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Sodium Chloride Induced Stress Screening of Indigenous Halophytic Grasses as Potential Candidates for Phytoremediation

Abubakar, M.[1, 2*], Mohammed, S. Y.[1], Abdullahi, S.[1], Namadi, S.[3]

[1]Directorate of Research and Development, Nigerian Institute of Leather and Science and Technology, Zaria.

[2]Department of Biochemistry, Faculty of Life Sciences, Ahmadu Bello University Zaria.

[3]Department of Botany, Faculty of Life Science, Ahmadu Bello University Zaria.

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Abstract: **Halophytes are salt tolerant plant species that thrive in high salinity and marshy areas, which serves as natural sink to industrial pollution loads. This study aimed at collection, identification and screening by Sodium chloride induced stress of six different halophytic grasses. Among the six collected, *Sporobolus pyramidalis, Sacciolepis africana, Sporobolus jacquemontii and Sacciolepis auriculata* were further selected based on the ability to possess an extensive root and shoot system. Relative growth rate (RGR) and increase in biomass of these halophytic grasses was used to evaluates their stress tolerance in response to NaCl induced stress experiment. The halophytes were acclimatized with Hoagland's nutrients solution for 9 days Prior to the NaCl-induced stress treatment. At the 10th day; 600, 400, 200 and 0 mM NaCl solution was simulated to the four experimental group (*n=3)* and was repeated on the 28th day. After the final stress treatment, physical observation was used to monitor the experiment for another 28 days. In order to ascertain their individual effects, all the grasses were harvested and oven dried at 80°C for 48 h to their constant weight for growth analysis. At 400, 200 and 0 mM NaCl concentrations, growth and increase in biomass of *S. africana, S. pyramidalis, S. jacquemontii and S. auriculata* were not significantly affected. However, at 600 mM NaCl concentration, the RGR indices yields 0.0129, 0.0092, 0.0051 and 0.0128 (gg^{-1}day^{-1}) respectively. This indicated that the halophytic grasses responded differently in terms of their growth to the high salinity stress treatment, hence their phytoremediation potential is in the order of *S. africana* > *S. auriculata* > *S. pyramidalis* > *S. jacquemontii*, respectively.**

Keywords: **Halophytic grasses, Hoagland's media, Induced stress, Relative Growth Rate, Stress tolerance, Phytoremediation.**

I. INTRODUCTION

Salinity as a major environmental problem is widespread all over the world. It is considered a challenging factor for plant growth in saline habitats. Soil salinity leads to numerous challenges to agricultural practice that includes poor soil physical conditions, plant growth and development inhibition, and reduced crop yields due to nutrional inbalance and high osmotic stress generated by excess salt concentration in the soil [1]. Also, heavy metals pollution due to industrial activities is a serious issue all over the world because of its detrimental effect to the ecosystem [2].

Interestingly, some plant species of poaceae family and genus sporobolus, can survive and complete their life cycle in a salt concentration ≥ 200 mM NaCl, as they adapt and actively control the uptake, storage, exclusion, and secretion of ions under saline conditions. Their adaptive mechanism to such environments includes salt tolerance and avoidance mechanisms [3], [4]. Among the halophytes, some have potentially evolved specific salt excretory glands in their leaves, which originate from their epidermis and is responsible fo excess salt secretion to enable them tolerate high salinity. Based on their structure, salt secretory glands are classified into four, which are; salt bladders, multicellular salt glands, bicellular salt glands and unicellular vacuole secretory hairs [5], [6]. Also, halophytes have also been found to evolved a defence mechanism that involve accumulation of osmo-protectants, such as proline, glycine betaine, polyphenols, and soluble sugars, in the cytosol to reduce and balance the osmotic pressure [7].

Soils with high salinity present a strong environmental stress to plants that grow on them, and hence plants that are able to tolerate or withstand the stress are termed halotolerant or halophytes and are suitably applied in phytoremediation. Phytoremediation therefore exploits these features plus the photosynthetic machinery of this halotolerant species to remediate the contaminated environment. Most importantly, the success of phytoremediation relies on the ability of plant species to tolerate a wide range of pollutants at high concentration and also to have fast growth rate and high biomass production [3].

Various conventional methods have been employed to remediate high salinity and heavy metals from the environment such as precipitation, filtration, oxidation, reduction, ion exchange, reverse osmosis [8], but prove in-effective because of their high operational cost, complexity and secondary pollution generation [9]. However, phytoremediation provides alternative solution to this menace in an environmentally friendly and economically viable way, were by plants are employed to reclaim the high salinity and heavy metals contaminated soil [10].

In order to achieve a successful remediation using plants, one of the most important criteria is the characterization and determination of pollution index of the target site, this points out the type of pollutant(s), the number of different heavy metal species and their amounts whether above or below the permissible limits. The next critical step is determining the best plant species for the target site based on their capacity to tolerate and detoxify the heavy metal species present in the polluted soil, in this case grasses (specifically halophytes) are considered ideal, because of their dense mass of roots system with extensive surface for pollutants uptake. Another important aspect is the effect of pollutants on the plant species used in remediation, because tolerance capacity of different plant species varies with different heavy metals based on their toxicity threshold, target site, translocation and accumulation in various plant parts [11]. Therefore this study aims to identify, screen and evaluate the tolerance ability of the halophytic grasses in response to NaCl induced stress treatment based on their relative growth rate (RGR) indices, to ascertain their stress tolerance ability towards their field application to remediate high salinity and heavy metals contaminated soils.

II. ECOLOGY AND TAXONOMIC CLASSIFICATION OF PLANT SPECIES USED IN THE STUDY

Sporobolus pyramidalis

This plant is commonly known as giant rat tail`s grass, and is a species of grass native to Africa, with a taxonomic classification; Kingdom: Plantae, Division: Tracheophytes, Order: Poales, Family: Poaceae, Genus: *Sporobolus* and Species: *Pyramidalis.* It is an erect, perennial tussock grass that usually grow up to 2 m in height. It changes shape from a 'rat's tail' when young to an elongated pyramid shape when mature, and it can establish in a wide range of soils and weather conditions. The potential distributions of this grass was predicted using climate modelling software. The specie has a C4 photosynthetic pathway of the phosphoenolpyruvate carboxycarbonate subtype, and are most abundant in tropical and subtropical areas of intermediate rainfall receiving about 500 mm annually. The plant does not produce more leaf in response to elevated CO2 but did produce a greater non leaf shoot biomass. Presumably the plant will therefore be even less palatable in the future when there will be higher global CO2 levels [12].

Sacciolepis africana

This plant is commonly known as cupscale grass, it is a species of grass widespread in tropical and warmer temperate region. Many are native to Africa, with a taxonomic classification; Kingdom: Plantae, Division: Tracheophytes, Order: Poales, Family: Poaceae, Genus: *Sacciolepis,* Species: *africana.* The plant has range of up to 49 specie synonyms, it is an annual C3 grass and highly variable in size, spikelet glabrous and pubescence, and flowers sporadically that persist throughout the year or even up to 24 months in tropical regions. It is a weed of rice, as it's called 'deep water rice grass', and hence may compete for resources with rice, it is also found in shallow, slow moving water where roots may form a coarse and dense mass [13].

Sacciolepis auriculata

This specie was named and refer as a synonym of *Sacciolepis* indica, it is a straggling grass of wet places habitat with light green spikelet, with a taxonomic classification; Kingdom: Plantae, Division: Tracheophytes, Order: Poales, Family: Poaceae, Genus: *Sacciolepis*, Species: *auriculata.* The native range of this species is tropical and subtropical old world to north and east Australia. It is an annual and grows primarily in the seasonally dry tropical biome [14].

Sporobolus jacquemontii

This specie was identified and named as synonym to *Sporobolus* pyrimidalis, commonly known as American Cat`s tail grass, are species of grass native to the southeastern united states, with a taxonomic classification; Kingdom: Plantae, Division: Tracheophytes, Order: Poales, Family: Poaceae, Genus: *Sporobolus,* Species: *Jacquemontii.* The native range of this species is tropical and subtropical America, Africa to Arabian peninsula. It is a perennial and grows primarily in the seasonally dry tropical biome. It has environmental and social uses, also used as animal food and medicine [14].

III. MATERIALS AND METHOD

Collection and identification of the plants species

In this studies, six different indigenous halophytic grasses which are; *S. pyramidalis, S. africana, S. jacquemontii, Sporobolus festivus, Sporobolus virginicus and S. auriculata* were collected from their natural habitat around Zaria LGA in Kaduna state and were taking to the Herbarium, Department of Botany, Faculty of Life Sciences, Ahmadu Bello University Zaria for identication. Among the six halophytic grasses collected, *S. pyramidalis, S. africana, S. jacquemontii and S. auriculata* were selected based on their physical characteristics such as extensive root and shoot systems, fast growth and increase in biomass etc. for the NaCl induced stress experiment.

The rhizomes of *Sporobolus pyramidalis* was collected at Tudun Mallam Kufeana swamp area, Sabon Gari Zaria in Kaduna State, identified at the Department of Botany Ahmad Bello University, Zaria and allocated a voucher number ABU090061. Where as, rhizomes of *Sacciolepis africana*, *Sacciolepis auriculata*, and *Sporobolus jacquemontii* was collected at Kubanni swamp area, Sabon Gari Zaria in Kaduna State and identified at the Department of Botany Ahmad Bello University, Zaria and allocated voucher numbers ABU0860061, ABU0930641, and ABU07081 respectively.

Plate 1: *Sacciolepis africana* (ABU0860061)

Plate 2: *Sporobolus pyramidalis* (ABU090061)

Plate 3: *Sporobolus jacquemontii* (ABU07081)

Plate 4: *Sacciolepis auriculata* (ABU0930641)

Experimental design

The experimental pots are randomly punched plastic containers of 250ml capacity filled with approximately 2kg Gardening soil (GS) (loamy) obtained from the greenhouse with 18:42:40 percent ratio of clay, silt and sand respectively. The pots were arranged and grouped into four groups (each in triplicate) for each experiment. The experimental groups are 600 mM NaCl simulated group, 400 mM NaCl simulated group, 200 mM NaCl simulated group and 0 mM control group. At the greenhouse, rhizomes measuring 6 – 8 cm in length of *S. pyramidalis*, *S. africana, S. jacquemontii and S. auriculata* were washed under running tap and transplanted to the experimental pots. The plants species were acclimatized for nine (9) days by irrigating with 100ml of Hoagland's media at room temperature, 14 hour light and 10 hour dark circle. After the acclimatization, 100 ml of 600, 400, 200 and 0 mM NaCl solution was added to each of the experimental group (*n=3)* and was repeated on the 28th day. After the final stress treatment, the experiment was monitored and watered daily to maintain the moisture content at approximately 60% for another 28 days, where growth and increase in biomass was observed and recorded in all the grasses.

NaCl molar Solutions Preparation

The preparation of different NaCl molar concentrations was carried out according to standard methods as follows: Mass in grams of NaCl needed to prepare a certain volume of solution is weight using a balance (which is obtained by multiplying the molecular weight/molar mass of NaCl, which is 58.44g/mol by the desired Molarity and by volume), placed in a volumetric flask, a volume of distilled water is then added while stiring to dissolved the salt and finally filled up to desired volume mark.

Hoagland Media Preparation

Hoagland media (HM) is specially formulated for plant cell, tissue and organ growth/cultures. The Hoagland media used in this study was prepared according to Hoagland and Arnon [15] and it contains the following constituents in milligram per litre (mg/l) of distilled water as shown in the table I below:

TABLE I: HOAGLAND MEDIA COMPOSITION

S. No.	Composition	mg/l
1.	Potassium nitrate	606.60
2.	Calcium nitrate	656.40
3.	Ammonium phosphate monobasic	115.03
4.	Manganese chloride.4H2O	1.81
5.	Boric acid	2.86
6.	Molybdenum trioxide	0.016
7.	Zinc sulphate.7H2O	0.22
8.	Copper sulphate.5H2O	0.08
9.	Ferric tartrate	5.00

Note: 1.63 grams of dehydrated basal salt mixture comprising the above proportions was weighed after vacuum drying, dissolved in 1L and autoclaved at 121˚C for 15 minutes.

Relative Growth Rate (RGR) analysis

Four plants were harvested before the experiment (one plant per pot), and four more were harvested (one from each pot) after the stress experiment (47 days). The harvested plants wer oven dried at 80ºC for 48 h and weighed. Dry mass of the shoot and root samples was used to determine the Relative growth rate (RGR) indices, which expresses the total increase in the plant dry mass within a time interval relative to the initial dry mass [16]. The relative growth rate (RGR) of whole plants was calculated using the formula:

$$RGR = (\ln Bf - \ln Bi)\, D^{-1}\ (g\ g^{-1}\ day^{-1})$$

Where Bf = final dry mass, Bi = initial dry mass (an average of the four plants dried at the beginning of the experiment) and D= duration of experiment (days).

Data Analysis

All data were collected in triplicates, analysed by one way ANOVA and independent t-test and the results presented as mean ±SD. The level of significance ($p \leq 0.05$) was considered, and means separation was done using Duncan's posthoc test. All analysis was done with SPSS IBM Corporation (Version 22, NY, United States).

IV. RESULTS AND DICUSSION

The physicochemical analysis of the soil sample used in this study as presented in table II indicated that the pH of the soil sample is close to neutrality, with average organic matter and low electrical conductivity which is ideal for optimum growth and development of plants.

TABLE II: PHYSICOCHEMICAL CHARACTERISTICS OF THE GARDENING SOIL (GS)

Samples	%Clay	%Silt	%Sand	%OM	%Moisture	Texture	pH	EC (ds/m)
CGS	18	42	40	1.76±0.20	7.28±0.18	Loam	6.77±0.34	0.77±0.23

Note; All values except for %Clay, %Silt, %Sand and Texture are Mean (±SD) of the physicochemical parameters.

The results obtained (Table III) from the physical observation and data analysis of the initial and final dry weight (data not shown) indicated that the physiological response of all the grasses varied with the different simulations of the NaCl concentrations. In all the grasses, chlorosis and stunted growth was observed in the group treated with 600 mM NaCl concentration, while normal leaves coloration and growth was observed in the (control) group treated with 0 mM NaCl concentration. The response of *S. africana, S. pyramidalis, S. jacquemontii and S. auriculata* to the induced stress varied significantly with different NaCl concentrations (figures 1: a-d). The results indicated that for all the halophytic grasses tested, their growth and biomass yield is adversely affected at a high (600 mM) NaCl concentration that resulted in chlorosis and dried leaves, but generally at concentration of 200 mM except for *S. jacquemontii*, the plant was not adversely affected physiologically as documented in a previous study [4].

TABLE III: RGR INDICES OF HALOPHYTIC GRASSES TRANSPLANTED AND GROWN FOR 47 DAYS

Treatments	RGR indices (g/g/day)			
	S. africana	*S. pyramidalis*	*S. jacquemontii*	*S. auriculata*
600 mM NaCl	0.0129±0.0005^{c}	0.0092±0.0004^{b}	0.0051±0.0001^{a}	0.0128±0.0002^{c}
400 mM NaCl	0.0374±0.0002^{h}	0.0131±0.0003^{c}	0.0093±0.0000^{b}	0.0153±0.0000^{cd}
200 mM NaCl	0.0370±0.0000^{h}	0.0307±0.0001^{g}	0.0096±0.0000^{b}	0.0163±0.0000^{d}
0 mM NaCl	0.0515±0.0070^{i}	0.0307±0.0002^{g}	0.0244±0.0000^{f}	0.0208±0.0001^{e}

Note: All values are mean (±SD). Different letters (a-i) indicates significant difference between the groups (ANOVA and Duncan's Posthoc, $p \leq 0.05$). RGR indices expresses the total increase in the plant dry mass relative to the initial dry mass per day (g/g/day).

This implies that conc. < 200 mM is within tolerable threshold of NaCl concentration for *S. africana, S. pyramidalis, S. jacquemontii and S. auriculata*. Also, *S. africana and S. auriculata* were able to tolerate a concentration of 400mM NaCl, hence this is within their tolerable threshold and probably may be due to production of excess compatible osmolytes such as proline, glycine betaine, polyphenols, soluble sugars etc. in their cytosol to reduce and balance the osmotic pressure [7], [17]. However, at high NaCl concentrations, the adverse effects on the plants is severe because the glands for salt excretion and other salt regulatory mechanisms could not mitigate the salt concentration. Also, presence of excess NaCl could leads to reduced potassium concentration gradient in plants and this affects chlorophyll biosynthesis, which leads to reduced photosynthetic activities and hence reduced growth [4]. This is in agreement with similar findings by [18], [19], and [20] which demonstrated that biomass yield decreased with a high salinity of NaCl treated plants. Also, the trends of the effects of different NaCl simulations on growth and increase in biomass of the halophytic grasses is depicted on fig. 1 below, which shows that as the concentration of NaCl increases, the RGR index decreases (i.e inverse relation) of all the halophytic grasses. However, concentrations of 400, 200 mM NaCl appears to cause similar effects on growth and increase in biomass of *S. africana, S. jacquemontii,* and *S. auriculata* as shown in the trends of fig. 1: (a), (c) and (d) respectively, where as the trends in fig. 1: (b) depicts that concentration of 200 mM NaCl appears to have no effect on the growth and increase in biomass of the *S. pyramidalis* species, which probably may be within its tolerable threshold.

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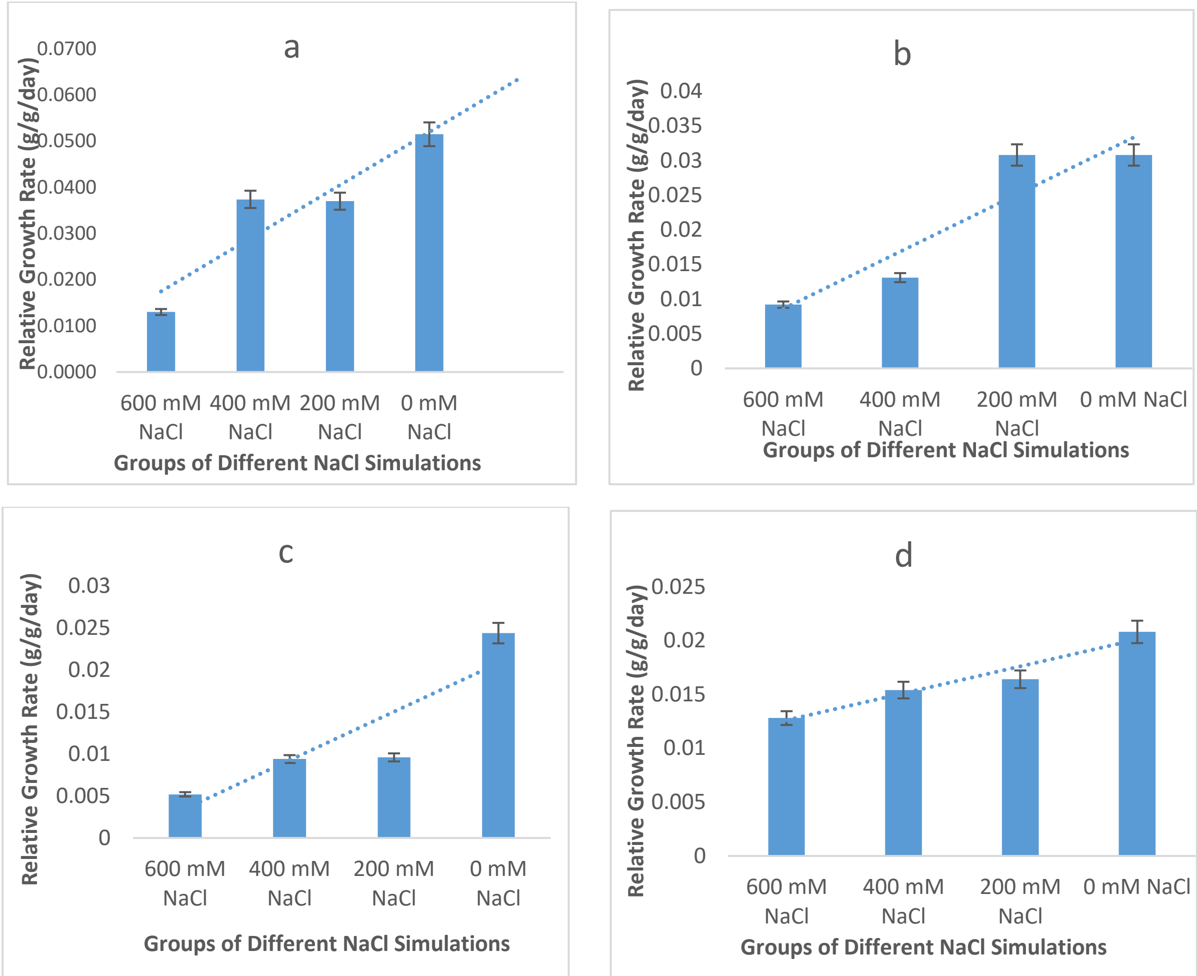

Figure 1: Trends of Different NaCl Simulations Effects on RGR Indices for (a) *S. africana,* (b) *S. pyramidalis,* (c) *S. jacquemontii,* (d) *S. auriculata*

V. CONCLUSIONS

In this study, all the halophytic grasses tested for salt stress tolerance shows the potential to tolerate salinity stress (except at 600 Mm NaCl concentration which adversely affected their growth), and hence their potential use in remediating saline soils [21]. This may be due to various mechanisms such as extrusion of excess salt through salt excretory glands, adjustment of osmoregulatory mechanism through alteration in proline, total amino acids and soluble sugars production in their cells [1], [22]. The result indicated that high salinity stress treatment significantly affect the growth and biomass yield of all the four grasses studied, hence at 600 Mm NaCl concentration the RGR indices of the halophytic grasses is in the order of *S. africana* > *S. auriculata* > *S. pyramidalis* > *S. jacquemontii*, hence their potential to be used in remediation of high salinity and heavy metals contaminated soils.

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