World Journal of Pharmaceutical Science and Research
www.wjpsronline.com
Research Article
ISSN: 2583-6579
SJIF Impact Factor: 3.454
Year - 2024
Volume: 3; Issue: 2
Page: 21-37

GENETIC DIVERSITY STUDIES ON WILD AND CULTIVATED GENOTYPES OF TIGER NUTS (*CYPERUS SPECIES*) USING MOLECULAR AND MORPHOLOGICAL MARKERS

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Article Received: 31 December 2023 | | ***Article Revised: 21 January 2024*** | | ***Article Accepted: 12 February 2024***

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DOI: https://doi.org/10.5281/zenodo.10940575

ABSTRACT

Research was carried out to investigate into the diversity existing between wild and cultivated Tiger nuts genotypes using morphological and molecular markers. Three genotypes of cultivated (Black, Brown and Yellow) Tiger nuts and two of wild (Black and brown) gentypes were sourced from ARTI, Zaria, Kaduna state. The genotypes were subjected to molecular analysis using apical leaf portions for extraction of DNA using the CTAB method, amplification of the rbcl gene using PCR and gel electrophoresis using nanodrop spectrophotometer. The Tiger nuts genotypes were also planted out in the field using CRD. Growth and yield data were obtained for all genotypes. The data generated from molecular and morphological attributes were subjected to statistical and genetic analysis using GENALex software and GENSTAT v 12. The results of the present study had shown that the wild and cultivated Tiger nuts differed significantly in their growth and yield attributes as well as in their molecular attributes. Three principal components contributed to 100 % of total variations that existed in the population of wild and cultivated total nuts. The molecular bands showed that the wild and cultivated black Tiger nuts were amplified by 800bps while other genotypes were amplified at 100bps by the rbcl primer. The polymorphic information content also varied and high in the rbcl primer. Heterozygousity was also high and as well as the Nei's gene diversity informativeness of the genotypes was greatly revealed by the markers.

KEYWORDS: Wild, cultivated-tiger nuts, Microsatellite markers. Morphological and yield traits.

INTRODUCTION

The tiger nuts (*Cyperus esculentus*) also called chufa sedge, nut grass, yellow nutsedge, tiger nuts sedge, edible galingale, water grass or earth almond is a perennial c4 plant of the sedge family(*Cyperaceaa*) is widespread in tropical and temperate zones and is also present in cooler regions.[1] It is a crop, but also grows wild very often as a weed.[2]

It is found in most of the Eastern Hemisphere including Southern Europ, Africa and Madgascar as well as Middle East and the Indian subcontinent.[3] In Spain, *Cyperus esculentus* is cultivated for its edible tubers called earth almond or tiger nut for the preparation of *hochata de chufa*, a sweet, milk-like beverage.

Tiger nut is an obligate outcrosser[4]; its base chromosome number n is 54 or 108[5] and no hybrids are known in nature[6], although it's been recognized a possible hybridization with *C. rotundus L.*[7]

The plant produce abundant seeds, but strong vegetative propagation through rhizomes and tubers is much more important than seeds in the diffusion of the species.[8,9]

The tuber are also a source of food for several animals (birds and mammals).[10] The origin of the cultivated type *(C. esculentus var. Sativus Boeckeler)* was located in the Mediterranean area, where it has been cultivated for its edible tubers since pre-dynastic Egypt (fourth Millennium BC).[11,12] At present, it is still cultivated for food and medicinal use in Southern Europe, Africa and Asia.[13]

The prehistoric tools with traces of tiger nut tuber starch granules have been recovered from the early Archaic period in North America, from about 9,000 years ago at the sandy Hill excavation site at the Mashantucket pquot Reservation in Mashantucket Connecticut. The tubers are believed to have been a source of food for those paleo-Indians.[14]

Zohary and Hopf estimate that *Cyperus esculentus* ''ranks among the oldest cultivated plants in Ancient Egypt''. Although nothing that chufa was not doubt an important food element in ancient Egypt during dynastic times, its cultivation in ancient time seems to have remained (total or almost totally) an Egyptian specialty.[15]

The crop of the tiger nut is very old, tubers of the tiger nuts were found in Sarcophagi and Egyptian tombs of the first dynasties. It was a appreciated food by the ancient Egyptians as the Theophrastus narrative proves on the Sandy Land not far from the river bed; the Malniathalle round in shape, boneless and skinless, grows on the earth. The citizens collect the tubers and cook them so they become very sweet, then eat them as a dessert.[16]

From Egypt, the cultivation of the tiger nut spread to North Africa, reaching the Iberian Peninsula and Sicily with the Islamic waves of the middle ages. The reasons that probably justify the introduction of its cultivation were on the hand, the prohibition of wine consumption by the Muslim religion which would certainly correlate with the proliferation of soft drinks. The tiger nuts dry tubers have been found in tombs from predynastic times about 6,000 years ago; in those times *Cyperus esculentus* tubers were consumed either boiled in beer, roasted or as sweets made of ground tubers with honey,[17] the tubers were also used medicinally, taken orally, as an ointment or as an enema and used in fumigants to sweeten the smell of homes or clothing.[18] There are almost no contemporary records of this plant in other parts of the old world.

Besides Egypt, at present tiger nuts cultivated mainly in Spain, where it is extended for common commercial purposes in mild climate areas. The plants were introduced by Arabs first in the Valencia region. They are found extensively too in California and were grown by the Paiute in Owens valley. Tiger nut is also cultivated in countries such as Brazil, the United States, Saudi Arabia, Iraq, India Ghana, Nigeria etc; where they are used primarily as animals feed or uncooked as a side dish; but in Hispanic countries they are used mainly to make horchata, a sweet, milk-like beverage. In Northern Nigeria, it is called aya and it is usually eaten fresh. It is sometimes dried and later rehydrated and eaten.

Tiger nuts are also a rich source of antioxidants, which are beneficial compounds that protect our body against aging and disease. It helps to activate brain cells, increases energy, and has positive impact on immune system and much more. Tiger nut tea cleans the body from toxins. It's a source of natural protein, vitamins, and minerals. Tiger nut oil is used in cooking and in cosmetic industry too. The high nutritive profile of tiger nuts has triggered several efforts by farmers and the likes to increase its production and consumption.[18]

The study was aimed to unveil the diversity in wild and cultivated genotypes of Tiger nuts using morphological and molecular markers and specifically design to compare the variation existing within the wild and cultivated species of tiger nuts using morphological markers and to compare the genetic diversity in wild and cultivated tiger nuts using molecular markers.

Unfortunately, the nuts-edge only exists as wild weeds in this rainforest ecology in lieu of the cultivated genotypes. The diversity in taste and colour between the wild and cultivated genotypes accounts greatly for the non- utilization of the wild type.

Hence, this study seeks to unveil the variability in morphology and molecular attributes between the wild and cultivated tiger nuts to identify innate contents responsible for the non- utilization of the wild tiger nuts-edge.

MATERIAL METHOD

Experimental Site

The experiment was conducted at the green house of the Department of Genetics and Biotechnology, Faculty of Biological Sciences, University of Calabar from July to November, 2022 for the morphological work while the molecular work was carried out in the Molecular Biology Laboratory of the same Department located.

Sources of experimental materials

Seeds of the yellow, brown cultivated species of Tiger nut (*Cyperus esculentus*) was sourced and obtained from the Institute for Agricultural Research/Training (IAR) Zaria, Kaduna state while the seeds of the dark and brown wild Tiger nuts (*Cyperus rotundus*) was sourced and obtained locally from the Botanical Garden of the University of Calabar.

Plastic cups were purchased at Watt market and filled with garden soils collected locally after sterilization using dry heat method.

Garden soil was collected from the A-horizon top soil (0 – 25 cm) which is known to contain plant growth nutrients using a soil augur.

Seed germination and planting

Five (5) seeds of each of the yellow and brown cultivated species and dark and brown wild species were sown directly into the plastic cups containing the soils and was nurtured using all prescribed agronomic practices for tiger cultivation.

Experimental Design and Treatment

The experiment was arranged as a simple complete randomized design (CRD) with four treatments. This was replicated three times. A total of 12 experimental units was used for the study.

The treatments was as follows

- Yellow Cultivar of Tiger nuts (TGN 4013) was designated as YCTN.
- Brown Cultivar of Tiger nuts (TGN 4331); was designated as BCTN.
- Dark wild species of Tiger nuts was designated as DWTN.
- Brown wild species of Tiger nuts was designated as BWTN.

Experimental layout

TREATMENT	TGN 4013 (A)	TGN 4331 (B)	DWTN (C)	BWTN (D)
REPLICATE 1	A	B	C	D
REPLICATE 2	D	C	A	B
REPLICATE 3	C	D	B	A

Data collection and analysis for morphological studies

Data was collected for some growth attributes as follows: Plant height, number of leaves per plate (cm), leaf length (cm), leaf width (cm), leaf area (cm^2) and petiole length (cm)

Data was collected for yield parameters as follows: Number of tubers per plant, 100 tuber weight per plant (g), fresh tuber weight per plant (g) and dry tuber weight per plant (g).

Data Analysis for morphological attributes

Data generated from the studies for morphological attributes was collated and subjected to the analysis of variance procedures (ANOVA) according the completely randomized design outlay. Attributes with significant treatment means was further separated using the Fischer's least significant difference (LSD) test at 5 % level of probability.

DNA Extraction for the molecular experiment

Total genomic DNA was extracted from young leaves (200mg per accession) of 4 to5 days old plants harvested from the morphological experimental following the cetyltrimethyl ammonium bromide (CTAB).[19] Extracted DNA was spectrophotometrically quantified and diluted to 25 mg $\mu 1^{-1}$ working solution.

SSR Analysis

SSR primers used by researchers/earlier workers on tiger nuts was selected. Primers with tri-nucleotides repeats were chosen. The total volume of PCR mixture was 25µl containing 12.5 of master mix 2.5µl template DNA, 1µl of each prime (forward and reverse) and 10.5 µl of sterilised water. The PCR reaction was performed in a thermal cycler using an initial 94^{o}C denaturing step for 5 min followed by 34 cycles of denaturing at 94^{o}C for 30 sec, primer annealing at 50-55^{o}C for 30sec and elongation at 72^{o}C for 1 min; followed by final elongation at 72^{o}C for 10 min and then at 4^{o}C until required for further processing.

Electrophoresis

PCR product was run in 1% agarose gel for about 30minites at 80V and viewed using UV illumination.

Data collection and analysis for molecular studies

SSR was scored for the presence (1) or absence (0) of band in the gel profile. Only clear and distinct bands were used to construct a binary matrix using Dice's coefficient in both cases. Data was analyzed using DARwin 5.0 software and then a final Neighbor joining (NJ) dendrogram was constructed by means of the Un Weighted Neighbor – Joining method.[20]

The percentage of polymorphic bands, major allele frequencies, number of alleles, heterozygousity and Nei's gene diversity indices was determined.[21,22]

Primers used for the study

Primers	Trinucleotides SSR	Source
RbcL F-IF	ATA TCA CCA CAA ACA GAA AC	Fay et al, 1998
RbcL R-1406R	TCC TTT TAG TAA AAG ATT GGG CGC AG	Fay et al, 1998
Rps16 –intron F	GTG GTA GAA AGC AAC GTC CGA CTT	Oxelmann et al, 1997
Rps R2 –	TCG GCA TCG AAC ATC AAT TGC AAC	Oxelmann et al, 1997
EST -1F	CTG TGC CGT CGT CGC ATG AGT TG	Starr et al, 2003
EST -18S-R	AGA CAA GCA TAT GAC TAC TGG CAG G	Starr et al, 2003

Scoring of polymorphic bands

Each band in the RAPD and SSR fingerprinting pattern was considered as a separate locus. Only distinct, reproducible, well-resolved fragments were selected and scored for presence (1) and absence (0) of a band. The binary matrix for SSR phenotypes was then assembled for analyses. A similarity matrix was constructed and subjected to cluster analysis following the un-weighted pair group method with arithmetical averages (UPGMA) of the computer program DARwin version 5.0. Measurement of genetic distance for pair-wise accessions was based on Nei's unbiased genetic distances using DARwin 12.0 software.

The Gene diversity for each selected primer was calculated as [1 - the ratio of number of bands per primer to the total bands informativeness of all the bands produced by the primers]. Gene diversity of a cultivar = [1 – NPB/TNB], where NPB (Number of polymorphic bands or band informativeness and TNB is the total number of bands produced by the 14 genotypes (Elite plantain cultivars evaluated) that contains the bands. The polymorphic information content (PIC) value was calculated using the formula $1 - \Sigma pi^2$, where *pi* is the frequency of the *nth* allele.[23]

The number of alleles here refers to the number of scored bands and the frequency of an allele was obtained by dividing the number of elite cultivars where it refers to the total number of elite cultivars present. Marker index (MI) for a primer was calculated as the product of PIC and the number of polymorphic bands obtained per primer.

Determination of soil physical and chemical properties

Soil samples from the experimental location behind biological science building were sampled using a soil auger to depth of 0-40 cm. The soil samples were bulked differently, air dried, crushed, sieved to pass through 2 mm mesh and analyzed for physico-chemical properties using standard laboratory procedures in the soil science laboratory as follows;

(a) Soil particle size distribution for percent silt, clay and sand using hydrometer method of Bouyoucos.
(b) Soil pH at 1:2.5 soil liquid ratio in water and KCl using electrode pH meter.
(c) Organic carbon was determined using Walkey and Black method of 1934.
(d) Total nitrogen was determined using Jackson procedures of 1965.
(e) Exchangeable Acidity was determined using the Mclean procedures of 1965.
(f) Exchangeable Bases Mg, K and Na were determined using Flame Photometry while Ca was determined by the Atomic Absorption Spectrophotometry.
(g) Available phosphorus was determined using the Bray and Kurtz procedures of 1945.
(h) Effective cation exchange capacity (ECEC) was obtained by summation.

Data analysis

Data that was generated from the study was analyze following procedure of analysis of variance for a randomized complete block design. Significant treatment means was separated using Fishers least significant difference (LSD) test at 5% probability.

Table 1: Physico-chemical properties of the soil used in the Experiment.

S/N	Soil properties	Values
1.	pH (H20)	5.3
2.	Organic C (%)	6.12
3.	Total N (%)	0.08
4.	Available P (mg/kg-1)	6.15
5.	Exch. Bases (cmol kg-1)	0.79
6.	Ca	0.42
7.	Mg	0.13
8.	K	0.16
9.	Na	0.08
10.	Exchangeable acidity	0.25
11.	CEC (cmol kg-1)	0.12
12.	Base saturation (%)	68.0
13.	% Sand	65.0
14.	% Silt	23.2
15.	% Clay	145
16.	Textural class	Sand loam

RESULTS

Electrophoregram of five genotypes of Tiger nuts

Figure 1 shows the electrophoregram of five genotypes, two (Brown and Black) obtained from wild Tiger nuts (*Cyperus rotundus*) and three (Black, Yellow and Brown) obtained from the cultivated Tiger nuts (*Cyperus esculentus*). The gel electrophoregram shows that the *rbcL* and *rps* genes were found wild and cultivated Tiger nuts genotypes

studied. The primers were able to amplify the genes in the Tiger nuts genotypes at different base pairs of the molecular ladder. The different genotypes containing the *rbcL* and *rps* genes were amplified at different sizes of the molecular ladder. The band sizes for amplicons range from 100bps to 800bps (Figure1). None of the genotypes genes was amplified above 800bps. As shown from the Figure 1, all the genotypes Samples 1 – 5) *rbcL* and *rps* genes were amplified at 100bps in all the alleles while only the black colour genotypes of both the wild and cultivated Tiger nuts amplified at 800bps respectively (Figure 1).

Genetic polymorphism among wild and cultivated species of Tiger nuts

The results of genetic polymorphism among wild and cultivated species of Tiger nuts are presented in Table 3. The results showed that polymorphism was attained by the different species at 800 bp while monomorphic condition was the case in the 100bps and other sites (Table 2).

Principal component analysis for the wild and cultivated Tiger nuts

The results of principal component analysis for the wild and cultivated Tiger nuts are presented in Table 3. The results showed that three principal components contributed a total of 100 % variability existing among the Tiger nuts species. Principal component one had an eigen value of 0.18359 and contributed the highest percentage variations of 58.02% to the total variation observed with major and principal loading values coming from number of tillers per plant (0.4285) and leaf area (0.4285). The second principal component has an eigen value of 0.125 and contributed 39.51% to the total variations observed in the population. The major loading values to this component is leaf area (0.5000). The third principal component has an eigen value of 0.00781 and contributed only 2.47% to the total variations observed in the population. The main loading value to the third principal component is plant height (0.125).

Genetic diversity indices among the wild and cultivated species of Tiger nuts

The results of genetic diversity indices among the wild and cultivated species of Tiger nuts evaluated are presented in Table 4. The results showed that the Rbcl primer performed better compared to EST and rps primers. Number of polymorphic bands ranged from 3 in Rps to 5 in Rbcl primers. Major allele frequency varied from 0.64 in Rbcl to 0.75 in EST primers. Number of allele ranged from 6 in EST to 10 in Rbcl primer. Heterozygusity decreases from 0.89 in Rbcl to 0.60 in Rps. Nei's gene diversity was 0.88 in Rbcl, 0.76 in EST and 0.72 in Rps. Polymorphic information content (PIC) of the primers varied from 0.61 in EST, 0.76 in Rps to 0.78 in Rbcl primers respectively.

Morphological markers for growth attributes the wild and cultivated tiger nuts

Table 5 and figure 3 presents the results of morphological markers for growth attributes the wild and cultivated tiger nuts. The results showed that some of the growth attributes like plant height (cm), number of leaves per plant, leaf length (cm) and leaf area (cm^2) differed ($p<0.05$) significantly among the evaluated tiger nut genotypes. Mean plant height among the tiger nut genotypes was 13.17±0.57 cm. Plant height varied from 16.27±1.33 cm in wild black tiger nut to 10.34±1.33 cm in cultivated brown tiger nut. Number of tillers per plant had a mean value of 3.93. Number of tillers varied between 3.33±0.57 in cultivated black tiger nut to 4.67±1.00 in wild black tiger nut. Average number of leaves per plant was 7.36±1.33. Leaves per plant showed 6.67±1.56 in wild black tiger nut and 8.17±1.31 in cultivated brown tiger nut genotype. Average leaf width obtained was 0.66±0.01 cm and varied from 0.45±0.01 cm in wild brown tiger nut to 0.72±0.57 cm. The mean leaf area for the tiger nut genotypes is 5.37±1.39 cm^2 and it ranged from 2.88±0.88 cm^2 in wild brown tiger nut to 7.88±0.57 cm^2 in cultivated black tiger nut genotype.

Table 6 presents the results of morphological markers for yield attributes the wild and cultivated tiger nuts. The results showed that some of the yield attributes like percent germination, number of tubers per plant, 100 seeds weight (g) , fresh and dry weight of tubers per plant differed ($p<0.05$) significantly among the evaluated tiger nut genotypes. Mean percentage germination among the tiger nut genotypes was 68.72±0.57 %. Percent germination varied from 53.33±13.33 % in cultivated yellow tiger nut to 86.67±13.33 % in the wild black tiger nut. Number of tubers per plant had a mean value of 3.47±1.39. Number of tbers varied between 0.00±0.00 in wild brown/black black Tiger nuts to 7.67±1.85 in cultivated brown tiger nut. Average weight of 100 seeds per plant was 4.56±1.33 g. Weight of 100 seeds weighed between 0.00±0.01 g in wild black brown Tiger nuts and 8.77±1.31g in cultivated brown tiger nut genotype. Average fresh weight of tubers obtained was 5.25±0.01 g and varied from 0.00±0.00 g in wild black/brown Tiger nut to 12.86±0.57 g in cultivated brown Tiger nuts genotypes. The mean dry weight of tuber for the Tiger nut genotypes is 3.93±1.39 g and it ranged from 0.00±0.00 g in wild black/ brown Tiger nuts to 8.03±1.52 g in cultivated brown Tiger nut genotypes.

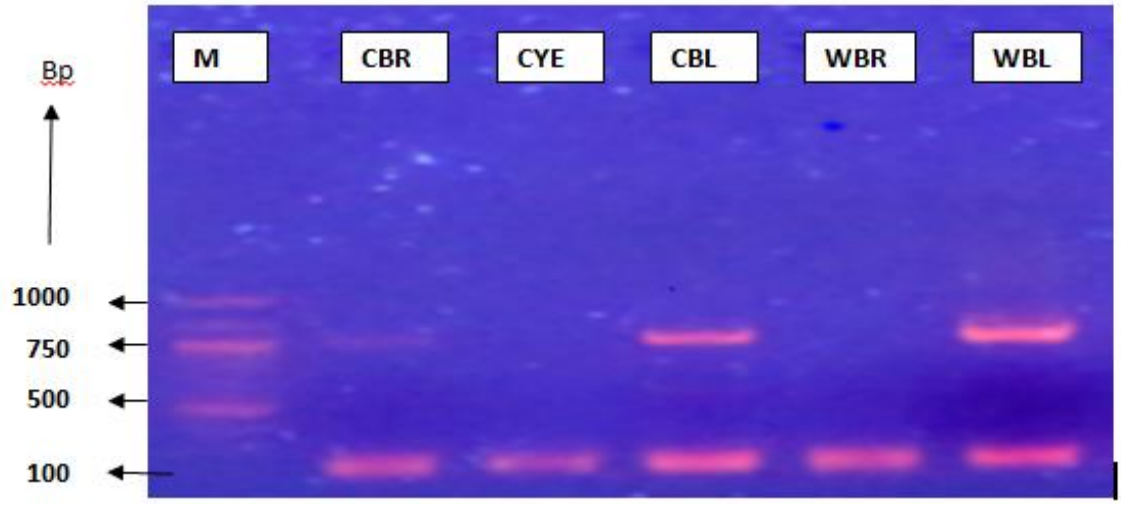

Figure 1: Electrophoregram showing band sizes of amplicons of rbcl and rps in wild and cultivated Tiger nuts.

M= DNA ladder, Bp = base pairs

CBR = cultivated Brown Tiger nuts

CYE=cultivated yellow Tiger nuts

CBL=cultivated black Tiger nuts

WBR =wild brown Tiger nuts

WBL = wild black Tiger nuts

Table 2: Genetic polymorphism among *Cyperus* species.

DNA size	CBR	CYE	CBL	WBR	WBL	Condition
100bp	1	1	1	1	1	Monomorphic
500bp	0	0	0	0	0	Monomorphic
800bp	1	0	1	0	1	Polymorphic
1000bp	0	0	0	0	0	Monomorphic

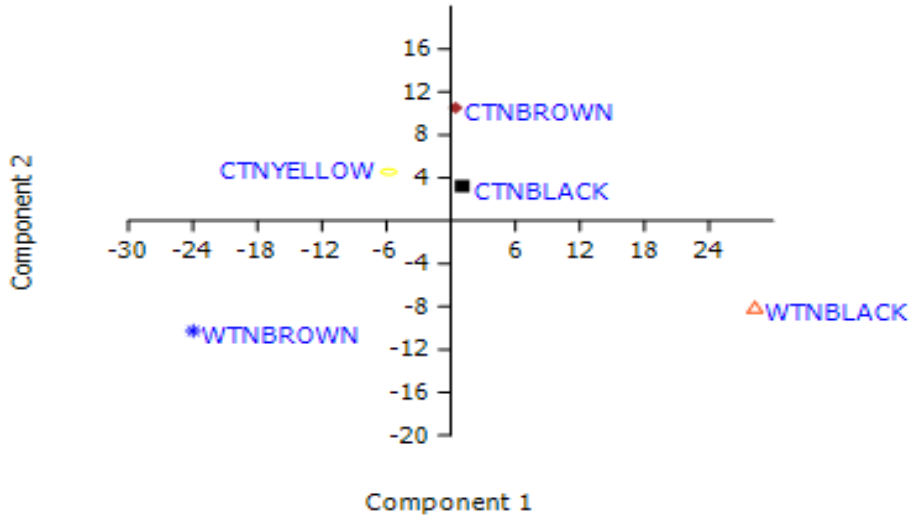

Table 3: principal component analysis of wild and cultivated Tiger nuts species.

PC	Eigenvalue	% variance
1	352.361	80.771
2	78.055	17.892
3	3.64658	0.8359
4	2.18456	0.50076

	PC 1	PC 2	PC 3	PC 4
CTNBROWN	0.42101	10.526	1.5574	-1.5606
CTNYELLOW	-5.7872	4.5531	0.75502	2.421
CTNBLACK	1.0632	3.2379	-3.3255	-0.25524
WTNBROWN	-23.977	-10.266	0.40952	-0.61336
WTNBLACK	28.28	-8.0508	0.60355	0.0082273

	PC 1	PC 2	PC 3	PC 4
Plant height	0.04328	-0.21346	0.24576	0.63313
Tillers/plant	0.025891	0.015581	0.21612	-0.14386
Leaves/plant	-0.012293	0.058501	0.20728	-0.30153
Leaf length	0.051496	0.0144	-0.068419	0.11322
Leaf width	0.0045214	0.0047478	-0.085999	0.032003
Leaf area	0.069331	0.033125	-0.72307	0.29589
% Germinatior	0.99392	0.048895	0.046304	-0.042781
Tubers/plant	-0.022833	0.38696	0.48023	0.39051
100 Tubers wei	-0.021428	0.47151	-0.2745	0.2067
Fresh tuber we	-0.022561	0.63172	-0.0419	-0.34168
Dry tuber weig	-0.021247	0.41942	0.071809	0.26896

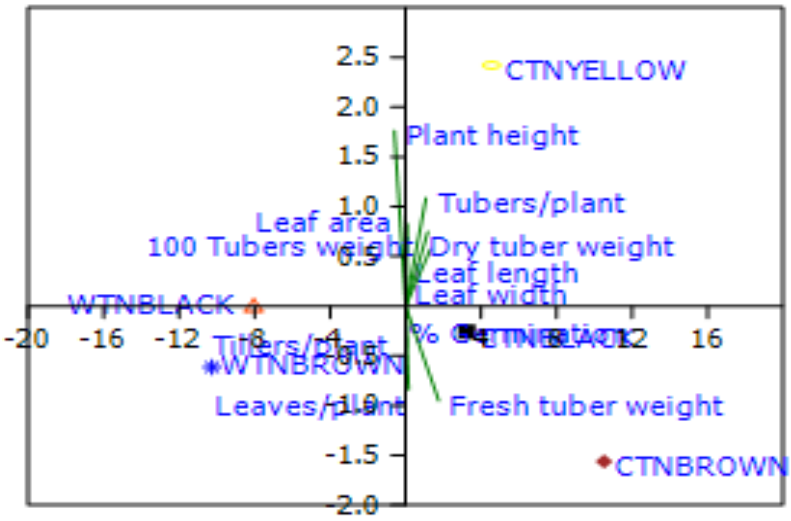

Table 4: Genetic Diversity indices among *Cyperus* species.

Primers	NPB	MAF	AL	He	NEI's GD	PIC
Rbcl	5	0.64	10	0.89	0.88	0.78
EST	3	0.75	6	0.64	0.76	0.61
Rps	3	0.68	6	0.60	0.72	0.76

The dendrogram in figure 2 shows that the wild and cultivated Tiger nuts genotypes belongs to two major clusters 1 and 2. Clusters 1 is made up of two wild (Black and Brown) Tiger nuts genotypes with same genetic distance. Cluster is made of the three cultivated (Black, Brown and Yellow) Tiger nuts genotypes also with same genetic distance and cluster one member. Each of the two clusters is a monophyletic group with only members of same species constituting members of the clusters.

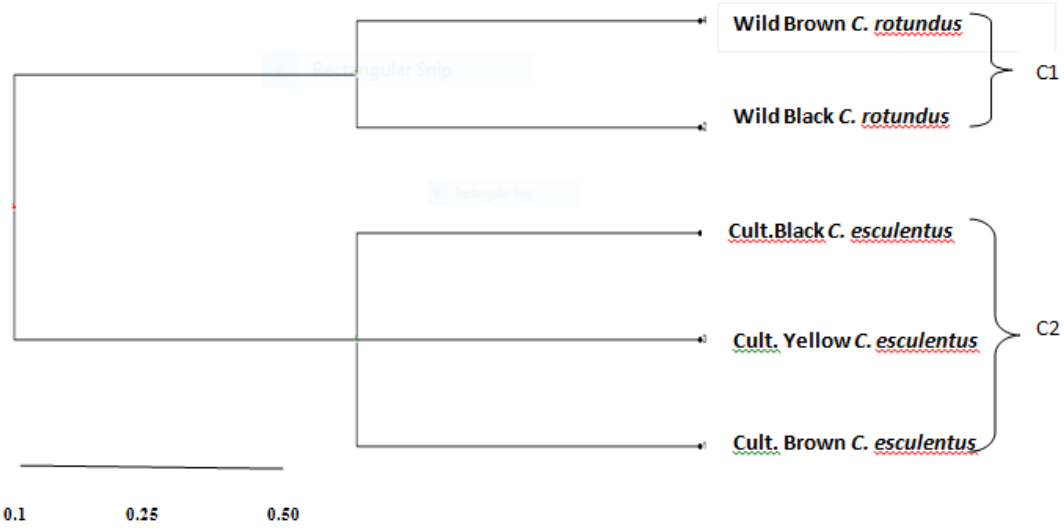

Figure 1: Dendrogram showing clustering pattern among wild and cultivated Tiger nuts species.

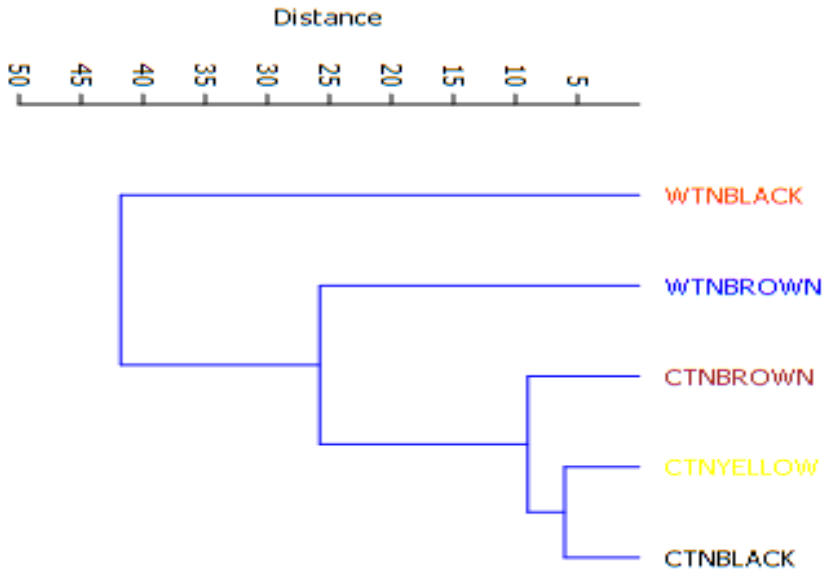

Figure 2: Dendrogram showing genetic distance among wild and cultivated Tiger nuts species.

A = Yellow cultivar **B = brown cultivar** **C = black cultivar**

Table 5: Growth Attributes of Cultivated and Wild Tiger nuts (*Cyperus species*).

	Plant height (cm)	No. tillers /plant	Leaves per plant (n)	Leaf Length (cm)	Leaf Width (cm)	Leaf Area (cm2)
CTN Brown	10.34^{d}±0.57	4.67±1.85	8.77^{a}±1.31	7.86^{b}±2.11	0.53±0.52	4.16^{d}±1.43
CTN Yellow	13.67^{b}±1.33	3.67±0.75	7.13^{b}±3.76	7.9^{b}±1.32	0.67±0.65	5.29^{c}±0.32
CTN Black	11.55^{c}±2.09	3.33±0.57	6.93^{b}±0.83	8.21^{a}±0.87	0.96±0.20	7.88^{a}±0.57
WTN Brown	14.04^{b}±1.00	3.33±1.00	7.33^{b}±2.42	6.43^{c}±0.00	0.45±0.03	2.89^{e}±0.88
WTN Black	16.27^{a}±1.33	4.67±1.00	6.67^{b}±1.56	9.21^{a}±0.00	0.72±0.00	6.63^{b}±0.12
Mean	**13.174**	**3.934**	**7.366**	**7.922**	**0.666**	**5.37**
LSD (0.05)	**1.04**	**NS**	**1.20**	**1.50**	**NS**	**1.25**

CTN = Cultivated tiger nuts; WTN = Wild Tiger nuts

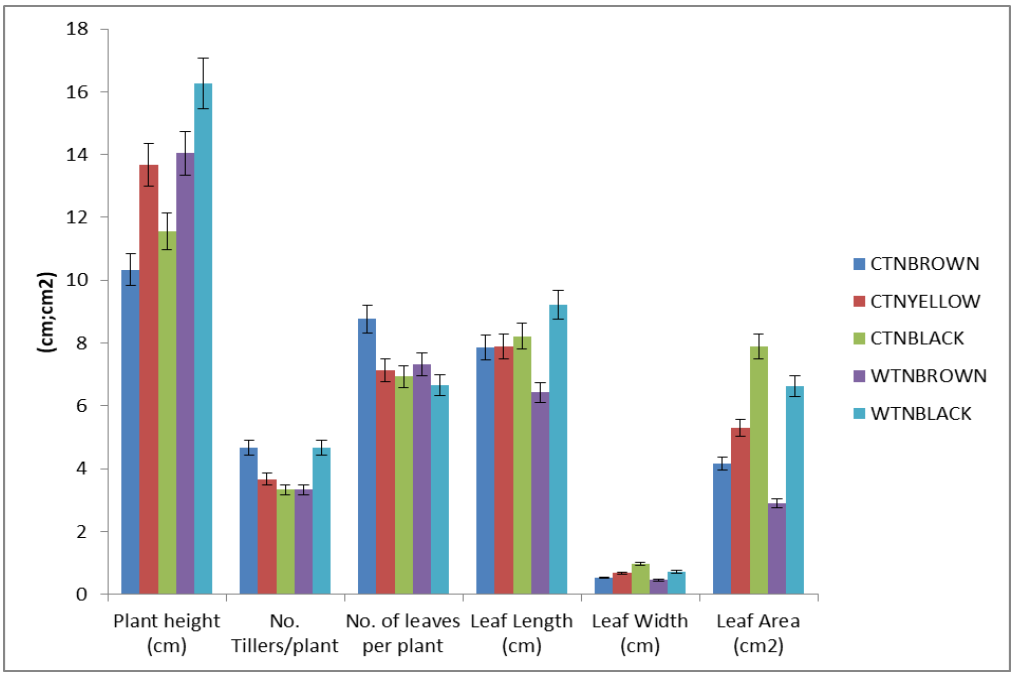

Figure 3: Morphological attributes/markers of wild and cultivated Tiger nuts.

Table 6: Yield attributes of cultivated and wild Tiger nuts (*Cyperus species*)

	% Germination	No. tubers/ plot	100 Tuber weight (g)	Fresh wt. of Tuber/plot (g)	Dry wt. of Tubers/plot (g)
CTN Brown	60.00^{a}±11.54	7.67^{a}±1.85	8.77^{a}±1.31	12.86^{a}±2.11	8.03^{a}±1.52
CTN Yellow	53.33^{a}±13.33	6.67^{a}±3.75	7.13^{a}±3.76	7.90^{a}±4.32	6.67^{a}±3.65
CTN Black	60.00^{a}±23.09	3.00^{a}±0.57	6.93^{a}±0.83	8.00^{a}±0.87	4.96^{a}±0.20
WTN Brown	34.64^{b}±20.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00
WTN Black	86.67^{a}±13.33	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00	0.00^{b}±0.00
Mean	68.00	3.47	4.56	5.25	3.93
LSD (0.05)	51.04	5.72	5.51	6.62	5.36

CTN = Cultivated tiger nuts; WTN = Wild Tiger nuts

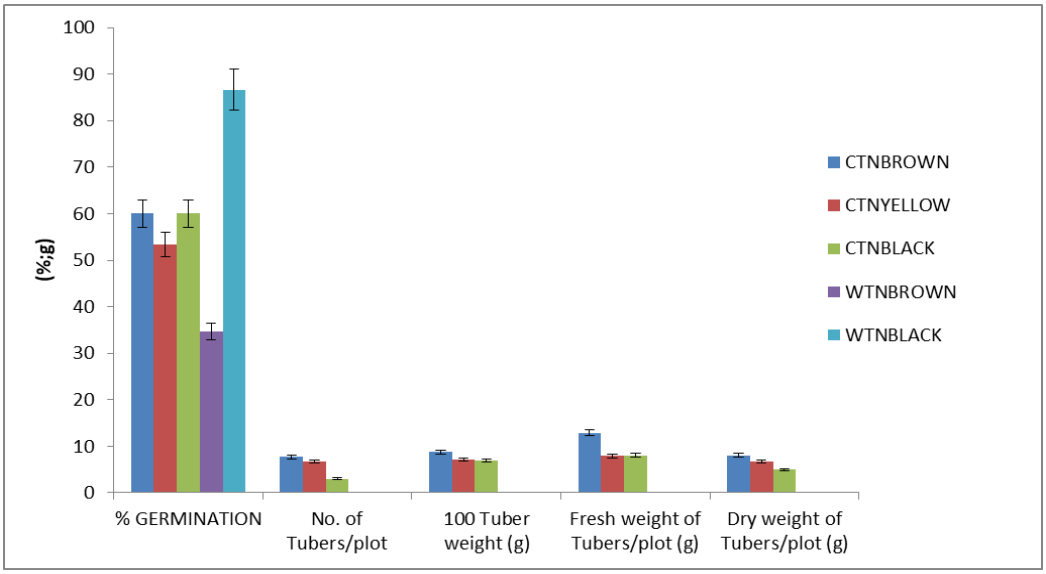

Figure 5: Yield attributes of Cultivated and wild Tiger nuts.

Table 4: Correlation coefficients among growth and yield attributes of cultivated and wild tiger nuts.

	Plant height	Tillers/plant	Leaves/plant	Leaf length	Leaf width	Leaf area	% Germination	Tubers/plant	100 Tubers wei	Fresh tuber we	Dry tuber weig
Plant height		0.8955	0.15666	0.64996	0.87872	0.92524	0.58395	0.18838	0.078132	0.049951	0.10763
Tillers/plant	0.082167		0.52219	0.30418	0.70993	0.99062	0.17308	0.73576	0.95758	0.79068	0.85762
Leaves/plant	-0.73549	0.38501		0.56869	0.36585	0.33664	0.6703	0.22156	0.33978	0.19969	0.2734
Leaf length	0.27857	0.5811	-0.34577		0.28589	0.11314	0.0061368	0.99908	0.92137	0.94857	0.95425
Leaf width	-0.095401	-0.22986	-0.52299	0.59888		0.0087532	0.46476	0.97065	0.63374	0.78375	0.79608
Leaf area	0.058748	0.0073655	-0.55018	0.7883	0.96225		0.22534	0.89787	0.76391	0.88834	0.90528
% Germination	0.33302	0.71674	-0.26197	0.97024	0.43446	0.66012		0.87788	0.90698	0.93369	0.89365
Tubers/plant	-0.69971	0.20907	0.66409	-0.00072142	-0.023056	-0.080298	-0.09606		0.024979	0.02159	0.005013
100 Tubers wei	-0.83543	0.03332	0.54723	0.061794	0.29186	0.18651	-0.073121	0.92376		0.0032301	0.0018832
Fresh tuber we	-0.87842	0.16515	0.68738	0.040404	0.17068	0.087814	-0.052106	0.93087	0.98062		0.0036312
Dry tuber weig	-0.79539	0.11206	0.61121	0.035939	0.16086	0.074464	-0.083624	0.974	0.98648	0.97904	

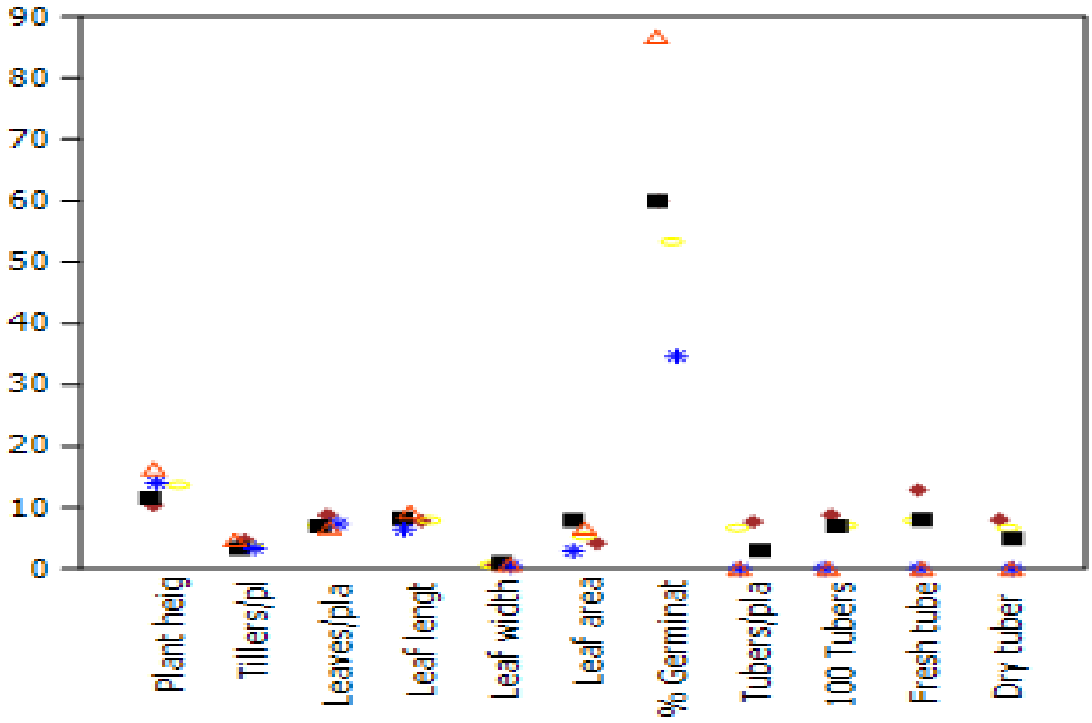

Figure 6: Jitter plot showing performance of growth and yield attributes of wild and cultivated Tiger nuts species.

DISCUSSION

The results of the present study had shown that the wild and cultivated Tiger nuts differed significantly in their growth and yield attributes as well as in their molecular attributes. Three principal components contributed to 100 % of total variations that existed in the population of wild and cultivated total nuts. The molecular bands showed that the wild and cultivated black Tiger nuts were amplified by 800bps while other genotypes were amplified at 100bps by the rbcl primer. The polymorphic information contents also varied and high in the rbcl primer. Heterozygousity was also high and as well as the Nei's gene diversity informativeness of the genotypes was greatly revealed by the markers. The study of the present findings is in line with the previous report.[24]

Cyperus esculentus is a sub-cosmopolitan weed, tolerating cold temperatures, but being more common in warmer zones[24]. It grows on several substrates and in numerous, usually not too dry habitats including fields and human-controlled environments.[18] As a consequence of its ecological plasticity and wide distribution, tiger nut is remarkable variable, with several morphotypes; this variability is exhibited as numerous specific and infraspecific taxa.

Also, according to a complete morphometric analysis performed Schippers and colleagues[25] that was not accepted in a researcher in a previous report[26]; four varieties of the wild type are currently recognized; which are *Cyperus esculentus var. esculentus, Var. heermannii (Buckley) Britton, Var. leptostachyus Boeckeeler* and *Var. macrostachyus Boeckeler.* These four varieties have definite geographical origin and distributions. They are considered as wild or non-edible tiger nuts.

The origin of the cultivated type *Cyperus esculentus Var. sativus Boeckeler* was located in the Mediterranean area, where it has been cultivated for its edible tubers since pre-dynastic Egypt.[11] At present, it is still cultivated for food and medicinal use in Southern Europe, Africa and Asia. It is important to note that *Cyperus esculentus Var. sativus* is also regarded as a cultivar of *Var. esculentus* (i.e. chufa).[27]

The present study results is in line with a previous study[28] that the phylogenetic position and biogeography of tiger nut with the objective of contributing new data to increase our understanding of evolutionary history of this invasive species. Our lateral goal is to understand possible relationships among its varieties. To address these goals, we employed the novel sequencing of molecular markers and data sets.[29,30] The molecular region chosen for this study are located both in biparaental nuclear DNA (nuclear ribosomal DNA (nrDNA) and in uniparental plastid DNA. The plastid genome of most poales is maternally inherited, reflecting gene flow by seeds (Harris and Ingram 1991); the chosen molecular regions include two genes (rbcL and nadhF) and one intron (rps16), which have already been employed in *Cyperus* and C4 phylogenies[31]. In the nuclear geome, the chosen sequence, which was a variable fragment of the external transcribed spacer 1 (ETSIf), has already been used to study phylogenetics of C3 and C4 *Cyperus.*[32,33]

CONCLUSION

The results of the present study had shown that the wild and cultivated Tiger nuts differed significantly in their growth and yield attributes as well as in their molecular attributes. Three principal components contributed to 100 % of total variations that existed in the population of wild and cultivated total nuts. The molecular bands showed that the wild and cultivated black Tiger nuts were amplified by 800bps while other genotypes were amplified at 100bps by the rbcl primer. The polymorphic information contents also varied and high in the rbcl primer. Heterozygousity was also high and as well as the Nei's gene diversity informativeness of the genotypes was greatly revealed by the markers.

Based on the findings of the study, the cultivated Tiger nuts can be used to improve the wild relatives and make them edible for increase food availability and security.

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