

Microsatellite fingerprinting, enzymes activity and chlorophyll profiling of local lines of air potato yam (*Dioscorea bulbifera* L.) for salt tolerance

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Abstract

Air potato yam (*Dioscorea bulbifera* L.) is an indigenous, edible, and energy-giving crop and used as herbal remedy for common ailments like diabetes, dysentery and conjunctivitis. This study sought to determine the tolerance of *D. bulbifera* to salt stress conditions through molecular, biochemical and physiological approaches. Results revealed that most of the local lines were less tolerant to sodium chloride showing reduced concentrations of chlorophyll a and b of the plants, and also showed increased activities of peroxidase, linolytic, xylanase, cellulase and glucose-6-phosphate dehydrogenase enzymes at increasing salt concentration and stress. Molecular analysis using Simple Sequence Repeats (SSR) revealed that 11 lines did not contain any trait related to the marker. Twenty eight (28) lines fingerprinted at 100 bp with the marker gene. Nine lines were fingerprinted at 300 bp with the marker gene while Dbor 1 fingerprinted at 100, 200, 300 and 400 bp with the marker gene. Three of the local lines which are round in shape, Dbor 1, Dbok 3 and Dbak 2 produced several bulbils at 300Nm of salt concentration and showed molecular bands at 400 and 300 bps indicating the presence of a salt tolerant gene at the locus and base pairs. Therefore, molecular analysis has revealed the presence of salt tolerant gene at 300 400 bp in the three local lines which can be further harnessed for salt-tolerance improvement in the less tolerant lines. Physiologically, chlorophyll contents and enzyme concentrations in the less tolerant local lines were critically affected with increasing salt concentrations.

Keywords: Enzymes assay, SSR markers, Chlorophyll a & b, Aerial yam, Salt tolerant lines

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Introduction

Air potato yam (*Dioscorea bulbifera*) is an indigenous edible crop propagated for its cheap source of the nutrient. It is fast-growing and can adapt to different soil types, weather and habitat conditions, therefore having the potential of tolerating salt stress thus providing food security in an era of famine occasioned by climate change (Clare and Jean, 1984).

Air potato yam is differentiated from all other species of *Dioscorea* by possessing specialized aerial bulbils at the base of petioles (Martin, 1974). The plant is a herb, growing vigorous with twining vine and non-spiny stems that grow up to 20m in length and branch freely above. The stems are round or slightly angled in cross-section. *D. bulbifera* is a perennial vine with broad leaves. The plant is dioecious, inflorescences are axillary and simple fasciculate. Flowers are white or greenish tinged, diminutive and sessile (Han and Hozio, 1993; Miller, 2003). Tuberization of this plant is solely aerial at the top (Miege and Demissew, 1997), with some varieties possessing edible underground bulbils.

Most of the varieties of *D. bulbifera* are cultivated for human consumption in tropical and subtropical countries of Asia, Africa and America, where their edible tubers are important human food sources for man and livestock (Malapa et al., 2005). Air potato yam is an economically important food crop (Hammer, 1998). Some cultivars of *D. bulbifera* have been reported to contain diosgenin, a steroid that is the major bioactive component employed in the synthesis of most steroidal hormones used in the production of birth control pills (Obboh et al., 2001; Dansi et al., 2005). Air potato yam has also been used in herbal medicine systems in Africa Asia and South America and has been reportedly utilized in the treatment of dysentery, diarrhea, conjunctivitis, fatigue and depression among other ailing health conditions (Dansi et al., 1991).

Notwithstanding its nutritional, medicinal and economic potentials of *D. bulbifera*, the crop is yet to receive the much-desired research attention it deserved. Commercial production and development of this aerial yam have not made any significant impact, probably because it is only utilized by rural resource-poor farmers. The development of Air potato yam has been reportedly hindered by poor genetic make-up in terms of resistance, which subjects most local cultivars to adverse biotic and

abiotic stress (Aggarwal et al., 2008).

Identification of resistance genes in local cultivars using molecular markers will be of immense benefit for the crop development since genetic markers are not influenced by environment and plant morphology and remain a basis for genetic improvement and discriminating among cultivars with similar morphological attributes. Molecular markers are also indispensable in determining the evolutionary relationship among local cultivars for plant identification (Aggarwal et al., 2008). Some of the genetic markers adopted in *Dioscorea* research included Amplified Fragment Length Polymorphism (AFLP) (Mignouna et al., 2003; Malapa et al., 2005), Random Amplified Polymorphism DNA (RAPD) (Asemota et al., 1996), and Simple Sequence Repeats (SSR) (Scarcelli et al., 2006). These markers have been widely used to detect and develop resistant genes in crops that enhance growth and productivity in the face of abiotic stress and challenges.

Recent studies by Kalu et al. (2021), revealed that abiotic stress contributes to the low productivity of crops (Shanker and Venkateswarly, 2011) and has been estimated to account for about 70% losses in total yields of crop plants (Rengasmy, 2010). According to Gómez-Bellot et al. (2013), salinity, as a major abiotic stress in agriculture has affected an estimated 45 million hectares of arable irrigated land and a projected continued increase as a result of climate change (Kumar et al., 2013). It has been estimated that >50% of the world Arable lands would be unproductive by the year 2040 if salinity is not checked (Wang et al., 2013).

Soil salinity is an environmental problem affecting agriculture (Jouyban, 2012) and has greatly contributed to low productivity of plants worldwide (Kumar et al., 2013). Saline soil causes physiological and metabolic imbalance resulting in poor development, growth and yield of plants (Jouyban, 2012). Salinity has been reported severally to negatively influence plant growth and reproduction. It also causes a reduction in chlorophyll concentrations, catalyzed or reduced enzymatic reactions and other physiological reactions occasioned by increased salt concentrations in the plant cell sap (Kalu et al., 2021).

This study, therefore, seeks to evaluate the tolerance of air potato yam to sodium chloride through molecular, biochemical and physiological studies.



Material and Methods

Collection of experimental materials

Bulbils of *D. bulbifera* with two distinguished shapes were obtained from local farmers in Abia State, Akwa Ibom State and Cross River State and established in the Biological Sciences Research Farm, University of Calabar, Calabar for the study. Forty (40) accessions of Air potato yam (*Dioscorea bulbifera*) were sourced and obtained. Ten (10) each of the following accessions were obtained from Ohafia in Abia State, Oron in Akwa Ibom State, Okobo in Akwa Ibom State and Akpabuyo in Cross River State, Nigeria. The accessions used and their source of germplasm are designated as Dboh for Ohafia, Dbak for Akpabuyo, Dbor for Oron and Dbok for Okobo lines respectively and their shapes as presented in Table 1;

Planting and treatment of the plant

Bulbils were planted in polythene bags and placed in a greenhouse in a randomized complete block design with three replicates. Three bulbils were planted per bag and watered with 250 ml of distilled water daily until sprouting was established after which the plants were thinned to one per bag. After four weeks of growth sprouting, the plants were treated with three different concentrations of sodium chloride solution (0, 100, 200 and 300mM), twice a week for six weeks during which agro-morphological data of vine length, number of leaves per plant, leaf area, petiole length and leaf length ascertained. Physiological parameters such as chlorophyll a and b concentrations, peroxidase and glucose-6 phosphate dehydrogenase enzymatic activities were measured using the atomic absorption spectrophotometer from leaf extracts of the plant samples.

Experimental site

The fieldwork was carried out in the screen house, University of Calabar. Enzymes activity profile, Chlorophyll a and b concentrations and molecular screening of the local lines were all carried out at the molecular biology laboratory in the Department of Biological Sciences, University of Calabar. Sodium chloride and other reagents were purchased from a commercial chemical store. All reagents were of analytical grade. The University of Calabar is on Longitude 004° E and Latitude 008°N.

Physiological characterization studies

Preparation of extracts

Leaf samples were weighed, frozen and ground using mortar and pestle. Grounded samples were hydrated with 20 ml of 80% acetone and phosphate buffer for Chlorophyll contents and enzyme's activities determination respectively. Each of the mixtures was incubated and centrifuged at 15000 rpm for four minutes. Supernatants were collected and stored on ice till further studies.

Table-1: Accessions of local lines of *D. bulbifera* used in the study, their origin and shape.

S/ N	LOCAL LINES /ACCESSIONS	DESIGNATION	LOCATION SOURCE	SHAPE
1	<i>Dioscorea bulbifera</i>	Dboh – 1	Ohafia	Round
2	<i>Dioscorea bulbifera</i>	Dboh – 2	Ohafia	Elongated
3	<i>Dioscorea bulbifera</i>	Dboh – 3	Ohafia	Round
4	<i>Dioscorea bulbifera</i>	Dboh – 4	Ohafia	Elongated
5	<i>Dioscorea bulbifera</i>	Dboh – 5	Ohafia	Round
6	<i>Dioscorea bulbifera</i>	Dbak – 1	Akpabuyo	Round
7	<i>Dioscorea bulbifera</i>	Dbak – 2	Akpabuyo	Round
8	<i>Dioscorea bulbifera</i>	Dbak – 3	Akpabuyo	Round
9	<i>Dioscorea bulbifera</i>	Dbak – 4	Akpabuyo	Elongated
10	<i>Dioscorea bulbifera</i>	Dbak – 5	Akpabuyo	Round
11	<i>Dioscorea bulbifera</i>	Dbor – 1	Oron	Elongated
12	<i>Dioscorea bulbifera</i>	Dbor – 2	Oron	Elongated
13	<i>Dioscorea bulbifera</i>	Dbor – 3	Oron	Elongated
14	<i>Dioscorea bulbifera</i>	Dbor – 4	Oron	Elongated
15	<i>Dioscorea bulbifera</i>	Dbor – 5	Oron	Round
16	<i>Dioscorea bulbifera</i>	Dbok – 1	Okobo	Elongated
17	<i>Dioscorea bulbifera</i>	Dbok – 2	Okobo	Round
18	<i>Dioscorea bulbifera</i>	Dbok – 3	Okobo	Elongated
19	<i>Dioscorea bulbifera</i>	Dbok – 4	Okobo	Round
20	<i>Dioscorea bulbifera</i>	Dbok – 5	Okobo	Elongated
21	<i>Dioscorea bulbifera</i>	Dboh – 6	Ohafia	Elongated
22	<i>Dioscorea bulbifera</i>	Dboh – 7	Ohafia	Round
23	<i>Dioscorea bulbifera</i>	Dbok – 6	Okobo	Round
24	<i>Dioscorea bulbifera</i>	Dbok – 7	Okobo	Elongated
25	<i>Dioscorea bulbifera</i>	Dbor – 6	Oron	Elongated
26	<i>Dioscorea bulbifera</i>	Dbor – 7	Oron	Elongated
27	<i>Dioscorea bulbifera</i>	Dbak – 6	Akpabuyo	Elongated
28	<i>Dioscorea bulbifera</i>	Dbak – 7	Akpabuyo	Round
29	<i>Dioscorea bulbifera</i>	Dbok – 8	Okobo	Round
30	<i>Dioscorea bulbifera</i>	Dbor – 8	Oron	Elongated
31	<i>Dioscorea bulbifera</i>	Dbak – 8	Akpabuyo	Elongated
32	<i>Dioscorea bulbifera</i>	Dboh – 8	Ohafia	Round
33	<i>Dioscorea bulbifera</i>	Dbak – 9	Akpabuyo	Elongated
34	<i>Dioscorea bulbifera</i>	Dbok – 9	Okobo	Elongated
35	<i>Dioscorea bulbifera</i>	Dbok – 10	Okobo	Elongated
36	<i>Dioscorea bulbifera</i>	Dbor – 9	Oron	Elongated
37	<i>Dioscorea bulbifera</i>	Dbor – 10	Oron	Elongated
38	<i>Dioscorea bulbifera</i>	Dbak – 10	Akpabuyo	Elongated
39	<i>Dioscorea bulbifera</i>	Dboh – 9	Ohafia	Elongated
40	<i>Dioscorea bulbifera</i>	Dboh – 10	Ohafia	Elongated



Chlorophyll a and b concentrations assay

Chlorophyll determination

The methods for the determination of chlorophyll contents from the *D. bulbifera* were adopted from Shanker and Venkateswarly, 2011. Five (5ml) suspension of algae was obtained at three (3) days interval from reaction prepared. Each algal suspension was centrifuged at 3500 rpm for 15minutes. The supernatant was easily discarded and the residual pellet was treated with 95% Dimethyl sulphate (DMSO). Five (5) ml of DMSO was then added to 5ml and homogenized. These were kept in a water bath at 70°C for 5minutes, removed and left to cool to room temperature. The extract was centrifuged at 3500 rpm for 5mins and the pigment read at a 660nm wavelength at photo spectrometer using distilled water as blank and readings recorded. Each of the sample extracts was transferred into a cuvette and absorbance was read at a wavelength of 645nm and 650nm. Chlorophyll concentrations were calculated from the formula as described by Kumar et al., 2013 and Jayeola and Oyebola, 2013.

Chlorophyll a contents of the leaves of yam varieties sown at the different concentrations were measured at the standard wavelengths of 645 and 650nm using the Atomic Absorption Spectrophotometer (AAS), Perkin Elmer, 7000- series, Machinio, USA. Chlorophyll b contents of the leaves of rice varieties sown at the different concentrations were measured at the standard wavelengths of 645 and 650nm using the AAS.

Peroxidase activity assay

The extract (0.6 ml), hydrogen peroxide (0.1 ml), guaiacol (0.1 ml) and buffer (2 ml) were added together in a cuvette for a reaction and the absorbance value was read at a wavelength of 436nm after one minute, two, three, four and five minutes. The values were used to determine the peroxidase activity.

Glucose- 6 phosphate dehydrogenase activity (Enzyme) assay

The reaction mixture for glucose-6 phosphate dehydrogenase activity included 0.6ml extract, 2.0 ml buffer, 0.1 ml glucose -6 phosphate and 0.1 ml beta Nicotinamide adenine dinucleotide phosphate (NADP). The absorbance values were read at a

wavelength of 340nm at one minute, two, three, four and five minutes. The values were used to determine the glucose-6 phosphate dehydrogenase activity.

Linolytic enzyme assay

This enzyme was assayed using Manganese peroxidase (MnP), Laccase (Lac) and Manganese-independent peroxidase (MIP). Each of these was determined through the measurement of the respective absorbance change at 470 nm relative to the rate of oxidation of 2,6-dimethoxyphenol in malonate buffer (pH 4.5). However, Lignin peroxidase (LiP) activity was measured by the oxidation of veratryl alcohol in succinate buffer (pH 3.0) at 310 nm.

Xylanase and Cellulase Enzymes assay

The modified procedures of Bisswanger, 2014 spiced with Oat spent xylem (1%) as substrate at 50 °C and a 5.3 pH was adopted for Xylanase and cellulase enzymes assay. Cellulase activity was measured using reducing sugars obtained from carboxymethyl cellulose (2% w/v, low viscosity). At the end of laboratory procedures, Xylose and glucose standard curves were used to calculate the xylanase and cellulase activities from the samples (Terauchi et al., 1992).

Data Analysis

The Analysis of variance (ANOVA) option of the Genstat V 12 of ICRAF of 2018 was used to analyze the data and mean with significant differences were separated using the Fishers least significant difference (LSD) at 5% level of probability.

Molecular Screening of *Dioscorea bulbifera* local lines using Simple Sequence Repeats (SSR) analysis

Collection of Plant materials

Five (5) g of apical fresh leaves of forty local lines of *Dioscorea bulbifera* representing entries from the different localities in Nigeria (Table 1) were used for the molecular study. The leaves were collected from Plants after two weeks of growth cultivated in 30cm size pots filled with sterilized top soil and maintained in a screen house at the University of Calabar research farm, Nigeria for genomic DNA extraction.

Table-2: Characteristics of 10 microsatellite primers used to characterize 40 accessions of *Dioscorea bulbifera*

Name	Forward and reverse sequences	Repeat motif	Ta (°C)
Db2	F: CACGACCTCCTGGAAGACAAC R: ATATAGCACGGGAGGCACAAAC	(GAC) ₄	53
Db3	F: TTTTACCCAGGATTTAGAAGAA R: GGACTGGAGCCACAAGATT	(CA) ₈	50
Db5	F: TGTCTATTATATTGCTCTTTCT R: CGTTTCTAATTCTGGGTAT	(GT) ₄	50
Db6	F: AAGCCGGTATCATTCAACAAAA R: CCCTCGCCAACATCAAGTAA	(AC) ₈	53
Db7	F: CCGCAAGGCTCAAAAAGTTAGG R: TCGTGGATGAAGATGGGTGGAC	(GA) ₄	53
Db8	F: TCCCAAGAAATCCAGAATA R: ATGCATGCCAAAACAAATA	(GAC) ₄	50
DBSSR1	F: ACACACACACAGAGAGAGAG R: AGAAGTTTGTGCCCCGTC	(AC) ₆ (AG) ₂₀ ... (GGA) ₃	54
DBSSR2	F: ACACACACACAGAGAGAGAG R: AACGCATCCCACCACTTC	(AC) ₆ (AG) ₁₃	54
DBSSR3	F: ACACACACACAGAGAGAGAG R: CACGATGGAGGAACACTT	(AC) ₆ (AG) ₁₉	54
DBSSR4	F: ACACACACACAGAGAGAGAG R: GAAAAGGAGAAGCCGAAT	(AC) ₆ (AG) ₉	54

Ta = annealing temperature

DNA extraction and quantification

Genomic DNA was extracted from fresh leaf apex of young leaves using modified cetyltrimethylammonium bromide (CTAB) procedure as described by Mignouna et al. (2003) in the molecular laboratory of the Department of Genetics and Biotechnology, University of Calabar. The quality and concentration of DNA was assessed by gel electrophoresis using 1% agarose with known concentrations of undigested lambda DNA (Sigma St. Louis, MO, USA). Quantification of DNA was done using a Nanodrop spectrophotometer (Beckman Coulter DU 530) at 260nm. Extracts were diluted in water to obtain a DNA concentration of 25ng/μL.

Polymerase chain reaction and fragment analysis

A total of ten SSR primer pairs were used in the study (Table 2). PCR reaction was conducted in a 20μL volume in a 96-well microtitre plate using an automated thermal cycler (Peltier Thermal Cycler 200).

The reaction volume contained 25ng of template DNA, 100μM each of dNTP, 2.5mM MgCl₂, 0.5μM each of fluorescently labeled forward primer and unlabeled reverse primer, 1X reaction buffer and 2 units of Taq DNA polymerase (Invitrogen). The forward primer was 5¹-labeled with one of the four fluorochromes PET, 6-FAM, NED and VIC. The PCR programme consisted of denaturation at 94°C for 5 mins, followed by 35 cycles of 94°C for 30s, 55

or 45°C for 20s and 72°C for 30 s, with a final extension step at 72°C for 7 min.

Capillary electrophoresis with a semi-automated system ABI/PRISM 3100 genetic analyzer was used to separate amplified PCR products. Samples for amplified product separation were prepared by adding 1μL of diluted PCR products to 9.4μL formamide and 0.1μL Gen size-500 LIZ. This was dispensed in ABI 96-well plates and denatured at 94°C for 5 min and allowed to cool down on the ice.

Primers used for the study

Ten (10) primer sets (forward and reverse) previously used by researchers working on *Dioscorea bulbifera* (Table 1) in the International Institute for tropical agriculture (IITA) Ibadan were sourced from Operon, USA and used for the study.

SSR fragment analysis

The fragment sizes in base pairs for each genotype across SSR markers were converted to binary data where alleles were transformed into presence (1) or absence (0) of an SSR band. Genetic diversity indices such as the number of alleles for locus; gene diversity and Polymorphic Information Content (PIC) were estimated using Power Marker version 3.25 software Genetica, The Netherlands. The cluster pattern of the genetic diversity was constructed using DARWIN 5.0 SIB, Switzerland.



Results

Genetic diversity indices analysis

Table 3 presents the summary of genetic diversity parameters generated by 10 SSR markers. DBSSR 2 recorded the highest number of alleles detected (12 alleles). This was followed by DB5 (10 alleles) DB3 and DBSSR4, respectively (Table 3). The discriminatory power of each SSR primer was assessed by calculating Polymorphic Information Content (PIC) values. The mean PIC value for all markers used was 0.74 and ranged between 0.37 and 0.85 in loci DB7 and DBSSR4, respectively (Table 3). Markers with more alleles but lower allele frequency had larger PIC as found in DBSSR4 (9 alleles and the highest PIC of 0.85, followed by DB5 with 10 alleles and PIC of 0.84, respectively (Table 3). Again, it can be established from the results that marker DB7 had the highest allele frequency of 0.60 while markers DB3, DB5 and DBSSR4 had the lowest frequency of the major allele (0.24). The average frequency of the major allele was 0.33 (Table 3).

Gene diversity was high, ranging from 0.48 in DB7 to 0.86 in DBSSR4 with a mean of 0.77. Generally, the allele frequency of all the primers was below 0.95, indicating that they were all polymorphic in character (Table 3). Plates 1& 2 show SSR marker (DBSSR2 & DB5) profiles generated for the *D. bulbifera* local accessions used in the study. Generally, the pattern of movement of the DNA bands in the gel distinguishes one accession from the other. Results observed from allelic frequency analysis proved that all the 10 primers were polymorphic (Table 3).

Table-3: Genetic diversity indices for the ten SSR markers used to analyze 40 accessions of *D. bulbifera*

Marker	Major. Allele. Frequency	Allele No	Gene Diversity	PIC
DB2	0.3600	8.0000	0.7936	0.7697
DB3	0.2400	9.0000	0.8512	0.8345
DB5	0.2400	10.0000	0.8544	0.8383
DB6	0.3200	6.0000	0.7520	0.7105
DB7	0.6000	2.0000	0.4800	0.3648
DB8	0.3600	6.0000	0.7488	0.7095
DBSSR2	0.2800	12.0000	0.8352	0.8176
DBSSR3	0.3200	6.0000	0.7680	0.7314
DBSSR5	0.3600	7.0000	0.7808	0.7521
DBSSR4	0.2400	9.0000	0.8608	0.8460
Mean	0.3320	7.4000	0.7725	0.7374

No rare alleles (alleles with allelic frequencies of less

than 0.1005) were obtained. This was possibly due to the genetic closeness of the local lines studied. Major allele frequency ranged between 0.2400 in DB3 to 0.3600 in DBSSR 5 with a mean value of 0.3320. The number of alleles per locus varied from as low as 2 obtained with DB7 to 12.00 obtained with DBSSR 2 markers with a mean number of 7.400. Nei's gene diversity was 0.4800 in DB7 and 0.8608 in DBSSR 4 with average gene diversity of 0.7725.

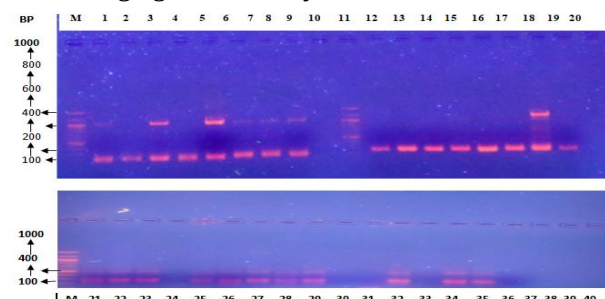


Figure-1: Electrophoregram showing bands of polymorphic loci 1 – 40

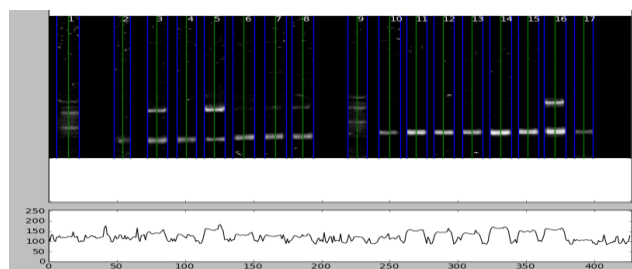


Figure-2: PyElph 1.4 Electrophoregram showing bands of polymorphic loci 1 - 20

Table 4 shows the approximate size of a gene (bps) that confers tolerance of the local lines of to sodium chloride. Eleven of the 40 local accessions or lines did not possess the complement of the tolerance gene found in the marker. Twenty-eight (28) of the local accessions showed low salt tolerance at 100 bps gene size (Fig 1 and 2). One (1) local accession Dbor – 1 obtained from Oron, Akwa Ibom State showed moderate to high tolerance to common salts with complementary bands at 100 bps, 200 bps, 300 bps and 400 bps (Fig. 1 and 2) respectively. This local accession also showed high phenomic attributes in the field at all concentrations of the common salts. The results also revealed that nine (9) of the local accessions were moderately tolerant to variable concentrations of common salt complementing the marker tolerance gene at 300 bps. Future research may be a focus on identifying the gene through next-generation sequencing and blasting at NCBI or other databases.

Table-4: Identification of potential size of salt tolerant gene in forty local lines of *Dioscorea bulbifera* based on electrophoregram of molecular markers showing non tolerant, tolerant and more tolerant local lines of *Dioscorea bulbifera* to Common salt

Local lines	0 bp	100 bp	200 bp	300 bp	400 bp
Non tolerant lines	Dbak – 5 Dbok – 5 Dbok – 7 Dbor – 8 Dbak – 8 Dbak – 9 Dbor – 9 Dbor – 10 Dbak – 10 Dboh – 9 Dboh – 10				
Tolerant lines		Dboh-1 Dboh -3 Dboh – 5 Dbak – 1 Dbak – 2 Dbak – 3 Dbak – 4 Dbok - 3		Dboh-1 Dboh -3 Dboh – 5 Dbak – 1 Dbak – 2 Dbak – 3 Dbak – 4 Dbok - 3	
Most Tolerant lines		Dbor - 1	Dbor – 1	Dbor - 1	Dbor - 1
	11	28	1	9	1

The AMOVA presented in Table 5 and figure 2 revealed that 100% of the genetic variations in molecular data was attributable to variations in the DNA molecules of the different lines while 0 % of the variability was attributable to environmental influences and variation among the *Dioscorea bulbifera* local populations. The variations is salt tolerance index observed among the local lines of *D. bulbifera* was totally attributable to molecular variance existing among the local populations.

Table-5: AMOVA table for SSR analysis of 40 accessions of *Dioscorea bulbifera* accessions evaluated for salt tolerance

Percentage molecular variation of the *Dioscorea bulbifera* local populations

Source	df	SS	MS	Est. Var.	%	p-vlaue	Phqt
Among Pops	1	0.417	0.417	0.000	0%	0.010	0.0014
Within Pops	38	18.600	0.493	0.493	100%		
Total	39	19.017		0.493	100%		

Key: phqt = estimate of the population genetic differentiation based on Permutation.

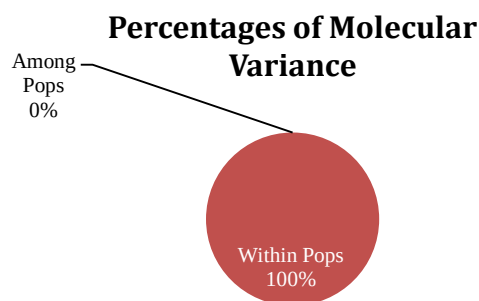


Figure-3: Percentage molecular variation of Air potato yam (*Dioscorea bulbifera*) population

The scattergram in figure 4 delineated the 40 local accessions of *D. bulbifera* into principal components. Most of the local accessions clustered within the central axis to principal component 1 while others were randomly distributed along the principal axes.

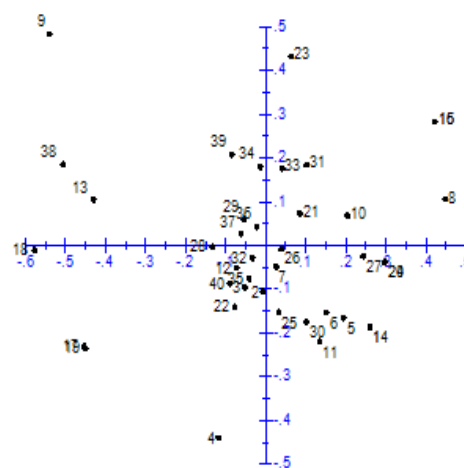


Figure-4: Scattergram showing distribution of 40 Air potato yam (*Dioscorea bulbifera*) accessions along the major principal coordinate axes

Table 6 presents the results of Five (5) principal components axes used to delineate 40 accessions of local *D. bulbifera* lines based on their contributions to the observed variations in their response to salt and physiological stress. The total variations observed among the local accessions to salt tolerance using this approach were 97.48% somewhat close to 100% obtained with the analysis of molecular variance. Principal component 1 showed an eigenvalue of 0.06581 with the highest percentage contribution of 48.63% of the total 97.48% variations in salt tolerance observed among all the accessions. The

major contributing local line to this principle component is Dbak - 3 from Akpabuyo, Cross River State with a loading value of 0.4479. Principal component 2 showed an eigenvalue of 0.03448 with a percentage contribution of 25.00% out of the total 97.48% variations observed in salt tolerance ability among the accessions. The major contributing local line to principal component 2 is Dbak - 4 from Akpabuyo, Cross River State with a loading value of 0.4832. Principal component 3 showed an eigenvalue of 0.02603 with a percentage contribution of 11.32% out of the total 97.48% variations observed in salt tolerance attributes of the local accessions. The major contributing local line to principal component 3 is Dbok - 3 from Okobo, Akwa Ibom State with a significant loading value of 0.3470. The results for principal component 4 showed an eigenvalue of 0.01583 with a percentage contribution of 6.89% out of the total 97.48% variations observed in salt tolerance attributes of the local accessions. The major contributing local accession to principal component 4 is Dbok - 8 from Okobo, Akwa Ibom State with a significant loading value of 0.3092. The results for the last Principal component 5 revealed an eigen value of 0.01296 with percentage contribution of 5.64% out of the total 97.48% variations observed in salt tolerance attributes of the local accessions. The major contributing local accession to principal component 5 is Dbor - 8 from Oron, Akwa Ibom State with a significant loading value of 0.2445.

The results of analysis of molecular data using DARwin 5.0 software delineated the 40 local lines of *Dioscorea bulbifera* into two (2) major clusters and ten (10) subclusters. Major cluster one has 20 local lines partitioned into 6 subclusters. Subclusters 1 has Dboh 4 and Dboh 2 local lines with the same genetic distance of 41. Sub-cluster 2 has 4 local lines of Dbak 10, Dboh 3, Dbor 3 and Dboh 4 respectively having the same genetic distance of 43. Sub-cluster 3 has 2 lines namely Dbok 1 and Dbor 5 with genetic distance of 47. Sub-cluster 4 has DBok 6 and Dbak 2 with the same genetic distance of 46. Sub-cluster five has five lines of Dbok 1, Dbor 5, Dboh 8, Dbok 8 and Dbor 8 all with the same genetic distance of 47. The last sub-cluster 6 in major cluster 1 has five lines including Dboh 9, Dbor 9, Dbor 6, Dbor 2 and Dbak 5 with genetic distance of 48 (fig. 5).

Major cluster 2 has four sub-clusters of 7, 8, 9 and

10. Sub-cluster 7 has two lines Dbok 7 and Dbok 5 with a genetic distance of 45. Sub-cluster 8 has two lines also, Dbor 4 and Dboh 1 with a genetic distance of 46. Sub-cluster 9 has the highest number of 15 local lines with a genetic distance of 44. Local lines in this sub-cluster includes Dboh 10, Dbok 10, Dbok 9, Dbak 8, Dbor 8, Dbak 6, Dboh 7, Dboh 6, Dbor 1, Dbak 2, Dbak 1, Dboh 5, Dboh 3 and Dboh 2 respectively. The sub-cluster with the least number of local lines is sub-cluster 10 with Dbak 4 line and has a genetic distance of 60 similar with the clade.

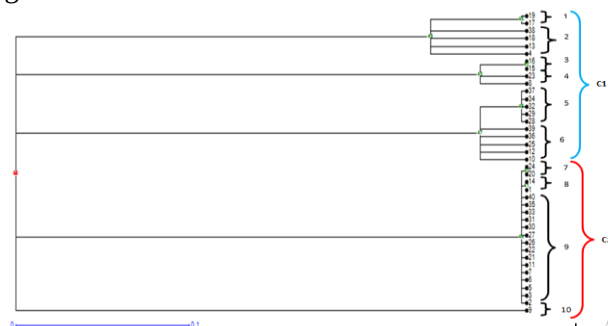


Figure-5: Dendrogram showing genetic distance of 2 major clusters and 10 sub-clusters from 40 accessions of Air potato yam (*Dioscorea bulbifera*) evaluated for salt tolerance using microsatellite markers

The network analysis of the 40 local lines of *Dioscorea bulbifera* clearly delineates the lines into ten groups with specific genetic distances and relatedness within and among all lines (Fig. 6).

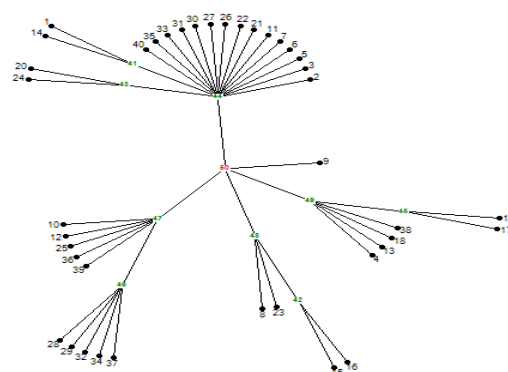


Figure-6: Network analysis of Air potato yam (*Dioscorea bulbifera*) accessions showing genetic similarity and 10 populations in 40 lines.

Table-6: Eigen value and percentage variations of five principal coordinates obtain from 40 Accessions of Air potato yam (*Dioscorea bulbifera*) evaluated for salt tolerance

Axis	PC1	PC2	PC3	PC 4	PC5
Eigen value	0.06581	0.03448	0.02603	0.01583	0.01296
Proportion (%)	48.63	25.00	11.32	6.89	5.64
Cum. Prop. (%)	48.63	63.63	74.95	81.84	97.48
	P- Axis 1	P- Axis 2	P Axis 3	P- Axis 4	P- Axis 5
	Coord.	Coord.	Coord.	Coord.	Coord.
Dboh 1	0.2607	-0.1864	0.2639	0.0190	-0.0926
Dboh 2	-0.0078	-0.1047	-0.0348	0.0282	-0.0560
Dboh 3	-0.0525	-0.0952	0.1077	-0.1885	0.0066
Dboh 4	-0.1187	-0.4393	-0.2851	0.0781	-0.2369
Dboh 5	0.1945	-0.1661	0.0272	-0.0896	-0.0406
Dbak 1	0.1515	-0.1529	-0.1077	0.1031	-0.0455
Dbak 2	0.0257	-0.0483	0.0466	-0.0764	-0.0980
Dbak 3	0.4479	0.1062	0.0743	-0.0041	-0.2117
Dbak 4	-0.5398	0.4832	0.0926	0.0470	-0.2465
Dbak 5	0.2042	0.0684	-0.2811	0.1280	0.1564
Dbor 1	0.1345	-0.2197	-0.0617	0.0474	0.0100
Dbor 2	-0.0725	-0.0511	0.1124	0.1056	-0.0887
Dbor 3	-0.4303	0.1050	-0.4564	0.1293	0.0015
Dbor 4	0.2607	-0.1864	0.2639	0.0190	-0.0926
Dbor 5	0.4225	0.2831	0.1376	0.0982	0.1016
Dbok 1	0.4225	0.2831	0.1376	0.0982	0.1016
Dbok 2	-0.4540	-0.2300	0.1355	0.0914	0.0568
Dbok 3	-0.5774	-0.0101	0.3470	0.0137	-0.0916
Dbok 4	-0.4496	-0.2349	0.1587	0.0214	0.1713
Dbok 5	0.2968	-0.0378	-0.0953	-0.1850	-0.0232
Dboh 6	0.0847	0.0730	-0.0361	-0.1420	-0.0899
Dboh 7	-0.0774	-0.1400	0.0166	-0.0731	0.0421
Dbok 6	0.0625	0.4317	0.1565	0.0714	-0.0100
Dbok 7	0.2968	-0.0378	-0.0953	-0.1850	-0.0232
Dbor 6	0.0318	-0.1518	0.1401	-0.1847	0.2445
Dbor 7	0.0387	-0.0081	-0.1165	-0.0086	-0.0465
Dbak 6	0.2429	-0.0244	-0.2597	0.0478	-0.0438
Dbak 7	-0.1332	-0.0035	-0.0185	0.0630	0.0947
Dbok 8	-0.0547	0.0594	0.0925	0.3092	0.0072
Dbor 8	0.1010	-0.1752	-0.0523	0.1016	0.1594
Dbak 8	0.1016	0.1843	-0.1109	-0.1077	-0.1887
Dboh 8	-0.0337	-0.0290	0.1339	0.1924	0.0580
Dbak 9	0.0417	0.1763	-0.0109	-0.1165	0.0837
Dbok 9	-0.0141	0.1794	-0.1829	0.1007	0.0497
Dbok 10	-0.0421	-0.0761	0.0958	-0.1523	-0.0489
Dbor 9	-0.0225	0.0427	-0.0939	-0.0174	0.1867
Dbor 10	-0.0620	0.0264	-0.0668	0.1343	0.0284
Dbak 10	-0.5069	0.1858	-0.1741	-0.3084	0.0201
Dboh 9	-0.0846	0.2072	0.0159	-0.1831	0.1803
Dboh 10	-0.0899	-0.0865	-0.0165	-0.0254	0.0145

Table-7: Chlorophyll a and b concentrations in leaves extracts of *Dioscorea bulbifera* treated with different concentration of Sodium chloride

Different concentration of Sodium chloride													
Parameter	0.00mM	Dboh 6 100mM	200mM	300mM	0.00mM	Dboh 7 100mM	200mM	300mM	0.00mM	Dboh 5 100mM	200mM	300mM	
Chlo.-a (mg g ⁻¹)FW	139.90 ^a	139.70 ^a	136.20 ^b	134.60 ^c	144.30 ^a	144.00 ^a	139.60 ^b	137.40 ^c	143.40 ^a	143.70 ^a	138.20 ^b	137.80 ^c	
Chlo.-b (mg g ⁻¹)FW	192.10 ^a	191.30 ^a	140.80 ^b	34.70 ^c	189.80 ^a	189.20 ^a	140.70 ^b	34.10 ^c	196.00 ^a	195.40 ^a	143.60 ^b	32.90 ^c	
		Dbak 1				Dbak 2				Dbak 3			
Chlo.-a (mg g ⁻¹)FW	144.20 ^a	144.00 ^a	139.40 ^b	138.00 ^c	146.20 ^a	146.00 ^a	142.60 ^b	139.70 ^c	146.80 ^a	146.4 ^a	143.3 ^b	139.2 ^c	
Chlo.-b (mg g ⁻¹)FW	187.70 ^a	187.10 ^a	140.20 ^b	31.20 ^c	204.40 ^a	203.60 ^a	166.70 ^b	73.40 ^c	206.90 ^a	206.70 ^a	168.20 ^b	75.20 ^c	
		Dbak 4				Dboh 1				Dbok 3			
Chlo.-a (mg g ⁻¹)FW	146.50 ^a	146.20 ^a	142.70 ^b	139.90 ^c	147.10 ^a	146.40 ^b	144.30 ^c	140.80 ^d	152.10 ^a	152.30 ^a	147.90 ^b	143.90 ^c	
Chlo.-b (mg g ⁻¹)FW	207.60 ^a	207.60 ^a	166.90 ^b	74.60 ^c	206.20 ^a	206.40 ^a	168.40 ^b	74.90 ^c	213.70 ^a	212.90 ^a	179.60 ^b	79.80 ^c	
Parameter	0.00mM	Dboh 2 100mM	200mM	300mM	0.00mM	Dboh 4 100mM	200mM	300mM	0.00mM	Dboh 2 100mM	200mM	300mM	
Chlo.-a (mg g ⁻¹)FW	151.90 ^a	151.50 ^a	147.90 ^b	143.40 ^c	152.40 ^a	152.50 ^a	148.50 ^b	144.30 ^c	152.70 ^a	152.80 ^a	148.30 ^b	144.20 ^c	
Chlo.-b (mg g ⁻¹)FW	212.90 ^b	219.90 ^a	182.30 ^c	82.20 ^d	217.30 ^a	216.10 ^a	179.90 ^b	87.10 ^c	214.20 ^a	214.00 ^a	176.80 ^b	85.70 ^c	
		Dboh 2				Dboh 3				Dboh 4			
Chlo.-a (mg g ⁻¹)FW	155.80 ^a	155.70 ^a	152.90 ^b	148.00 ^c	157.10 ^a	157.30 ^a	154.10 ^b	149.40 ^c	154.90 ^a	154.40 ^b	152.80 ^c	147.80 ^d	
Chlo.-b (mg g ⁻¹)FW	219.20 ^a	218.80 ^a	211.00 ^b	99.80 ^c	214.80 ^a	213.90 ^a	209.80 ^b	99.20 ^c	212.40 ^a	212.40 ^a	210.10 ^b	98.60 ^c	
		Dboh 5				Dbok 1				Dbok 2			
Chlo.-a (mg g ⁻¹)FW	155.20 ^a	155.60 ^a	153.40 ^b	149.00 ^c	204.10 ^a	204.40 ^a	199.60 ^b	192.40 ^c	204.20 ^a	204.00 ^a	199.00 ^b	193.70 ^c	
Chlo.-b (mg g ⁻¹)FW	216.20 ^a	215.80 ^a	210.70 ^b	99.20 ^c	230.90 ^a	230.20 ^a	223.30 ^b	102.20 ^c	232.40 ^a	231.90 ^a	223.80 ^b	103.70 ^c	
Parameter	0.00mM	Dhor 1 100mM	200mM	300mM	0.00mM	Dboh 3 100mM	200mM	300mM	0.00mM	Dboh 3 100mM	200mM	300mM	LSD
Chlo.-a (mg g ⁻¹)FW	204.40a	204.10a	201.1b	194.50c	204.20a	204.10a	199.40b	194.00c	0.51				
Chlo.-b (mg g ⁻¹)FW	232.80 ^a	232.70 ^a	225.80b	107.60c	232.50a	232.20a	224.40b	104.90c	5.40				

Values are mean of chlorophyll a and b determinations.

Means with the same superscripts on the same row are not significantly different (p<0.05).

Twenty (20) lines that showed tolerance for common salt at 400, 300 and 200 bps were selected and evaluated for Chlorophyll a and b profiles or contents. The results as presented in Table 8 and figure 7 revealed that all the evaluated lines had more Chlorophyll b contents compared to Chlorophyll a. The results also showed that the concentrations and profiles of both Chlorophyll a and b decreases with increasing concentration of common salt in soil solution. The local line Dboh 1 obtained from Oron, in Akwa Ibom state had the highest Chlorophyll a and b profiles at all the given concentrations and control while Dboh 6 showed the least profiles of Chlorophyll a and b contents in all the given concentrations and control.

Results of enzymatic activities for ten local lines with high salt tolerance potentials are presented in Table 8. The result shows that the activities of all evaluated enzymes in the local lines increased with increase in the concentrations of salts in the soil solution. The concentration of cellulase was highest in all the local lines evaluated while peroxidase was the least active enzyme in terms of concentrations. The results revealed that increase in the concentrations of enzymes was proportional to increasing stress

initiated by increasing salt concentrations. Enzymes activity showed increasing activity with increasing concentration of the salt in solution. All the enzymes G6DP, peroxidase, Xylanase, cellulase and lignolytic enzymes all showed increased activities with increasing salt concentrations.

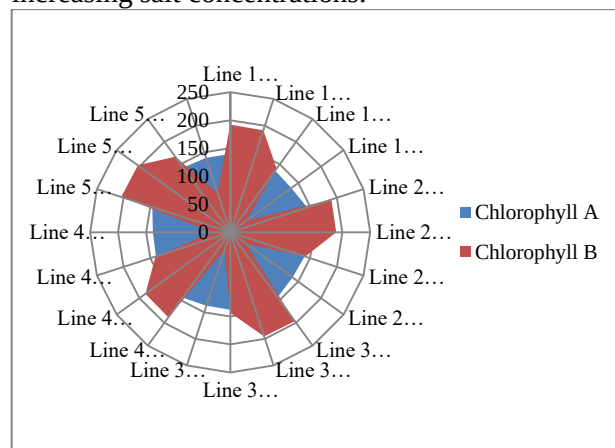


Figure-7: Radar plots showing Chlorophyll a and b in five lines of Air potato yam with high salt tolerance potentials

Line 1= Dboh 2; Line 2= Dbok 3; Line 3= Dboh 2; Line 4= Dbak 2 and Line 5= Dboh 1

Table-8: Enzyme bioactivities rate in obtained from Ten salt tolerant species of *Dioscorea bulbifera* treated with different concentrations of sodium chloride

	Control	100nM - Dbok 4	200nM	300nM
G6PD Enzyme	0.01	0.02	0.04	0.06
Peroxidase Enzyme	0.01	0.02	0.03	0.04
Xylanase Enzyme	0.05	0.1	0.14	0.22
Cellulase Enzyme	1.02	1.43	1.57	1.68
Lignolytic Enzyme	0.41	0.52	0.61	0.74
		Dbok 5		
G6PD Enzyme	0.01	0.03	0.05	0.06
Peroxidase Enzyme	0.01	0.02	0.03	0.03
Xylanase Enzyme	0.05	0.07	0.09	0.12
Cellulase Enzyme	1.1	1.13	1.15	1.16
Lignolytic Enzyme	0.52	0.54	0.55	0.56
		Dbok 2		
G6PD Enzyme	0.01	0.02	0.04	0.06
Peroxidase Enzyme	0.01	0.02	0.03	0.04
Xylanase Enzyme	0.06	0.08	0.12	0.15
Cellulase Enzyme	0.92	0.95	0.97	1.01
Lignolytic Enzyme	0.52	0.61	0.69	0.77
		Dbok 3		
G6PD Enzyme	1.51	1.53	1.54	1.56
Peroxidase Enzyme	0.01	0.02	0.03	0.03
Xylanase Enzyme	0.08	0.12	0.15	0.18
Cellulase Enzyme	1.04	1.07	1.18	1.2
Lignolytic Enzyme	0.26	0.33	0.45	0.52
		Dbak 3		
G6PD Enzyme	1.5	1.52	1.54	1.55
Peroxidase Enzyme	0.01	0.01	0.02	0.03
Xylanase Enzyme	0.1	0.14	0.18	0.22
Cellulase Enzyme	1.14	1.22	1.4	1.42
Lignolytic Enzyme	0.61	0.72	0.8	0.96
		Dbok 2		
G6PD Enzyme	0.01	0.03	0.04	0.06
Peroxidase Enzyme	0.01	0.04	0.04	0.05
Xylanase Enzyme	0.11	0.15	0.21	0.24
Cellulase Enzyme	0.96	1.31	1.57	1.66
Lignolytic Enzyme	0.72	0.74	0.75	0.75
		Dbak 2		
G6PD Enzyme	0.01	0.02	0.03	0.05
Peroxidase Enzyme	0.01	0.02	0.02	0.05
Xylanase Enzyme	0.05	0.06	0.08	0.15
Cellulase Enzyme	1.09	1.21	1.32	1.35
Lignolytic Enzyme	0.52	0.58	0.59	0.61
		Dbok 3		
G6PD Enzyme	0.01	0.03	0.04	0.05
Peroxidase Enzyme	0.02	0.02	0.05	0.05
Xylanase Enzyme	0.1	0.13	0.14	0.16
Cellulase Enzyme	1.02	1.13	1.15	1.16
Lignolytic Enzyme	0.42	0.47	0.5	0.51
		Dbok 6		
G6PD Enzyme	0.01	0.03	0.06	0.08
Peroxidase Enzyme	0.02	0.02	0.03	0.03
Xylanase Enzyme	0.06	0.07	0.08	0.11
Cellulase Enzyme	1.08	1.14	1.17	1.19
Lignolytic Enzyme	0.44	0.51	0.53	0.54
		Dbok 1		
G6PD Enzyme	0.01	0.02	0.04	0.04
Peroxidase Enzyme	0.01	0.03	0.03	0.03
Xylanase Enzyme	0.04	0.05	0.08	0.9
Cellulase Enzyme	1.2	1.41	1.5	1.5
Lignolytic Enzyme	0.51	0.53	0.55	0.55

Discussion

Sodium chloride was observed to cause an increase in cellulose, xylanase, lignolytic, glucose-6 phosphate dehydrogenase and peroxidase activities in *D. bulbifera*. An increase in these enzyme activities suggests the formation of excess hydrogen peroxide by the salt and consequent oxidative stress. Therefore, as a defense response to oxidative stress, the peroxidase and glucose-6 phosphate dehydrogenase activities were increased. This agrees with Terauchi et al., 1992, who reported an increase in anti-oxidative enzymes under salt stress. This suggests that enzymatic activities in *Dioscorea bulbifera* increase with increased salt stress or concentrations. The cellulase enzyme activity was found to be the most affected due to its involvement in the plant photosynthetic activity especially during the day. Despite the reduction in growth and yield of crop plants by salinity, some species of plants such as rice and maize are reported to have the capacity of tolerating salinity of various concentrations. These abilities to tolerate salinity is made possible through an increase in the activities of enzymes associated with salt stress (Terauchi et al., 1992). The increased enzyme activity acts as a defensive mechanism against cell damage and contributes to the tolerance response of plants (Mignouna et al., 2003).

The profile of Chlorophyll a and b was found to decrease with an increase in salt concentrations in soil solutions. Not many variations in chlorophyll contents were obtained relative to the control experiment with zero concentration of salt. The present study also revealed higher concentrations of Chlorophyll b compared to Chlorophyll a. It also revealed a decreasing total chlorophyll content with increasing concentrations of common salt in solution. This finding is in line with the reports of Kalu et al., 2021 who posited that chlorophyll contents increase with decreasing abiotic stress (Ashraf et al., 2014; Asiedu et al., 1998).

Sodium chloride reduced the phenomic parameters in *Dioscorea bulbifera* as enlisted in Table 2. Specifically, it decreases the vine length, number of leaves and number of bulbils of the *D. bulbifera*. The decrease increases with increasing concentration of sodium chloride and was more significant at 300mM concentration of the sodium chloride. At 100mM, the height and number of leaves of *D. bulbifera* of some of the lines was at higher value, although not significantly different from that of the controls.

From this, it can be deduced that a higher concentration of sodium chloride (>100mM) caused a reduction in the growth of *D. bulbifera* and lower concentration of sodium chloride ($\leq$ 100mM) tend to stimulate the growth of *D. bulbifera*. The reduction in the growth of *Dioscorea bulbifera* by sodium chloride agrees with authors who reported a reduction in growth and yield of plants exposed to salt stress (Ben and Yene, 2013; Karp et al., 1997. Malapa et al. (2005) reported a reduction in the growth of *Euryae marginata* by salt stress as evidenced by reduction in fresh weight, leaf water content and Chlorophyll a and b content.

Gónmez-Bellot et al. (2013) reported a decrease in total root length of *E. japonica* treated with salt. Jouyban (2012) also reported the same decrease in *Echium amoenum*. Sodium chloride was observed to cause a loss in fresh root weight of maize, an effect that increases with a higher level of salinity (Jouyban, 2012). Jampeetong and Brix (2009) reported decrease in leaf area and root length with salinity in *Salvinia natans*. Salinity also causes a reduction in chlorophyll concentrations of plants.

The leaf yellowing was more conspicuous at a 300mM concentration of sodium chloride. This was an indication of a deficiency of some essential nutrients (sulphur, iron, magnesium and nitrogen) in the plants. Saline soils of higher concentration (above 200mM) could lead to interference in the absorption of nutrients by the root hairs of the plants. Bulbils with spindle or elongated shapes could be said to be less tolerant to the salt treatment compared to the round-shaped lines which were more tolerant to salt treatments.

The results of principal component analysis of molecular attributes studied revealed five principal components, which explained 97.48% while analysis of molecular variance attributes explained 100 % of variations in the population to molecular attributes with most of the loading score coming from Dbor , Dbok and Dbak. Cluster patterns based on squared Euclidean distance, 0.05, using Ward's method revealed two major clusters each with 6 and 4 sub clusters respectively with varying genetic distances indicating a close genetic relationship and relatedness among the local lines. The results also showed that all the local lines developed from a common ancestor. The results are in consonance with the positions of Brisibe et al., 2021 who reported similar results with Musa species AAB genome.

Assessing the genetic diversity of *D. bulbifera*



germplasm using DNA based markers like SSR is important for efficient conservation and utilization. The results demonstrated genetic polymorphism in *D. bulbifera* germplasm from all local sources in the study area.

Variations observed in the cluster groups suggest that the mutation rate is high and many of the mutations are not neutral showing and having an effect on phenotype. This is expressed in morphological variation among the cultivars presenting different colours and shapes for both aerial and underground organs (Obidiegwu et al., 2009).

Conclusion

Thirty (30) local lines of *Disocorea bulbifera* showed fingerprinted at 100, 200, 300 and 400 bps respectively. Ten of the local lines did not show any affinity to the salt-tolerant marker of gene and this was reflected in their low Chlorophyll a and b profiles and reduced enzyme activities. The forty (40) lines of *D. bulbifera* were also assessed for genetic diversity using ten microsatellite markers or loci. A total of 74 alleles were detected with an average allele number of 7.4 per locus. Polymorphic Information Content (PIC) mean value of 0.74 showed the existence of variability among the accessions. Cluster and principal component analysis showed 2 major and 10 sub-cluster groups. There was 97.48 % relationship between the relatedness of the lines and their local area of collection. Ultimately, microsatellite fingerprinting markers proved to be effective in identifying salt-tolerant lines of the studied *D. bulbifera* germplasm. The study could help in improving the available local lines of *D. bulbifera* for salt tolerance, breeding programs and cultivar development. The study had revealed the size of the possible genes which can be harnessed and developed as a salt-tolerant marker for *Dioscorea* species in this locality and beyond. The study revealed that *D. bulbifera* lines secrete more enzymes with increasing concentration of salts. Cellulose enzymes being the major enzyme involved in photosynthesis was at the highest concentration and activity. Chlorophyll b showed the highest-profile compared to Chlorophyll b across all the salt-tolerant lines.

A combination of biochemical, physiological and molecular approaches in this study could prove a viable tool in the determination of genetic diversity and relationships among *D. bulbifera* accessions

which is critical to the genetic improvement of the crop.

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