynthetic capacity, resulting in the obstruction of cell division and expansion. The total biomass of variety Gana significantly (P<0.05) decreased starting at 100 mM NaCl compared to Kwete and Bagaa which significantly (P<0.05) decreased starting at 50 mM NaCl. This can be explained according to Menguekam et al. (2014), she ascribed the diminution in plant biomass to the loss in water absorption by plants and disintegration of food reserves for variety Gana, follow by Kwete and Bagaa. The Root/Shoot dry weight generally decreased in the course of experimentation. For Hand et al. (2022), the Root/Shoot may enhance NaCl tolerance by limiting the movement of harmful ions to the shoot, retarding the commencement of the tolerance.

Nutrient uptake

The three varieties of white yam where significantly influenced by NaCI. The Na⁺ contents of plant partitioning (shoots and roots) of three cultivars greatly increased

while the ratio (K⁺/Na⁺), K⁺ and Ca²⁺ significantly (p < 0.05) dropped with salinity in the cultivar organs (Table 4). These data are in line with those of Benito et al. (2014) who stated that, the decrease of the study mineral can be directly associated to the uptake of Na⁺ by roots. But Shabala and Pottosin (2014) explained that K⁺ and Ca²⁺ are required in the intracellular K⁺ balance which is important for excellent functioning of the photosynthesis activities and stomatal opening. Our results are in consonance with those obtained by Parvez et al. (2020) on Chenopodium quinoa (genotype A7) and Cichorium spinosum in salty soil stress, respectively. He demonstrated that, higher transport of K⁺ and Ca²⁺ in the leaves participated to weakening noxious ions in leaf cells. The minerals (K⁺ and Ca²⁺) were more in the plant parts of the Gana and Kwete varieties compared to Bagaa variety and for Bai et al. (2019), they play a key role in the preservation of osmotic adjustment and pressure exerted by fluid in a cell. In this study, potassium and calcium are dropped from the membranes and cell walls of Gana and Kwete compared to Bagaa variety, in the presence of sodium and could be recommended among saline stress responses tolerance. Several workers like Al-Karaki (2000) and Rahneshana et al. (2018) have related plant salt tolerance mechanisms

Variatio	Concentration	Dry weight (g)			
variety	(mM NaCl)	RDW	SDW	TDW	RDW/SDW
Gana	0	19.50±1.45 ^a	79.81±5.63 ^a	99.31±7.35 ^a	0.25 ^a
	50	17.86±1.95 ^a	78.88±4.19 ^a	96.74±5.42 ^a	0.22 ^b
	100	12.86±1.50°	62.39±5.46 ^c	75.21±6.25 ^c	0.21 ^b
	200	10.44±0.89 ^c	48.31±3.19 ^d	58.75±4.60 ^d	0.20 ^b
Kwete	0	18.27±1.04 ^a	74.18±9.65 ^e	92.54±5.66 ^e	0.24 ^a
	50	16.34±1.43 ^a	70.97±9.44 ^f	86.93±3.15 ^f	0.23 ^a
	100	9.47±0.69 ^c	51.70±6.71 ^d	60.68±4.62 ^g	0.18 ⁹
	200	7.89±1.72 ^h	42.14±6.50 ^h	49.67±3.52 ^h	0.18 ^g
	0	17.06±1.50 ^a	68.49±5.89 ⁱ	85.58±4.67 ^f	0.24 ^a
Bagaa	50	15.18±2.32 ^j	65.81±6.66 ^j	70.58±5.72 ^j	0.23 ^a
	100	7.08±2.31 ^h	46.65±6.11 ^d	53.61±3.92 ^k	0.15 ^k
	200	5.84±2.05 ¹	40.73±1.50 ^h	46.25±2.85 ^h	0.14 ^k
		Two way ANOVA result			
Cultivar (C)		*	*	*	ns
Salinity treatments (S)		*	*	*	*
Interaction C×S		ns	*	*	ns

Table 3. Variation of dry weight of three varieties of white yam cultivars at different salt concentrations.

Data are means (n=5) \pm SD; Data of each column followed by the same letter designate no significant difference (p < 0.05), Duncan's test. The punctuation mark designates the interactions among varieties and salt treatment. *Significant (p < 0.05), ns= Not significant.

Source: Experimental data analysis.

with the ability to stabilize a high leaf K^+ /Na⁺ this approach corroborate with our results.

It was observed that Cu2+, Mn2+, Fe3+ and Zn2+ concentrations were significantly (p < 0.01) decreased with increasing salinity in all study plants with different magnitude related to their salinity-tolerance (Table 4). Our results corroborate with the work of Dai et al. (2014). He noted that Fe³⁺, Zn²⁺, Mn²⁺ and Cu²⁺ were generally decreased with elevated salinity. These micronutrients were significantly lower in Bagaa compared to Gana and Kwete. Our findings are in line with Grattan and Grieves (1999) who pointed out that with regard to handiness of study micronutrients to plants building up in salty areas they may grow, drop, or have no results. We also observed in this experiment that, Fe³⁺, Zn²⁺, Mn²⁺ and Cu²⁺ in shoots were accumulated more in the salt-tolerant Gana, followed by Kwete and then Bagaa. In the same line, Marschner (1995) and Nouck et al. (2016) observed different responses of genotype plants and their capacity to effectively metabolize micronutrient in salty stress conditions.

Chlorophyll (a+b)

Salinity significantly (p<0.01) reduced chlorophyll (a+b)

content starting at 100 mM NaCl for all varieties (Figure 1). These findings were in line with those of Mostafa Heidari (2012) on two basil (Ocimum basilicum L.) genotypes; Alzahrani et al. (2019) on two genotypes of Vicia faba and Najar et al. (2019) on Medicago truncatula. These authors attributed decrease in chlorophyll to the increased activity of cholorophyllase which induces breaking up of the chlorophyll, plastid forms and salt causing fluctuations of pigment protein complexes. The chlorophyll contents were higher in variety Gana, followed by Kwete and then Bagaa. It was observed in our research that, the chlorophyllase are responsible of photodamages which affected the Bagaa more than Gana and Kwete varieties. Jaleel et al. (2007) attributed this intrusion of salt ions with the combination of proteins affecting the architectural chlorophyll elements, preferably the breakdown of chlorophyll.

Osmolyte contents

A significant (p<0.01) grow of osmolytes was observed in all varieties in Figure 2. The Proline (PRO) content, Soluble proteins (PR), (FAA) and total soluble carbohydrates (CH) significantly (p<0.01) increased with varying concentrations of NaCl during the experiment.

Mineral in plant partitioning (µg g⁻¹ DW) Treatment Varietv Plant organs Zn²⁺ K+ Ca²⁺ Fe²⁺ Cu²⁺ Mn²⁺ (mM NaCl) Na⁺ K⁺/Na⁺ 0 71.60±2.48ª 37.74±142^a 33.47±1.12^a 235.35±7.16^a 141.28±10.85^a 207.88±12.27^a 105.29±4.43^a 0.52±0.01^a 50 89.20±3.18^b 218.29±8.22^b 132.17±12.26^b 31.25±1.16^b 29.68±1.39^b 201.17±7.33^b 96.77±5.18^b 0.35±0.01^b Roots 100 26.26±1.75^b 191.27±6.17° 81.58±3.93° 0.27±0.02° 110.81±4.25° 26.46±1.11° 115.81±13.26^c 163.89±8.2121° 200 136,30±6.03d 19.30±1.04^d 20.78±2.07^d 172.58±7.55^d 91.72±11.43^d 141.44±6.36^d 74.19±2.37^d 0.16±0.01^d Gana 0 102.39±3.4° 88.62±3.82^e 389.66±11.75^e 0.55±0.02^a 182.51±7.13^e 548.75±12.16^e 265.18±14.13^e 201.57±10.38e 50 211.82±7.71^f 91.22±2.72^f 81.31±2.54^f 512.67±10.14^f 251.25±12.27^f 364.19±8.43^f 193.29±8.56^f 0.43±0.01^f Shoots 100 274.84±13.719 76.48±3.129 75.37±3.189 481.89±10.17^g 215.81±15.18^g 319.28±10.389 162.37±6.829 0.33±0.01^b 200 310.31±9.06^h 70.68±1.95^h 64.75±2.14^h 428.72±112.22^h 187.29±11.24^h 301.16±13.27^h 143.59±6.31^h 0.22±0.01^h 0 67.44±2.21ª 33.22±1.17ⁱ 30.12±1.01^a 221.18±5.17^b 132.48±13.81ⁱ 201.46±9.12^b 93.27±4.12^b 0.49±0.01^a 50 78.66±2.14j 28.41±1.03^c 26.34±1.15^b 202.59±6.13j 117.52±14.35° 186.58±5.18j 85.13±4.47° 0.36±0.01^b Roots 100 97.31±3.23k 23.55±0.72k 21.17±1.23^d 182.87±5.35k 101.86±16.63k 156.29±4.21k 70.19±2.69^d 0.24±0.01° 200 16.46±0.851 123.87±4.251 15.15±1.16 164.23±±6.511 83.27±19.391 128.85±5.32l 68.39±3.55^d 0.13±0.011 Kwete 0 93.88±2.18^f 362.81±8.27^f 172.48±4.19^m 81.53±1.38m 521.89±10.16^m 243.56±16.12^m 192.75±5.20^f 0.54±0.01^a 50 198.28±5.58ⁿ 82.27±2.13ⁿ 73.28±2.179 348.55±6.18ⁿ 0.41±0.01^f 509.46±9.21^f 233.49±13.64ⁿ 181.56±6.45ⁿ Shoots 0.30±0.01° 100 215.69±7.37^f 68.32±2.24° 64.31±2.24^h 462.17±11.11° 191.27±14.66° 308.17±8.47^h 148.33±5.33^h 200 289.29±8.13^p 54.17±1.56^p 52.18±1.88^p 391.84±13.28^p 173.17±12.27^p 295.28±10.29^p 121.89±5.89^p 0.19±0.01^d 0 30.24±1.03^b 27.19±1.03^b 207.81±9.27^j 124.36±11.21° 198.29±7.38^b 82.58±3.38° 63.84±1.03^q 0.47±0.02^q 50 73.27±2.09^a 25.87±1.01° 22.13±1.21^d 193.19±4.28° 105.42±10.83^k 173.38±6.23r 76.91±2.99^d 0.35±0.01^b Roots 100 91.88+1.55^b 20.18±0.52^k 17.11±1.17¹ 171.89+6.49^d 91.87±8.36^d 141.17±6.29^d 63.80±3.16^s 0.21±0.01^h 200 13.15±1.01^t 11.24±0.75^t 54.73±2.12t 112.36±3.26° 143.67±5.18^t 71.36±5.42^t 115.43±4.37^t 0.11 ± 0.01^{1} Bagaa 0 167.27±3.73^m 90.26±3.85^f 74.12±2.169 341.67±6.12ⁿ 183.29±3.13ⁿ 0.53±0.02^a 501.17±7.73^u 223.18±11.28^u 50 185.92±4.39e 73.11±2.43^h 62.28±±2.87^h 489.48±8.89^v 206.87±13.27^v 323.23±5.679 172.37±4.48^v 0.39±0.02^b Shoots 100 205.77±5.46^f 61.15±2.07^w 53.14±2.81^p 451.82±10.05^w 172.29±14.73^p 301.81±7.12^h 131.66±4.31^w 0.27±0.01° 200 261.83±7.57[×] 46.71±1.41× 43.19±1.16^x 372.13±8.24^x 154.13±15.16^x 283.28±6.18× 0.17±0.02^d 103.48±4.16^a **Two way ANOVA result** * * * Varieties (V) ns ns * * * * Salinity treatments (S) * Interactions V×S * * * * ns ns ns

Table 4. Variation of mineral uptake of three varieties of white yam cultivars at different salt concentrations.

Data are means (n=5) \pm SD; Data of each column followed by the same letter designate no significant difference (p < 0.05), Duncan's test. The punctuation mark designates the interactions among varieties and salt treatment. *Significant (p < 0.05), ns= Not significant. Source: Experimental data analysis.



Figure 1. Variation of chlorophyll (a+b) of three varieties of white yam cultivars under saline conditions. Data are means (n=5) \pm SD; Data of each bars followed by the same letter designate no significant difference (p < 0.05), Duncan's test. The punctuation mark designates the interactions among varieties and salt treatment. *, **, *** Significant (p < 0.05; p < 0.01 and p < 0.001), respectively, ns= Not significant.

Our data are similar to the results obtained by Gouveitcha et al. (2021), Nouck et al. (2022) and Hand et al. (2022) on Triticum aestivum, Abelmoschus esculentus L. Moench, V. unguiculata (L. Walp) and Talinum triangulare (JACQ.), respectively. They concluded that the accumulation of certain osmolytes in the cytoplasm such as PR, PRO, CH and FAA allows the plant to maximize the storage of sufficient reserves that can support the detrimental effects caused by salinity. This can be metabolite pointers to the identification of tolerant plants to salinity stress. The results of this work showed an increase of PRO in the leaves of Gana and Kwete varieties with increased salinity levels compared to Bagaa. The variety Gana showed a marked increase, proof that it is more tolerant than the two other varieties to salinity. Previous workers explained that, Proline plays a key role in osmotic adjustments, acts as an enzyme inhibitor, stabilizes membranes and cellular structures under salinity stress, and detoxifies free radicals (Larher et al., 1993; Amini and Ehsanpour, 2005). In the same soluble the total carbohydrates increased line, significantly in Gana and Kwete varieties. This was caused by the regulatory osmotic adjustment during stress conditions. Irannejad and Shahbazian (2004) and Kumar et al. (2021) explained that CH contributes in stabilizing osmotic stress in plant cells the protection of biomolecules and membranes. These findings also illustrated that, the soluble proteins grow significantly with varying salt concentrations in the culture medium. According to the results of Kosová et al. (2013), the production of soluble protein improves plant salinity tolerance during osmotic adjustment and it is suggested like a tolerance strategy of plants in salty stress conditions. The increase of FAA in Gana and Kwete compared to Bagaa according to Cusido et al. (1987) is due to the deficiency of K^+ caused by NaCl which improved the levels of FAA, particularly aspartic acid, glutamic acid and PRO.

Antioxidant compounds

The results showed various responses of superoxide dismutase (SOD), peroxidase (POD), total phenol (TP) and flavonoids (FLA) (Figure 3). Significant (p<0.01) increase of SOD, POD, TP and FLA was observed in the leaves of Gana and Kwete varieties compared to Bagaa. These results are similar with findings of Sevengor et al. (2011); Kahrizi et al. (2012), and Hand et al. (2017). According to them, plants developed an antioxidant defense mechanism which includes the storage of SOD and POD (Foyer and Noctor, 2005). In the same line, Nouman et al. (2012) argued that, in the cytosol and chloroplast, POD can perfectly scavenge H₂O₂ and SOD purifies superoxide anion free radicals which increases the production of H₂O₂ and has a detrimental effect in plant cells. The increase of SOD, POD, TP and FLA in Gana and Kwete is indicative of greater ROS scavenging and it is a proof that both varieties are more tolerant compared to Bagaa. For Menguekam et al. (2014) and Kumar et al. (2021), the storage of both non-enzymatic antioxidants is a cellular adaptive process for clean oxygen free radicals, and for the protection of photosynthetic activities.



Figure 2. Variation of osmolyte contents of three varieties of white yam cultivars under salinity stress. Data are means (n=5) ± SD. Source: Experimental data analysis.

Agronomic parameters

The studied agronomic parameters (tuber yield and harvest index) under salinity stress showed a significant (p < 0.05) decrease of the three varieties crop plants (Table 5). These results are similar to those of Alam et al. (2004) who explained that, under salinity stress, the toxicity of NaCl hamper crop yields by disrupting plant nutrition and reducing photosynthetic activities. The results of this work show that Gana and Kwete varieties improved more in all the parameters examined more than Bagaa. These results are in consonance with those of Kargar and Kareh (2017) and Nouck et al. (2022). For

those workers, the reduction in crops growth and yield under salinity is due to the decrease in turgor in plant partitioning (roots and shoots) which reduced water potential. In the same line, Villora et al. (2000) and Nouck et al. (2022) explained that, the decrease in some growth parameters (leaf area and stem height) and biomass (Dry and fresh) in salty soils, could not be affected by tolerant varieties.

Conclusion

The studied white yam varieties were influenced by NaCl



Figure 3. Variation of antioxidant compounds of three varieties of white yam cultivars under salinity stress. Data are means $(n=5) \pm SD$. Source: Experimental data analysis.

in the culture medium and showed different responses. The varieties Gana and Kwete exhibited higher growth parameters (SH, LA and ND), dry weight partitioning roots, shoots and ratio (roots/shoots), chlorophyll contents, ions repartition (K⁺, Ca²⁺, Zn²⁺, Fe²⁺, Mn²⁺ and K⁺/Na⁺) compared to Bagaa. In all varieties, the decreased of study parameters under salinity in the culture medium was observed from 50 mM. An increase of primary (total free amino acids, total soluble carbohydrates, soluble proteins and proline and secondarv (total phenols, flavonoids. Superoxide dismutase and peroxidase) metabolites, respectively started at 50 mM, although the increase was less in Bagaa compared to Gana and Kwete. The tuber yield and harvest index obtained in the farm improved at 50 mM NaCl in Gana and Kwete. The strategy used by salt-tolerance Gana and Kwete varieties was to improve in osmotic adjustment, proof of an increased in storage of study metabolites. Therefore, their biochemical accumulation in Gana and Kwete is pointed as initial indicators of salt-tolerant plants and osmotic adjustment ability under saline conditions. The three varieties could be recommended in moderate saline soils with preference given to the Gana and Kwete.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

Veriety	Treatment	Yield parameters		
Variety	NaCI (mM)	Tuber yield (t/ha)	Harvest index (%)	
Cana	0	1.42±0.04 ^a	0.76 ± 0.02^{a}	
Gana	50	1.31±0.02 ^b	0.70±0.01 ^b	
Kwete	0	1.35±0.01°	0.72±0.03 ^c	
	50	1.24±0.03 ^d	0.66±0.01 ^d	
Desse	0	1.27±0.02 ^e	0.68±0.02 ^e	
Bagaa	50	1.03±0.02 ^f	0.60±0.03 ^f	
		Two way ANOVA Result		
Varieties (V)		**	*	
Salinity treatment (s)		*	*	
Interactions (V×S)		*	ns	

Table 5. Changes of tuber yield and harvest index of white yam at the mature stage under saline conditions.

Data are means (n=5) \pm SD; Data of each column followed by the same letter designate no significant difference (p < 0.05), Duncan's test. The punctuation mark designates the interactions among varieties and salt treatment. *, ** Significant (p < 0.05 and p < 0.01) respectively, ns= Not significant. Source: Experimental data analysis.

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