The Journal of Agricultural Sciences - Sri Lanka
Vol. 19, No 2, May 2024. Pp 316-335
https://doi.org/10.4038/jas.v19i2.10091

Genetic Diversity of Tiger Nuts (*Cyperus esculentus* L.) Grown in Ghana Based on Morpho-Descriptors and SSR Markers

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Received: 29th December 2022 / Accepted: 14th March 2024

ABSTRACT

Purpose: *Limited information is available on the diversity of tiger nuts in Ghana for improvement. Therefore, evaluating the genetic diversity via the use of morphological descriptors and molecular markers (SSR) should generate substantial evidence to reveal the variability among and within the crop.*

Research Method: *Forty-two (42) accessions of Cyperus esculentus Lativum were collected from major tiger nut cultivation areas (phytogeographic zones) in Ghana and evaluated using 11 morpho-descriptors and 9 SSR markers to assess variability among and within the tiger nut accessions and underlined causes.*

Findings: *Significant differences were observed among the accessions in terms of percentage inflorescence ($p \leq 0.01$), distance of the last tiller from the main plant ($p \leq 0.05$) and the number of tubers per stand ($p \leq 0.05$). Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster analysis grouped the accessions into seven clusters including one large heterogenous cluster at 0.94 Euclidean distance, and genetic distance ranged from 0.8 to 1.00. The SSR profiling revealed 141 alleles across 41 of the accessions used for the molecular study with a mean PIC value of 0.78 ± 0.15 and mean observed heterozygosity of 0.12 ± 0.16 coupled with four heterotic UPGMA cluster groupings including one large heterogenous cluster at a similarity coefficient of 0.88. The high variability among the accessions for some of the traits, and the low genetic variability within the accessions in cluster groupings for both morphological and SSR evaluations suggested some level of admixture of genes over years of cultivation.*

Originality/ Value: *The current study has identified some accessions for improvement. Also, it has contributed to knowing some SSR markers that would amplify tiger nuts for future molecular study for breeding enhancement.*

Keywords: *Accession, Cyperus esculentus, Genetic diversity, Morpho-descriptors, PIC, SSR markers*

INTRODUCTION

Tiger nut (*Cyperus esculentus* L.) is a perennial weed in the sedge family (*Cyperaceae*). The crop is known to many as chufa, nutgrass, earth almond, water grass, rush nut, yellow nutsedge and northern nutgrass (Shilenko *et al.*, 1979). The origin of *Cyperus esculentus* is generally uncertain.

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While some believe that tiger nut is a native to the Old World (mainly Africa and Asia) (DAISIE, 2014), others are of the view that it originated from tropical and subtropical regions of the World, including Africa, Asia, Europe and North America (Govaerts, 2014; USDA-ARS, 2014). After this, it spread around the globe (Holm *et al.*, 1977).

The plant is tough, erect, growing from a perennial tuber-bearing rhizome, and has fibrous roots. It grows to a height of 30-90 cm tall, producing seeds with many slender rhizomes forming runners above the ground. The tips of the underground stems produce tubers that are of economic importance and appear in three different colors; black, brown, and yellow.

The tuber (nut) is a source of feed, food, medicine and perfumes (De Vries, 1991; Gambo and Da'u, 2014). It can be eaten raw, roasted, baked, or made into a refreshing beverage called *Horchata De Chuf* (in Spain), '*kunnu aya*' (in northern Nigeria), and '*atadwe milk*' (in Ghana). In Ghana and some African countries, such as Nigeria and Sierra Leone, tiger nut is available in the market in fresh, semi-dried and dried forms for sale, the form in which it is consumed uncooked. The tuber is valued highly for its functional nutrient components, such as 26–30% starch and 21–25% fat, providing about 400–450 kcal energy 100 g^{-1} (Sánchez-Zapata *et al.*, 2012). It is also rich in dietary fibre, protein (7–8%) (Mokady and Dolev, 1970; Oderinde and Tairu, 1988), fibre (8–10%) (Umerie and Enebeli, 1996), vitamins (C and E), and minerals (sodium, calcium, potassium, magnesium, zinc and traces of copper) (Sanful, 2009). The tiger nut is an aphrodisiac, has carminative and diuretic effects, and is used as a stimulant and tonic to moderate the incidence of colon cancer, coronary heart diseases, obesity, diabetes, excessive thirst, and gastrointestinal disorders (Adebayo-Oyetoro *et al.*, 2017; Chukwuma *et al.*, 2010; Sánchez-Zapata *et al.*, 2012) as well as constipation, high blood pressure and diarrhea (Oladele and Aina, 2007).

Though tiger nut is an underutilized and largely unexploited crop, it has high export potential (Tetteh and Ofori, 1998) and if adequately resourced, would provide Ghana with foreign exchange. Ghana exports tiger nuts to many countries, such as the Netherlands, Germany, the United Arab Emirates, Austria, Canada, the United Kingdom, the United States, and France among others. In 2017, Ghana's export of tiger nuts was 63, 300 mt worth US$ 535,390. A drop however occurred in 2019 with an export of 61, 980 mt worth US$ 434, 310 (Tridge, 2022). Currently, the record available for the year 2021, showed an export of 39,560 mt worth US$ 93,960 (Tridge, 2022). Tiger nut farming in Ghana is localized in very few areas of the Northern, Bono East, Bono Ahafo, Eastern, Central, and Volta regions. Its cultivation provides about 85% of jobs for the youth and women in these cultivation areas (Tetteh and Ofori, 1998).

Despite the nutritional, medical, and economic benefits of tiger nut, the crop remains an orphan (Donkor *et al.*, 2019), probably, due to its invasiveness and obnoxious weed character coupled with minor scientific and technological studies to promote its enormous potential for use.

Unlike other crops such as maize and cowpea, tiger nut cultivation has received little agronomic and breeding attention for improvement. Very few studies have been conducted on its production globally. Research available had been focused on its morphological studies and nutritional value with scanty information on its molecular survey. Limited studies in Ghana revealed low genetic diversity among the 64 genotypes (Asare *et al.*, 2020). Yield and phenotypic traits show that cultivars in Ghana are unimproved. The use of these locally unimproved cultivars over the years accumulates pests and diseases and, consequently low yields. There is therefore the need to study the diversity existing in the accessions available for parental selection for improvements in Ghana. Hence the main objective was to evaluate the genetic diversity among and within the forty- two (42) accessions based on 11 morphological traits and 9 SSR markers.

MATERIALS AND METHODS

Collection of Germplasm

Accessions were collected from major growing areas in Ghana. A total of 42 accessions made up of 24 browns, 12 blacks and 6 yellows (Fig.02) were collected from farmers in the Northern, Upper East, Upper West, Bono East, Eastern, and Central regions of Ghana (Fig.01).

Accessions collected had no passport data, hence, they were named using the first three letters of the name of the town or community where the collection was done and the color of the tuber. Accessions of the same color collected from the same community/ town were differentiated with numbers in addition. For example, YEN-b1, YEN- b2 and YEN- b3 for Yendi brown 1, Yendi brown 2 and Yendi brown 3 respectively (Table 01). The tubers were bagged and labeled in zip-locked polythene bags and transported to the University of Cape Coast Teaching and Research Farm.

Figure 1: Map of Ghana showing geographical locations where tiger nuts (*Cyperus esculentus* L.) accessions were collected

Experimental Site and Culture

The experiment was conducted at the Teaching and Research Farm of the University of Cape Coast from May to September 2021 during the major growing season. The experimental site lies between $5°6'$ N and $1°15'$ W with a bimodal rainfall and an amount of rainfall of 773.7 mm per annum. The major rainy season is usually experienced from April to June and the minor season occurs from September to October with dry spells (harmattan) commencing from November to February. The site usually records a temperature of 25.8°C and an average humidity of 86.3%. The land, which previously was cropped with eggplant, before the cultivation was ploughed and harrowed to a depth of about 30 cm. The soil was sandy loam with a pH of 6.1. The total land area was 85 m^2 (thus; 5 m x 17 m). There were 126 plots in all with each plot measuring 0.6 m x 1 m in a Randomized Complete Block Design with 3 replications. Each replication had 42 plots with each plot having 10 tubers planted per genotype at a spacing of 15 cm x 20 cm. Genotypes were hydro-primed (soaked in water) for 6 hours in disposable Styrofoam cups before planting. This was done to remove unviable tubers (tubers that floated) and also to enhance sprouting. Planting was done after priming and harvesting was done 98 days after planting (DAP). However, some genotypes showed early signs of maturity at 85/86 DAP. Weeding and irrigation were regularly done when needed.

Germplasm for Molecular Profiling

A total of 42 accessions were pot- -planted in a screen house at the Biotechnology Laboratory at the CSIR-Crops Research Institute, Fumesua in the Ashanti Region of Ghana. The accessions were planted using the tubers harvested from the field at the Teaching and Research Farm of the University of Cape Coast. Forty-

Table 1: Accession, place of collection and color of the tuber of tiger nuts (*Cyperus esculentus* L.)

S/N	Accession	Color	Place/ Area of collection	Region
1	ADU- b	Brown	Aduamoa	Eastern
2	ADU- B	Black	Aduamoa	Eastern
3	ADU- Y	Yellow	Aduamoa	Eastern
4	APR- b	Brown	Assin Praso	Central
5	ASU- b	Brown	Asukese No.2	Eastern
6	BAJ- b	Brown	Bawjiase	Central
7	BAJ- B	Black	Bawjiase	Central
8	BAJ- Y	Yellow	Bawjiase	Central
9	BAW- b	Brown	Bawku	Upper East
10	BEP- B	Black	Beposo	Central
11	BEP- Y	Yellow	Beposo	Central
12	BUO- b	Brown	Buoyam	Bono East
13	BUO- B	Black	Buoyam	Bono East
14	DED- b	Brown	Dedeso	Eastern
15	DEM- b	Brown	Demso	Eastern
16	ENK- b	Brown	Enkroful	Central
17	ENK- B	Black	Enkroful	Central
18	GYI- b	Brown	Gyilli	Upper East
19	KAL- b	Brown	Kaleo	Upper West
20	KAL- B	Black	Kaleo	Upper West
21	MBA- b	Brown	Mbaabaasa	Central
22	MBA- B	Black	Mbaabaasa	Central
23	NEB- b	Brown	New Ebu	Central
24	OBR- b	Brown	Obratwawu	Central
25	OBR- B	Black	Obratwawu	Central
26	OBR- Y	Yellow	Obratwawu	Central
27	OEB- b	Brown	Old Ebu	Central
28	OEB- B	Black	Old Ebu	Central
29	OFF- b	Brown	Offinso	Eastern
30	PUT- b	Brown	Putobio	Central
31	PUT- B	Black	Putobio	Central
32	SAN- b	Brown	Sankana	Upper West
33	TAK- b	Brown	Takpo	Upper West
34	TAK- B	Black	Takpo	Upper West
35	TWI- b	Brown	Twifo Praso	Central
36	TWI- Y	Yellow	Twifo Praso	Central
37	WIO- b	Brown	Wiomua	Central
38	WIO- B	Black	Wiomua	Central
39	YEN- b1	Brown	Yendi	Northern
40	YEN- b2	Brown	Yendi	Northern
41	YEN- b3	Brown	Yendi	Northern
42	ZEB- Y	Yellow	Zebila	Upper West

one (41) accessions (Table 02.) were successful for DNA extraction as accession ADU-b did not germinate.

DNA extraction: Young leaves of a two-week-old plant were harvested for genomic DNA extraction. The DNA was isolated using a CTAB extraction buffer. The quality of DNA was tested using agarose gel electrophoresis and the relative DNA quantity was measured following the method described by Sambrook *et al.* (1989).

SSR markers and PCR amplification: Table 03. shows the sequences of 20 SSR primers (Arias *et al.*, 2011) and expected product sizes used for the *Cyperus rotundus* marker transferability studies. Polymerase chain reactions (PCR) were carried out in a Techne

Table 2: Tiger nuts (*Cyperus esculentus* L.) accessions were used for the molecular study

S/N	Genotype	S/N	Genotype	S/N	Genotype	S/N	Genotype
1	APR-b	12	OBR-Y	23	DED-b	34	OEB-b
2	BUO-b	13	NEB-b	24	SAN-b	35	TWI-b
3	ZEB-Y	14	OBR-b	25	OFF-b	36	ENK-B
4	ASU-b	15	BAJ-Y	26	TWI-Y	37	WIO-B
5	MBA-b	16	BEP-Y	27	YEN-b1	38	TAK-B
6	GYI-b	17	PUT-B	28	ENK-b	39	MBA-B
7	TAK-b	18	WIO-b	29	DEM-b	40	BAJ-B
8	YEN-b3	19	ADU-Y	30	OEB-B	41	ADU-B
9	BAW-b	20	PUT-b	31	BUO-B		
10	YEN-b2	21	BEP-B	32	KAL-B		
11	BAJ-b	22	KAL-b	33	OBR-B		

Thermal cycler (TC- 412) in a 10L reaction mixture in 96-well plates. A PCR master mix kit was used for the amplification. Genomic DNA (1 µL) and 0.5l each of forward and reverse primers were added to the PCR kits for DNA amplification. PCR was subjected to initial denaturation at 95°C for 3 min, followed by cycles of 95°C for 10 sec, 52°C for 10 sec and 72°C for 10 sec. The reaction was repeated for 35 cycles and a final extension at 72°C for 10 minutes. The reactions were then held at 4°C until electrophoresis. A volume (2 µL) of Bromophenol blue dye (6X) was added to the products generated after the PCR. Amplified products were separated on 6% polyacrylamide gel in boric acid EDTA (TBE) buffer, stained with ethidium bromide and captured using Alphaimager HP (protein simple, USA).

Data Collection and Analysis

Agro-morphological data: In all, data on eleven (11) morphological traits (1 qualitative and 10 quantitative traits) were collected. Percentage germination (PG), number of tillers per stand (NT), distance from the last tiller to the mother plant (DTP), plant height (PH), percentage inflorescence (PI), number of tubers per stand (NTS), number of rings per tuber (NRT), length per tuber (LT), width per tuber (WP), tuber shape (TS) and tuber size in terms of 100 tuber weight per genotype (WT) were among data collected. Data were taken from 5 randomly selected plants of each genotype per plot. The number of tillers per stand (NT), distance from the last tiller to the mother plant (DTP), plant height (PH), and percentage inflorescence (PI) was taken at the time of flowering (inflorescence). The number of tubers per stand (NTS) was determined at harvest while measurements of NRT, LT, WP, TS and WT were carried out 4 days after harvest. Tuber shapes were calculated based on the ratio of length to width (L/W) and were considered oval (<1.3), ovoid (1.3-1.8) and oblong (>1.8) (Pascual *et al.*, 2000). Tuber weight was taken by weighing 100 tubers selected randomly per genotype using an electronic balance.

Univariate analysis for analysis of variation (ANOVA) and descriptive statistics were performed using Genstat version 11.1 and Minitab version 21.1.1.0 statistical packages. Multivariate analysis for clustering and principal component analysis were done using Genstat, Minitab and PAST version 4.03 statistical packages.

Molecular data: Bands were scored as '1' for present and '0' for absent based on the expected band sizes (Fig.03). A cluster analysis was performed on the scored binary data using the Simple Matching Coefficient method, with arithmetic average (UPGMA) in NTSYS Ver. 2.2.0. Genetic diversity parameters such as major allele frequency (Na), allele number (Ne), gene diversity or expected heterozygosity (He), observed heterozygosity (Ho) and polymorphic information content (PIC) were determined with the Nei (1983) method using Power Marker Software Ver. 3.25.

RESULTS AND DISCUSSION

Variability in Agro-morphological Traits for the Genotypes Evaluated

Among the qualitative traits studied, twenty- five (25) accessions among the 42 studied were oval. The majority of these were accessions collected from the Central Region of Ghana and possessed all three tuber colors (black, brown and yellow). Ten were ovoid where the majority were brown and were also Central Region accessions. The remaining 7 accessions had oblong and brown tubers with the majority (99%) made up of Eastern Region collection (Table 01).

Table 3: SSR primers, sequences and expected product size were used for the study

Primer Name	Repeat Motifs	Primer Sequence	Expected band
StvCyR_1a	(AG)	GCATTTCGTCACCTTCCATTAAAC (F)	193
StvCyR_27b	(TGG)	TTACTTTGTTTGCAGTTGCAGAGG (R)	95–444
		GAGTGAGGGAGTGAGAGAGGGAC (F)	
		TATAGCAAAGTCAGCAGCGCAC (R)	
StvCyR_64a	(GA)	GTACAAACCACAAACCGTAGACCC (F)	114–138
		ACTCTCCTCCTCCATCGTAAGCTC (R)	
StvCyR_126a	(GA)	GGCCTCCGTAAGAAGAAGAATGAC(F)	165-166
		AAGTTCAAGGCAGACGTTAAGCAC (R)	
StvCyR_142a	(GA)	CACGGTAAATTAAACATCACACGG (F)	112
		TTGATCTAACACTTGTACTGCGCC (R)	
StvCyR_181a	(GATA)	TCAATAGAAGAATCCCACTCAGCC (F)	135-157
		GTGGAGGTAAAGATCAGCAACCAG (R)	
StvCyR_156a	(AT)	TGCCCAGCTTTACATCTTAATTGC (F)	182-191
		CTGAACAACTGGCACATACAGAGC (R)	
StvCyR_254a	(TA)	AATCATGAAGGTGATTGGACAAGG (F)	104-149
		CATCCATCCTCTTCTTTGTTCTCG (R)	
StvCyR_483a	(GA)	TGTGTTGTGTGGAAGAGAGGAGAG (F)	117-455
		AACAAACCTCAGAACTCAACTGCC (R)	

Source: Arias *et al.* (2011)

Figure 2: Color of tiger nut tubers: A - black-coloured tiger nut, B- brown-coloured tiger nut, C- yellow-coloured tiger nut

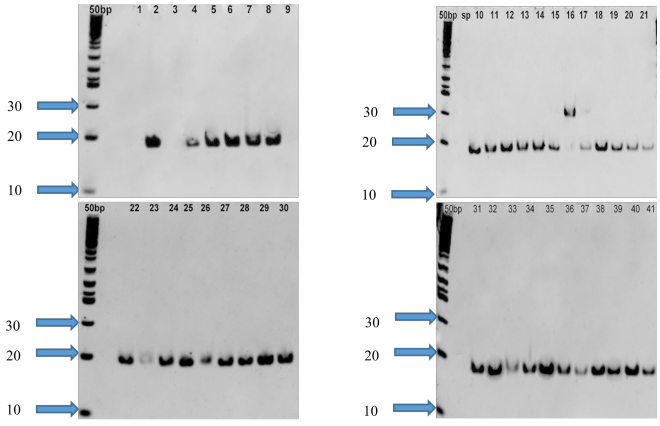

Figure 3: PAGE gel image of PCR products of the SSR marker 64A

50bp= Marker, Sample 1= APR-b, 2= BUO-b, 3= ZEB-Y, 4= ASU-b, 5= MBA- b, 6= GYI-b, 7=TAK-b, 8= YEN-b3, 9= BAW-b, 10= YEN-b2, 11= BAJ-b, 12= OBR-Y, 13= NEB-b, 14= OBR-b, 15= BAJ-Y, 16= BEP-Y, 17= PUT-B, 18= WIO-b, 19= ADU-Y, 20= PUT-b, 21= BEP-B, 22=KAL-b, 23= DED-b, 24= SAN-b, 25= OFF-b, 26= TWI-Y, 27= YEN-b1, 28= ENK-b, 29= DEM-b, 30= OEB-B, 31= BUO-B, 32= KAL-B, 33= OBR-B, 34= OEB-b, 35= TWI-b, 36= ENK-B, 37= WIO-B, 38= TAK-B, 39= MBA-B, 40= BAJ-B, 41= ADU-B.

This affirms an earlier observation made that a greater number of brown oblong tiger nut accessions in Ghana are of Eastern Region origin (Asare *et al.*, 2020).Percentage germination ranged from 3.33% to 100% with a mean of 70.04. The mean squares were significantly different for all traits except for the number of tubers per stand (NTS) which on average recorded 39.00 and ranged from 8.20 to 123.60 per stand. A very large coefficient of variation (260.27%) indicating large phenotypic variation was observed for percentage inflorescence (PI). Yield (HTW) and yield components (LT, WT, TS) also varied widely variability as indicated by a high coefficient of variation values and highly significant ($p < 0.001$) mean squares (Table 04).

The least observed germination percentage was 3±7.7 and was recorded for the accession, OEB-b. Among the 42 accessions, only seventeen (17) produced inflorescences. The flowers observed were whitish-green (pale green) for all the accessions with different colors of tubers. Inflorescence was less frequently observed in the black-tuber genotypes (3) and yellow-tuber genotypes (3) as compared to the brown-tuber genotypes (11). This is in agreement with earlier reports that not all tiger nut accessions produce flowers (Yang *et al.*, 2022). Those that even produce flowers rarely do so on the field (De Vries, 1991). Therefore the assertion that tiger nuts produce copious seeds for propagation (De Castro *et al.*, 2015; Larridon *et al.*, 2013; Stoller and Sweet, 1987) may not apply to all accessions. Among the 42 accessions, the highest mean weight (HTW) of 201.7g± 0.4 was recorded in OFF-b from the Eastern Region. This was followed by another brown accession, APR-b from the Central Region with 186.0g ± 0.1. BUO-B from the Bono East Region ranked 7th with HTW of 175.4g ± 0.4 and was the highest recorded yield for the black accessions. Next to this was ENK-B from the Central Region, another black accession that produced an HTW of 174.4g±0.4 (Table 05).

Cluster analysis: The hierarchical UPGMA cluster analysis based on the traits evaluated grouped the accessions into seven major clusters on the Euclidean similarity matrix at a distance measure of 0.94 within a range of 0.8 to 1.0 (Fig.04). The high-ranged similarity index indicated low genetic variability within the genotypes as indicated by Asare and co-workers (Asare *et al.*, 2020). A similar observation was made when twenty-four local tiger nut accessions from Ghana were grouped into six major clusters based on agro-morphological traits (Donkor *et al.*, 2019).

Cluster I was made up of 8 accessions (19.0% of the total accessions). These accessions were clustered on the premise of similarity in yield and its related characteristics as the length of tuber, tuber shape and hundred tuber weight (Fig.05). Their tubers were oblong longer and characterized by high tuber weight (hundred tuber weight) (Table 05). The accessions were all brown-coloured with four from the Eastern Region, three from Central Region and one from Bono East Region.

Cluster II was the largest and heterogeneously consisted of 28 accessions with three sub-clusters. It contained 67% of the accessions being a mixture of brown, yellow and black-coloured accessions and the tubers were oval or ovoid. Accessions here were grouped based on percentage germination, number of tillers, distance of the last tiller from the main plant, plant height, number of rings per tuber and tuber width. Sub-cluster A had 13 of the accessions, which were characterized by tall plant height, a high number of rings per tuber and production of inflorescences. The tubers were mostly oval with few ovoid shapes and from the Upper East, Upper West, Northern, Eastern and Central Regions. Sub-cluster B had 11 accessions that were characterized by very high numbers of tiller numbers (Table 05). They were dominated by accessions with black-colored tubers; and made up of about 90% of Central Region collections, except for TAK-B which was collected from Takpo in the Upper West Region of Ghana. These findings agree with earlier observations that black tiger nuts in Ghana are mostly found in the Central Region (Asare *et al.*, 2020). TAK-B might have found itself in the Upper West Region probably due to migration by traders. Sub-cluster C had four accessions consisting of two browns, one black and one yellow accession which produced oval-shaped tubers. These accessions were similar in traits such as having moderately low germination percentages and producing medium numbers of tillers (Table 05). However, three accessions (BAJ-b, WIO-B, BEP-Y) were Central Region collections with the remaining (GYI-b) from Upper West.

Clusters III and IV comprised only one accession each (BAW-b and YEN-b3) which produced brown-colored and oval in shaped tubers, and from the Upper East and Nothern regions respectively. YEN-b3 produced the highest number of tubers and was followed by BAW-b. Though YEN-b3 produced the highest number of tubers, it had the least in hundred-tuber-weight among all the accessions; thus, the smallest. Cluster V was made up of only one accession (OFF-b), an Eastern Region collection. It produced oblong-brown tubers, the highest number of inflorescence and the highest

Table 4: Means, standard errors, mean squares, standard deviations, coefficient of variations and range of 11 morpho-descriptors of the 42 tiger nut genotypes used for the study and levels of significance differences

Variable	Mean	SE	MS	SD	CV	Min	Max	Sig. Level (p-value)
PG	70.04	2.47	1323.50	27.70	39.54	3.33	100.00	***
NT	13.094	0.512	53.72	5.752	43.93	1.000	33.000	***
DTP	9.452	0.238	9.53	2.673	28.27	1.400	17.200	*
PH	91.77	1.97	806.10	22.10	24.08	15.80	132.40	***
PI	0.3254	0.0754	1.02	0.8469	260.27	0.0000	5.0000	**
NTS	39.00	1.82	504.70	20.46	52.45	8.20	123.60	ns
NRT	5.7540	0.0446	0.62	0.5010	8.71	5.0000	7.0000	***
LT	19.460	0.530	76.12	5.950	30.58	8.450	38.700	***
WT	14.218	0.212	11.33	2.376	16.71	6.750	19.750	***
TS	1.3777	0.0360	0.36	0.4039	29.32	0.9169	2.7942	***
HTW	133.46	3.44	4554.42	38.65	28.96	30.98	201.87	***

PG= percentage germination; NT= number of tillers per plant,
DTP= distance of the last tiller from the main plant, PH= plant height,
PI= percentage inflorescence, NTS= number of tubers per stand,
NRT= number of rings per tuber, LT= length of tuber,
WT= width of tuber, TS= tuber shape; HTW= hundred tuber weight.

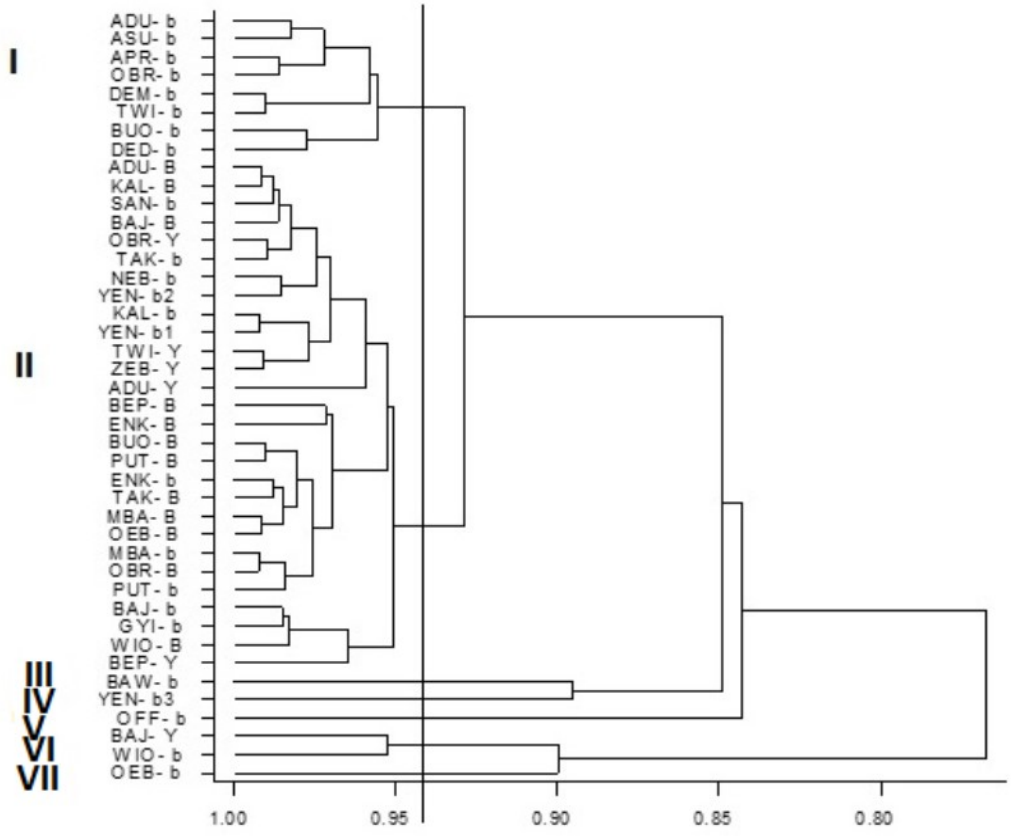

Figure 4: Relationship among the 42 tiger nut genotypes based on 11 morpho-descriptors. Clusters were generated using the Euclidean similarity matrix and UPGMA cluster method

hundred-tuber-weight (Table 05). Cluster VI had two accessions from the Central Region, which produced oval-type, yellow and brown tubers (Fig.04). They were grouped because they had low percentage germination and were characterized by short plant height (Table 05). Cluster VII was made up of only one oval-brown accession which hailed from the Central Region of Ghana. It had the lowest germination percentage

and was the shortest in height with very short tiller distances (Table 05).

Principal component analysis: Variability among the 42 tiger nut accessions for the traits (qualitative and quantitative) by the PCA biplot revealed a scattered distribution of the accessions for all the traits indicating large variations. However, the biplot topography exhibited clustered coverage for many accessions with only three of them largely dispersed from the others inferring less genetic diversity among the accessions. Accessions within tight angles of < 900 and angles >2700 are closely related while those 180^0 apart are uncorrelated and could be considered in heterotic groups for future improvement. The three accessions that separated from the others were (18: GYI-b), (28: OEB-B) and (30: PUT-b) (Fig.05).

The first five PCs accounted for a total of 84.55% variability among the 42 genotypes (Table 06). PC1 accounted for 39.629% of the variability with an Eigenvalue of 4.359 (Tables 06. & 07.). Traits that made a major contribution to the variability included percentage germination, plant height, number of tillers, the distance of the last tiller from the main plant, number of rings per tuber and width of the tuber (Table 06.). PC 2 contributed to the variation by the length of the tuber, tuber shape and hundred tuber weight (Fig.06).

Correlation among the traits: The highest significant positive correlation of 0.899*** was observed between the tuber shape and length of the tuber. This was followed by the association between plant height and percentage germination recording 0.844***. A strong significant positive correlation existed between the distance of the last tiller from the main plant and the number of rings per tuber (0.636***) on one side and also between the width of the tuber (0.584***) on another (Fig.07). Tillering and its effects on yield and its related traits in tiger nuts have not fully been exploited (Asare *et al.*, 2020) as appears in rice and wheat. However, the strong significant positive correlation of the current study is suggestive that, selecting a genotype for tuber characteristics such as length/width ratio (tuber shape) and number of rings per tuber will not be deleterious to the selection of tillering distance, as certain traits could be selected as proxies for others (Adu *et al.*, 2018). Again, tillering distance (DTP) recorded a significant positive correlation with percentage germination (0.543***). On yield and its related traits, a strong positive correlation was observed between hundred-tuber-weight and length of tuber (0.593***). On plant growth characteristics, positive correlations were recorded between hundred tuber weight and the number of tillers (0.407**), hundred-tuber-weight and distance of the last tiller from the main plant (0.474**) and hundred-tuber-weight and plant height (0.429**). Hence in tiger nut breeding, imposing selection of a genotype for tuber weight can be based on plant architectural characteristics such number of tillers, tillering distance and plant height (Asare *et al.*, 2020).

Diversity among 41 Ghanaian Tiger Nut Accessions using SSR Markers

A total of 141 alleles were generated across the nine SSR loci of the 41 tiger nut genotypes and the number of alleles per loci ranged from 9 to 22 with an average of 15.67 ± 3.97 per loci (Table 08).

The lowest and highest number of alleles were recorded for StvCyR_483a and StvCyR_254a loci respectively. Allele frequency ranged from 0.12 for StvCyR_156a to 0.63 for StvCyR_142a and StvCyR_483a with an average of 0.34 ± 0.21.The gene diversity or expected heterozygosity ranged from 0.58 to 0.93 with a mean of 0.79 ± 0.15 exceeding 0.50, indicating the existence of rich variability for the SSR loci assessed among the tiger nut accessions. The observed heterozygosity however, ranged from 0.00 to 0.54 with the mean of 0.12 ± 0.16, an indication of low variability among the genotypes evaluated. Again based on the Chi-square (X^2) value of 5.38 at 8 degrees of freedom, the Ho was not significantly different from the critical Chi-square value of 15.5 at $P<0.05$, signifying low genetic diversity for the population. This is not surprising as vegetatively propagated crops such as tiger nuts are usually self-sterile (Nybom and Lācis, 2021) and rarely hybridize naturally. In contrast, the high values of H_e over H_o are indicative of the admixture effect of genes among tiger nut populations in Ghana, genetic drift, and the high mutation reproducibility rate of SSR primers (Mukhopadhyay and Bhattacharjee, 2016). The Polymorphic information content recorded an average of 0.78 ± 0.15, with StvCyR_483a having the lowest (0.56) and the highest (0.93) recorded for StvCyR_254a.

The expected heterozygosity values for all loci were greater than 0.50, with over 84% having PIC values greater than 0.60, which indicates a high discriminating ability of the SSR loci among the accessions. The most discriminative marker was StvCyR_254a with a PIC value of 0.93 followed by StvCyR_156a (0.92) and StvCyR_64a (0.91) (Table 09).

Table 5: Mean, minimum, maximum and standard deviations of morpho-descriptors and yield traits of the 42 tiger nut genotypes

Genotype	PG (%)	NT	DTP (cm)	PH (cm)	PI (%)	NTS	NRT	LT (mm)	WT (mm)	TS	HTW (g)
ADU- b	86.7	14.9	11.5	103.6	0	28.7	6	26.5	14	1.9	183
	(60-100)	(11-20)	(10-15)	(93-117)	(0-0)	(11-46)	(6-6)	(22-34)	(12-16)	(2-2)	(183-183)
	23.1	4.4	2.8	12.5	0	17.1	0	6.8	2.1	0.3	0.1
ADU- B	94.4	10.1	8.4	101.6	0	42.1	6	18.8	16.7	1.1	132
	(87-100)	(8-13)	(7-10)	(91-114)	(0-0)	(29-64)	(6-6)	(17-20)	(15-19)	(1-1)	(132-132)
	6.9	2.9	1.3	11.83	0	18.9	0	1.4	1.9	0.1	0
ADU- Y	91.1	8.1	9.6	95.1	0.3	12.9	6	22.5	13.1	1.7	113.4
	(80-100)	(7-10)	(9-10)	(86-111)	(0-1)	(11-15)	(6-6)	(19-25)	(12-15)	(2-2)	(113-114)
	10.2	1.2	0.3	13.8	0.6	2	0	3	1.2	0.3	0.4
APR- b	74.4	22.7	9.9	95.5	0.3	27.9	5	23.3	13.4	1.7	186
	(63-83)	(12-29)	(8-12)	(90-101)	(0-1)	(11-48)	(5-5)	(17-32)	(11-15)	(1-2)	(186-186)
	10.2	9.2	2	5.5	0.6	18.9	0	7.8	2.1	0.3	0.1
ASU- b	57.8	18.2	11.5	104.2	0.3	42.1	6.3	26.5	14.6	1.8	185.2
	(40-83)	(14-26)	(10-13)	(93-112)	(0-1)	(42-43)	(6-7)	(23-32)	(14-16)	(2-2)	(185-185)
	22.7	6.6	2.9	9.6	0.6	0.6	0.6	5.1	0.9	0.2	0.2
BAJ- b	41.1	12	10	96.6	0.3	34.1	5.3	18.3	16.1	1.1	114.1
	(20-63)	(5-18)	(5-13)	(34-108)	(0-1)	(16-48)	(5-6)	(15-22)	(15-17)	(1-1)	(114-114)
	21.7	6.7	4.5	38.1	0.6	16.3	0.6	3.7	0.9	0.2	0.1
BAJ- B	85.6	9.7	9.6	83.3	0.3	21.9	6	16.5	15.3	1.1	131.2
	(73-100)	(6-12)	(9-10)	(81-85)	(0-1)	(12-32)	(6-6)	(14-19)	(13-17)	(1-1)	(131-132)
	13.5	3.4	0.7	1.6	0.6	10	0	2.1	2	0	0.4
BAJ- Y	22.2	10.7	4.2	49.3	0	47.9	5	14.4	13.4	1.1	78.4
	(7-40)	(6-18)	(1-8)	(26-81)	(0-0)	(25-87)	(5-5)	(13-16)	(12-16)	(1-1)	(78-79)
	16.8	6.8	3.1	28	0	33.8	0	1.5	1.9	0	0.4
BAW- b	85.6	24.2	8.7	104.8	1.3	61.7	5	8.8	8.7	1	177.2
	(60-100)	(14-33)	(8-10)	(97-118)	(0-4)	(15-113)	(5-5)	(8-9)	(13-14)	(1-1)	(177-177)
	22.2	9.6	1.1	11.6	2.3	49	0	0.4	2	0.1	0.2
BEP- B	62.2	22.2	11.4	88.6	0	39.1	6	18	13.7	1.3	143.3
	(37-80)	(16-29)	(10-13)	(83-94)	(0-0)	(33-45)	(6-6)	(16-20)	(13-14)	(1-1)	(143-144)
	22.7	6.6	1.4	5.2	0	6.1	0	1.7	1.1	0.1	0.4
BEP- Y	47.8	21.7	10.5	82.6	0.7	20.6	5	15.5	13.4	1.2	86.1
	(37-57)	(14-33)	(10-12)	(81-85)	(0-2)	(8-28)	(5-5)	(14-17)	(12-16)	(1-1)	(86-86)
	10.2	10	1.2	2.5	1.2	10.8	0	1.5	2.3	0.1	0.1
BUO- b	88.9	13.6	8.5	113.3	0	43.1	6	30.8	15.4	2	126
	(77-97)	(11-18)	(7-11)	(96-126)	(0-0)	(40-46)	(6-6)	(27-34)	(15-16)	(2-2)	(126-126)
	10.7	3.8	2.2	15.8	0	3	0	3.3	0.6	0.2	0.1

Table 5: Mean, minimum, maximum and standard deviations of morpho-descriptors and yield traits of the 42 tiger nut genotypes

Genotype	PG (%)	NT	DTP (cm)	PH (cm)	PI (%)	NTS	NRT	LT (mm)	WT (mm)	TS	HTW (g)
BUO- B	74.4	16.6	9.2	100.1	0	45.9	6	19.9	17.2	1.2	175.4
	(50-100)	(10-22)	(7-11)	(92-104)	(0-0)	(29-71)	(6-6)	(18-23)	(16-20)	(1-1)	(175-176)
	25	5.6	2	6.6	0	22.4	0	2.6	2.2	0	0.4
DED- b	77.8	14.3	10.9	99.3	0	38.5	6	30.3	12.4	2.4	136
	(73-83)	(4-19)	(9-14)	(90-108)	(0-0)	(12-54)	(6-6)	(25-39)	(10-14)	(2-3)	(136-136)
	5.1	5	2.7	9.1	0	23.2	0	7.3	1.8	0.4	0.1
DEM- b	63.3	10.1	8.9	94.6	0	51.2	5.3	25.5	13.6	1.9	180.1
	(40-100)	(8-12)	(8-10)	(84-108)	(0-0)	(36-69)	(5-6)	(21-28)	(12-16)	(2-2)	(180-180)
	32.1	1.8	1.2	12.5	0	17.1	0.6	3.8	2.2	0.4	0.2
ENK- b	45.6	13.5	10.5	93.3	0	38.7	6	19.7	14.9	1.3	133.3
	(30-67)	(9-19)	(10-11)	(79-107)	(0-0)	(17-61)	(6-6)	(16-22)	(14-17)	(1-1)	(133-133)
	19.0	5.1	0.7	13.6	0.0	22.3	0.0	3.0	1.7	0.1	0.3
ENK- B	87.8	17.7	12.6	97.9	0	58.9	6	18.2	15.8	1.2	174.4
	(77-100)	(13-23)	(11-14)	(84-107)	(0-0)	(53-64)	(6-6)	(17-20)	(13-18)	(1-1)	(174-175)
	11.7	4.8	1.4	12.4	0	5.2	0	1.7	2.3	0.1	0.4
GYI- b	57.8	14.3	10.1	82.1	0	24.3	6	15.2	14.8	1	100.7
	(43-67)	(8-19)	(5-15)	(69-104)	(0-0)	(10-37)	(6-6)	(13-18)	(13-17)	(1-1)	(100-101)
	12.6	5.3	4.8	19.1	0	13.6	0	2.6	1.8	0.1	0.6
KAL- b	82.2	11.8	9.8	95.5	0	35.3	5	16.7	13.3	1.2	78
	(47-100)	(10-15)	(9-11)	(77-109)	(0-0)	(17-51)	(5-5)	(14-20)	(12-15)	(1-1)	(78-78)
	30.8	3	1.2	17	0	17.1	0	3.3	1.1	0.1	0
KAL- B	98.9	12	9.7	97.7	0.3	33.3	6	17.6	15.8	1.1	105.6
	(97-100)	(10-14)	(8-11)	(90-102)	(0-1)	(21-42)	(6-6)	(17-20)	(14-18)	(1-1)	(105-106)
	1.9	2	1.4	7	0.6	10.9	0	1.6	2.1	0.1	0.6
MBA- b	61.1	10.8	9.5	84.1	0	41.1	6	24.3	14.8	1.6	162
	(17-90)	(7-15)	(6-12)	(42-119)	(0-0)	(21-59)	(6-6)	(20-30)	(12-17)	(2-2)	(162-163)
	39.1	4.1	3.1	39	0	19	0	5.3	2.3	0.1	0.4
MBA- B	60	13.1	9.9	90.5	0	57.8	6	17.8	16.4	1.1	174.1
	(27-90)	(10-15)	(8-11)	(78-105)	(0-0)	(52-64)	(6-6)	(17-18)	(16-18)	(1-1)	(174-174)
	31.8	2.8	1.4	13.5	0	6	0	1	1	0.1	0.3
NEB- b	82.2	9.9	7.4	105.5	1	20	6	18	12.5	1.4	119
	(53-100)	(8-12)	(6-9)	(75-122)	(0-2)	(14-26)	(6-6)	(14-23)	(11-17)	(1-2)	(119-119)
	25.2	2.1	1.6	26.1	1	6	0	4.8	1	0.3	0.1
OBR- b	90	16.1	10.5	104	0.7	33.1	5	23.5	13.3	1.7	169.5
	(77-100)	(16-16)	(7-13)	(99-107)	(0-2)	(17-42)	(5-5)	(16-32)	(12-14)	(1-2)	(169-170)
	12	0.1	3.1	4.2	1.2	14.2	0	7.8	1	0.4	0.5

Table 5: Mean, minimum, maximum and standard deviations of morpho-descriptors and yield traits of the 42 tiger nut genotypes

Genotype	PG (%)	NT	DTP (cm)	PH (cm)	PI (%)	NTS	NRT	LT (mm)	WT (mm)	TS	HTW (g)
OBR- B	66.7	14.7	11.3	86.4	0	49.7	6	23	14.4	1.7	138.2
	(47-90)	(9-18)	(8-13)	(82-89)	(0-0)	(32-77)	(6-6)	(17-30)	(11-16)	(1-3)	(138-138)
	21.9	4.8	2.7	3.6	0	23.9	0	6.7	2.6	0.9	0.3
OBR- Y	95.6	11.7	9.9	110.3	1	33.3	5.3	18.2	15.5	1.1	118.1
	(87-100)	(10-14)	(7-11)	(104-121)	(0-2)	(30-35)	(5-6)	(15-23)	(15-17)	(1-1)	(118-118)
	7.7	2.3	2.2	9.2	1	2.7	0.6	4.5	0.5	0.2	0.3
OEB- b	12.2	2.6	3.4	27.8	0	13	6	12.6	9.9	1.3	43.4
	(3-17)	(1-3)	(2-4)	(16-34)	(0-0)	(12-14)	(6-6)	(12-13)	(10-10)	(1-1)	(43-44)
	7.7	1.4	1.6	10.4	0	1	0	0.2	0.2	0	0.4
OEB- B	48.9	11.5	10.3	77	0	46.9	6.3	19.4	16.3	1.2	163
	(27-83)	(10-14)	(8-12)	(67-82)	(0-0)	(43-53)	(6-3)	(15-24)	(14-20)	(1-1)	(163-163)
	30	2.5	1.9	8.8	0	5.1	0.6	4.8	3	0.1	0
OFF- b	76.7	11.6	8.9	112.8	3	33.6	6	28.7	14.4	2.1	201.7
	(43-100)	(8-15)	(7-10)	(99-132)	(1-5)	(22-49)	(6-6)	(27-31)	(11-18)	(2-3)	(201-202)
	29.6	3.3	1.4	17.4	2	13.9	0	2	3.2	0.6	0.4
PUT- b	63.3	14.7	12.3	83.4	0	41	6.7	23.4	16	1.5	159.1
	(40-97)	(11-17)	(10-14)	(82-86)	(0-0)	(37-43)	(6-7)	(21-28)	(15-17)	(1-2)	(159-159)
	29.6	3.3	1.7	2.3	0	3.6	0.6	3.7	1.2	0.1	0.2
PUT- B	85.6	11.5	9.9	104.1	0	38.6	6	17.3	16.2	1.1	171.2
	(70-97)	(10-12)	(7-11)	(93-117)	(0-0)	(24-52)	(6-6)	(17-18)	(16-17)	(1-1)	(171-171)
	13.9	1.5	1.2	12.3	0	13.8	0	0.9	0.9	0.1	0.2
SAN- b	76.7	8.7	8.8	101.7	0	30.4	6	19.5	14.8	1.3	113.1
	(67-97)	(7-11)	(8-10)	(92-109)	(0-0)	(23-43)	(6-6)	(18-21)	(14-16)	(1-1)	(113-114)
	17.3	2	1.3	8.6	0	11.1	0	1.3	1.3	0.1	0.3
TAK- b	81.1	12	10.5	96.1	1	28.7	6	19.7	15.5	1.3	135.1
	(63-93)	(8-16)	(8-13)	(83-105)	(0-3)	(22-41)	(6-6)	(15-24)	(15-17)	(1-1)	(135-135)
	15.8	3.8	2.7	11.2	1.7	11	0	4.4	1.3	0.2	0.3
TAK- B	71.1	12.5	10.1	92.6	0.3	47.7	6	18.6	15.8	1.2	119.4
	(50-87)	(9-18)	(8-12)	(89-99)	(0-1)	(26-63)	(6-6)	(16-20)	(15-17)	(1-1)	(119-120)
	19	4.5	2.1	5.4	0.6	19	0	2.2	0.8	0.1	0.4
TWI- b	41.1	11.7	8	92.7	0.3	54.3	5.3	25.7	14.6	1.7	167.1
	(27-57)	(8-17)	(5-10)	(62-108)	(0-1)	(35-91)	(5-6)	(17-36)	(13-17)	(1-2)	(167-168)
	15	4.5	2.5	26.4	0.6	32	0.6	9.8	2.2	0.4	0.5
TWI- Y	73.3	10.5	9.3	86.9	0	51.3	5.7	14	13.5	1	119
	(20-100)	(8-14)	(8-12)	(50-109)	(0-0)	(32-80)	(5-6)	(13-15)	(13-14)	(1-1)	(119-119)
	46.2	2.9	2.2	32.1	0	25.2	0.6	1.1	0.9	0.1	0.1

Table 5: Mean, minimum, maximum and standard deviations of morpho-descriptors and yield traits of the 42 tiger nut genotypes

Genotype	PG (%)	NT	DTP (cm)	PH (cm)	PI (%)	NTS	NRT	LT (mm)	WT (mm)	TS	HTW (g)
WIO- b	36.3	11.6	7.3	81.7	0	44.5	5	19.6	13.7	1.4	129.4
	(13-50)	(7-14)	(4-9)	(48-99)	(0-0)	(21-67)	(5-5)	(19-21)	(13-15)	(1-1)	(129-130)
	20	3.8	2.5	29.5	0	23.2	0	1	1.1	0	0.5
WIO- B	43.3	14.4	7.9	60.6	0	40.4	6	16.1	14.5	1.1	120.3
	(10-77)	(6-21)	(3-11)	(16-85)	(0-0)	(18-57)	(6-6)	(15-18)	(14-16)	(1-1)	(120-121)
	33.3	7.3	4.1	38.5	0	20.2	0	1.7	1	0	0.3
YEN- b1	100	10.3	10.1	111.7	0	37.9	5	15.5	12.5	1.2	93.4
	(100-100)	(8-14)	(7-12)	(105-118)	(0-0)	(29-54)	(5-5)	(14-17)	(12-13)	(1-1)	(93-94)
	0	2.9	2.7	6.2	0	14	0	1.5	0.5	0.2	0.4
YEN- b2	85.6	9.2	8	99.3	1.3	27.4	5.3	16	14.2	1.1	97.4
	(60-100)	(5-12)	(6-10)	(94-109)	(0-2)	(25-31)	(5-6)	(14-20)	(14-15)	(1-1)	(97-98)
	22.2	4	2.1	8.1	1.2	3.5	0.6	3.4	0.6	0.2	0.4
YEN- b3	76.7	14.1	7.7	89.2	1	75.7	6	9.8	8	1.2	31
	(30-100)	(6-20)	(5-10)	(52-110)	(0-2)	(14-124)	(6-6)	(9-11)	(7-9)	(1-1)	(31-31)
	40.4	7.1	2.1	32	1.7	55.8	0	1.1	1.1	0.1	0.1
ZEB- Y	88.9	8.3	10.4	97.1	0	44.5	6	14.5	14.9	1	120.5
	(67-100)	(6-11)	(7-17)	(93-129)	(0-0)	(33-51)	(6-6)	(13-17)	(13-18)	(1-1)	(120-121)
	19.2	2.3	5.9	28.8	0	9.8	0	2.5	3.1	0.1	0.2

PG= percentage germination; NT= number of tillers per plant, DTP= distance of the last tiller from the main plant, PH= plant height, PI= percentage inflorescence, NTS= number of tubers per stand, NRT= number of rings per tuber, LT= length of tuber, WT= width of tuber, TS= tuber shape; HTW= hundred tuber weight

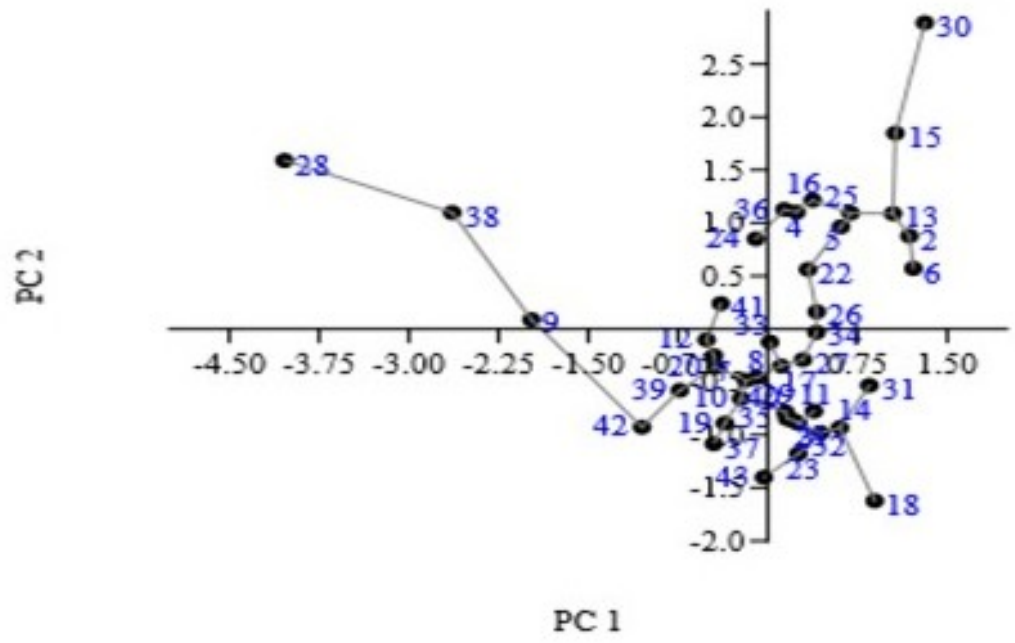

Figure 5: PCA biplot showing groupings of the 42 tiger nut accessions based on 11 morpho-descriptor variables evaluated on the Eigenvalue scale

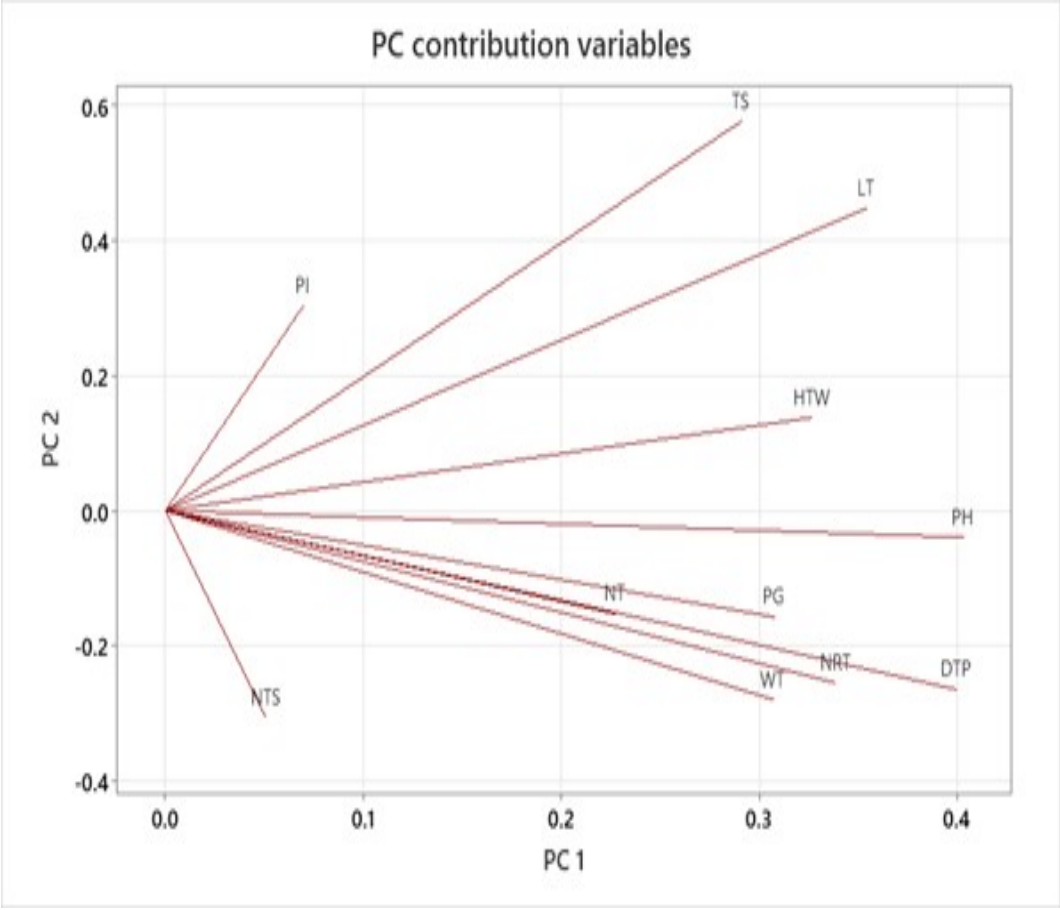

Figure 6: PC biplots of variables indicating the contributions of the various traits of the tiger nut accessions

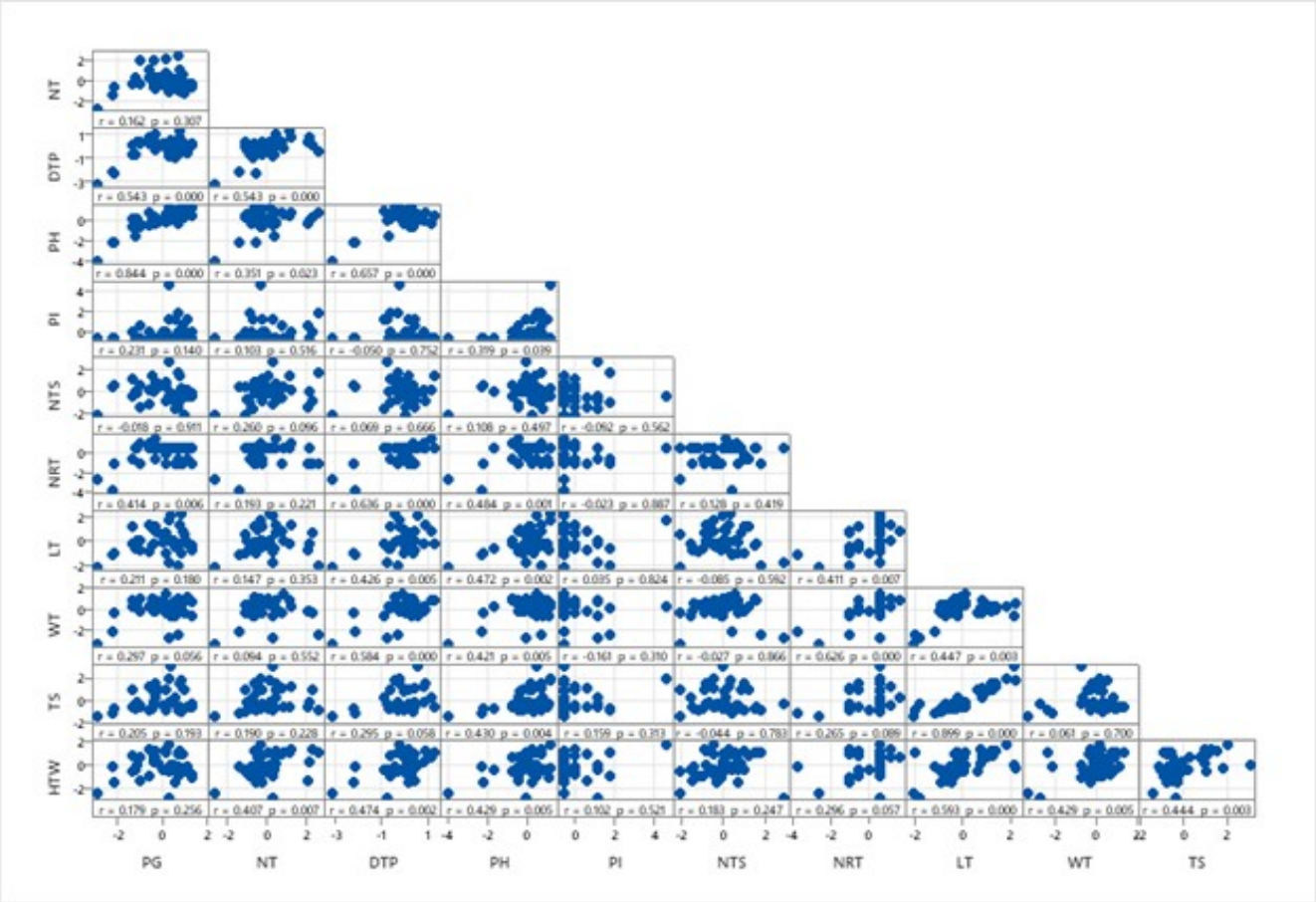

Figure 7: Pearson correlation matrix among the 11 morpho-descriptors of the 42 tiger nut accessions

Table 6: Eigenvectors of the first five principal components of morphological trait evaluated

Variable	PC1	PC2	PC3	PC4	PC5
PG	0.307	-0.157	0.465	0.261	-0.183
NT	0.227	-0.153	0.108	-0.562	0.536
DTP	0.399	-0.265	-0.024	0.001	0.273
PH	0.403	-0.040	0.357	0.068	-0.153
PI	0.070	0.303	0.623	-0.061	0.064
NTS	0.051	-0.306	-0.037	-0.607	-0.677
NRT	0.338	-0.255	-0.136	0.199	-0.202
LT	0.354	0.447	-0.297	0.049	-0.107
WT	0.307	-0.280	-0.313	0.310	0.089
TS	0.291	0.577	-0.114	-0.090	-0.186
HTW	0.327	0.138	-0.193	-0.304	0.154
Eigenvalue	4.359	1.492	1.389	1.291	0.770
% Variance	39.63	13.56	12.63	11.73	7.00
% Cumulative	39.63	53.19	65.82	77.55	84.55

PG= percentage germination; NT= number of tillers per plant,
DTP= distance of the last tiller from the main plant, PH= plant height,
PI= percentage inflorescence, NTS= number of tubers per stand,
NRT= number of rings per tuber, LT= length of tuber,
WT= width of tuber, TS= tuber shape; HTW= hundred tuber weight

Using 191 microsatellite markers, five of them were found to distinguish *Cyperus esculentus* from 12 accessions of *Cyperus rotundus* (Arias *et al.*, 2011). However, the markers used in the current study were not part of the 191 microsatellites used by Arias *et al.* (2011). Okoli and others found a wide range

Table 7: Principal component analysis of 11 morphological traits evaluated on the 42 tiger nut accessions cultivated in Ghana based on correlation coefficient similarity matrix

PC	Eigenvalue	% Variance	Proportion	Cumulative
1	4.359	39.629	0.396	0.396
2	1.492	13.564	0.136	0.532
3	1.389	12.630	0.126	0.658
4	1.291	11.734	0.117	0.776
5	0.769	6.995	0.070	0.846
6	0.674	6.131	0.061	0.907
7	0.522	4.743	0.047	0.954
8	0.249	2.263	0.023	0.977
9	0.172	1.561	0.172	0.016
10	0.074	0.670	0.007	1.00

Table 8: Summary statistics on the SSR markers analyzed with Power marker software Ver. 3.25.

Marker	Na	Ne	He	Ho	PIC
StvCyR_1a	0.27	14.00	0.84	0.12	0.82
StvCyR_27b	0.59	19.00	0.65	0.10	0.64
StvCyR_64a	0.17	18.00	0.91	0.07	0.91
StvCyR_126a	0.24	14.00	0.87	0.07	0.86
StvCyR_142a	0.63	12.00	0.58	0.05	0.57
StvCyR_156a	0.12	18.00	0.92	0.02	0.92
StvCyR_181a	0.24	15.00	0.87	0.10	0.85
StvCyR_254a	0.15	22.00	0.93	0.54	0.93
StvCyR_483a	0.63	9.00	0.58	0.00	0.56
Total	3.04	141	7.15	1.07	7.06
Min	0.12	9	0.58	0	0.56
Max	0.63	22	0.93	0.54	0.93
Mean	0.34	15.67	0.79	0.12	0.78
SD	0.21	3.97	0.15	0.16	0.15
X2cal				5.38	
X2tab				3.84	
Probability				0.05	

Major allele frequency (Na), Number of alleles (Ne), Gene diversity or expected heterozygosity (He), Observed heterozygosity (Ho), and Polymorphic information content (PIC).

of variability in purple and yellow nutsedge with RAPD (Okoli *et al.*, 1997). High genetic variability in cultivated and wild yellow nutsedge was also detected using RAPD (Abad *et al.*, 1998). On the contrary low genetic diversity was observed in *Cyperus esculentus* when AFLP markers were used (Dodet *et al.*, 2008). They then suggested that the application of microsatellite genetic diversity studies could be more effective in determining genetic variability in a crop. Akabassi *et al.* (2021), in a systematic review, also suggested the use of a more robust tool, such as microsatellite markers to the study of the genetic diversity of *Cyperus esculentus* to elucidate the crop's diversity, especially in Africa, where studies had tailored more on the morphological characteristics and food use. The discriminatory power of SSR markers has been demonstrated in this study.

Cluster analysis using molecular data: The genetic relationship among the estimated 41 tiger nut genotypes using similarity coefficients ranged highly from 0.85 – 0.96. The pairwise associations among the genotypes at 0.96 were found between BAJ-B/ APR-b, PUT-B/ BUO-b and OEB-B/ KAL-b. The least similarity groupings (0.85) were between NEB-b/ TAK-b, BEP-Y/ BUO-b, BEP-Y/ GYI-b, ADU-Y/ GYI-b, PUT-b/ BUO-b, PUT-b/ GYI-b, and PUT-

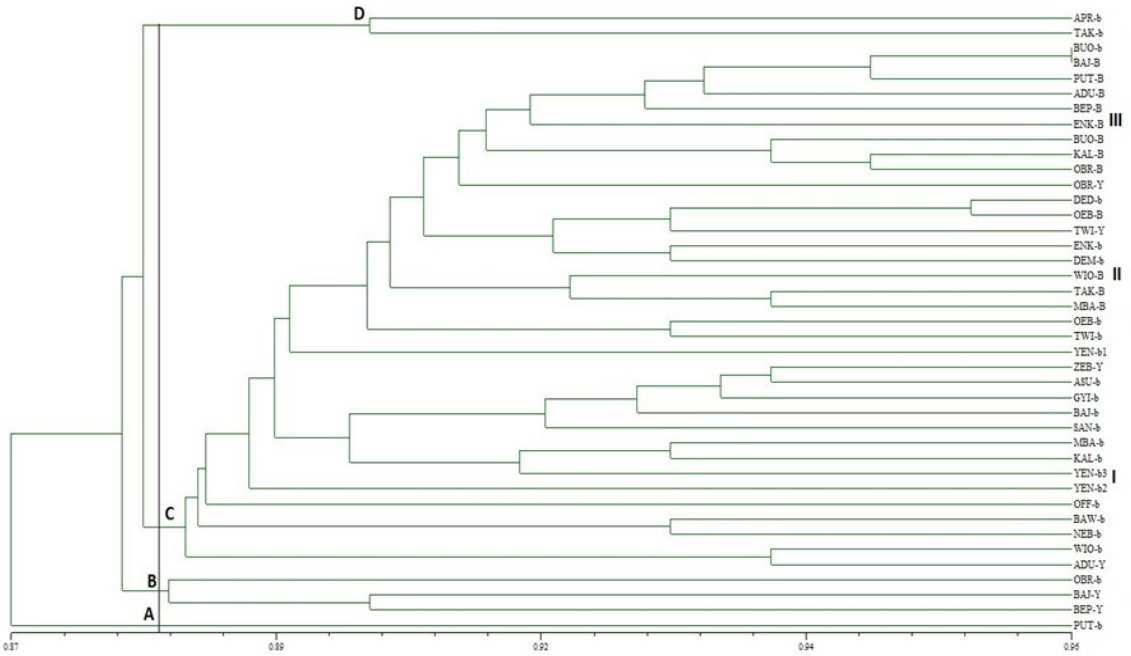

Figure 8: UPGMA cluster analysis (Nei, 1983) shows the relationship among the 41 tiger nut genotypes generated from NTSYS version 2.01

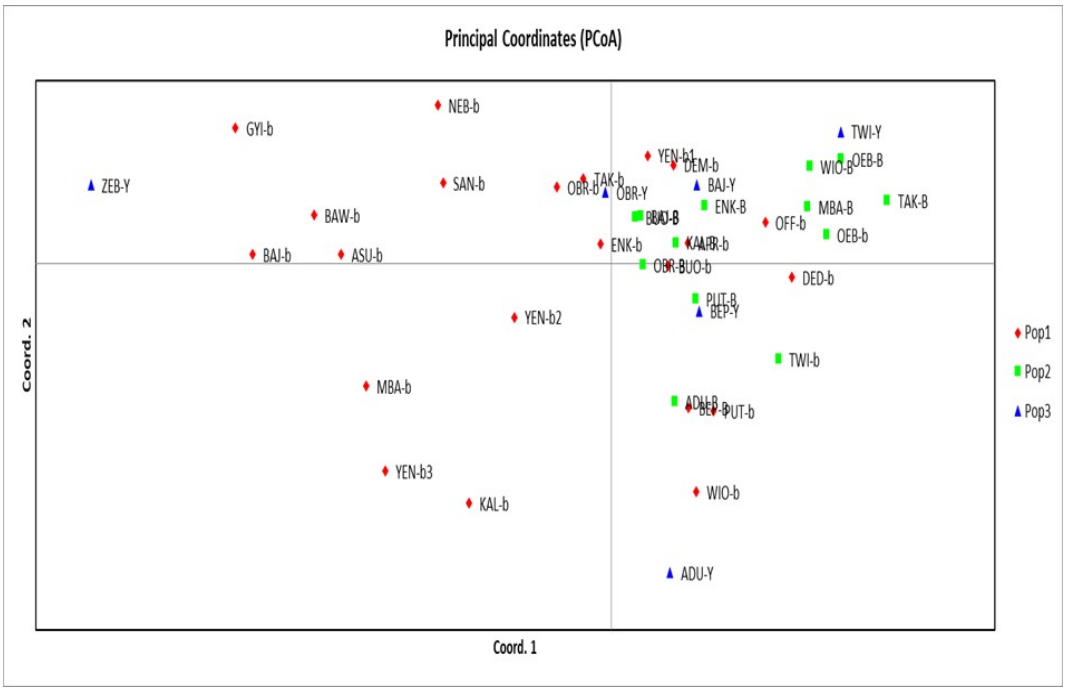

Figure 9: Groupings of 41 tiger nut accessions based on principal coordinate analysis of SSR data

b/ NEB-b. The UPGMA analysis grouped the 41 genotypes into four heterotic groups (clusters); A, B, C and D at a similarity coefficient of 0.88. Cluster A was distinct and was made up of only one genotype, PUT-b as an outlier. Cluster B was made up of three genotypes, all of which were Central Region collections; one brown (OBR-b), and two yellow (BAJ-Y and BEP-Y). Cluster C was the most heterogeneous cluster with 35 accessions of a mixture of brown, yellow and black colors and had three sub-clusters: I, II and III. However, each intrasubcluster accession had more genetic elements in common than the others, evidence of less genetic variability within the population. This is evident by the observation of one type of color dominating the others in each subcluster. For example, subcluster III had the accessions dominated with black color while subcluster I was dominated with brown. Cluster D, however, was made up of two genotypes; APR-b and TAK-b (Fig.08), both brown but from different regions of cultivation in Ghana.

Principal coordinate analysis: The principal coordinate analysis is a multivariate technique used to describe the variance relationship existing among or within individuals of a population (Khayyam Nikoyie *et al.*, 2009). The relative variance of each coordinate indicates the genetic distances within or among populations.

All nine SSR loci generated some level of genetic variability for the population on basic coordinate analysis using a simple matching coefficient similarity matrix across the 41 tiger nuts accessions. Three populations were identified, populations 1, 2 and 3, indicating the presence of genetic diversity among the genotypes. Whiles majority of the accessions (brown and few yellow), were from Upper West (4), Upper East (2), Northern Region (2), and Central Region (6), with few of Eastern Region (1) accessions were skewed to one-half of the quadrants, the remaining accessions (black, yellow and few browns) from Upper West (2), Eastern (5), Central (16), Bono East (2) and Nothern (1) Regions separated to the other side of the quadrant (Fig.04). The findings, therefore, confirm the existence of genetic variability among the accessions on one side but high genetic similarity within. This aligns with the observations made in the morphological characterization of the current study, and also in support of earlier agro-morphological characterization of tiger nut accessions in Ghana by Donkor *et al.*, (2019).

CONCLUSION

Cyperus esculentus is a cross-pollinated crop which is mainly propagated vegetatively using tubers. Vegetative propagation produces true-to-type offspring which result in low genetic diversity over generations. The 42 accessions collected in various locations in Ghana differed highly significantly in all 11 morphological characteristics. However, the morphological UPGMA cluster analysis grouped the accessions into seven main clusters with one large heterogenous cluster using the Euclidean similarity index of 0.94. The heterogeneous cluster contained more than half the study population (28 accessions) and had three sub-clusters, an indication of low genetic variability within the population. On SSR molecular evaluation, a total of 141 alleles were obtained for the nine SSR loci across 41 accessions with a mean of 15.67 ± 3.97 per loci and a mean PIC value of 0.78 ± 0.15, indicating the high discriminating power of the markers. Nonetheless, molecular UPGMA cluster analysis partitioned the population into four heterotic groups with one large heterogenous cluster, suggesting evidence of low genetic variability within the accessions. This is coupled with a low mean heterozygosity value of 0.12 ± 0.16. The differences in variability among the studied accessions for some traits indicate some level of admixture of genes over generations. The exchange of planting material of tiger nut among farmers along the boundaries of the different geographical regions in the country and the subsequent selection of the accessions for planting could have also contributed to the narrow genetic base of the crop.

Therefore, enhancing the genetic diversity of tiger nut accessions for genetic improvement will demand mutation breeding as tiger nuts are seldom propagated sexually. Also, exotic accessions could be imported to support breeding and selection for improved varieties.

Conflicts of interest

There is no conflict of interest.

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