International Journal of Genetics and Genomics
2023; 11(1): 18-26
http://www.sciencepublishinggroup.com/j/ijgg
doi: 10.11648/j.ijgg.20231101.13
ISSN: 2376-7340 (Print); ISSN: 2376-7359 (Online)

Study of the Genetic Diversity of Tiger Nut (*Cyperus esculentus*) Ecotypes of Niger Using SSR Markers

Bori Haoua[1, *], Idi Saidou Sani[2], Zangui Hamissou[3], Adam Toudou[3]

[1]Irrigated Crops Department, National Institute of Agronomic Research of Niger, Niamey, Niger

[2]Faculty of Agronomic Science, University of Diffa, Diffa, Niger

[3]Faculty of Agronomy, Abdou Moumouni University, Niamey, Niger

Email address:

haoua82bori@gmail.com (Bori Haoua), zanguiagro@gmail.com (Zangui Hamissou), ssaidouidi@gmail.com (Idi Saidou Sani), ztoudouadam@yahoo.com (Adam Toudou)

[*]Corresponding author

To cite this article:

Bori Haoua, Idi Saidou Sani, Zangui Hamissou, Adam Toudou. Study of the Genetic Diversity of Tiger Nut (*Cyperus esculentus*) Ecotypes of Niger Using SSR Markers. *International Journal of Genetics and Genomics*. Vol. 11, No. 1, 2023, pp. 18-26. doi: 10.11648/j.ijgg.20231101.13

Received: February 9, 2023; **Accepted**: February 25, 2023; **Published**: March 9, 2023

Abstract: Tiger Nuts (*Cyperus esculentus* L.), a member of the Cyperaceae family, is a plant cultivated in Niger for its tubers. The present study focuses on the molecular characterization of 10 Tiger Nuts (*Cyperus esculentus* L.) ecotypes from Niger. These Tiger Nuts ecotypes were collected in the Tiger Nuts producing regions of Niger (Dosso and Maradi). DNA extraction and genetic diversity analysis were carried out at the molecular biology laboratories of ICRISAT, Hyderabad, India. Codominant nuclear and polymorphic microsatellite (SSR) markers were used. The genetic diversity parameters calculated are: polymorphism rate (P) at the 95% threshold, allelic diversity (A), observed (Ho) and expected (He) heterozygosity rate and the panmixy deviation in a subpopulation (Fis) under the Hardy-Weinberg hypotheses. The results showed that genetic diversity ranged from 0.03 (StvCyR_181a and StvCyR_327ska) to 0.60 (StvCyR_93ska) with a mean of 0.28 and. For the Tiger Nuts producing regions, the average deficit in heterozygotes is highly significant, with a mean Fis of 0.415 (Fis, 1000 permutation test, $P<0.001$). A significant amount of variability (27%) from differentiation between the ecotypes studied was observed in the analysis of molecular variance. These results clearly show a genetic differentiation between the populations of the large, small and wild Tiger Nuts, grouping them into three distinct groups. The study also showed that the genetic structure of the Tiger Nuts is not linked to a particular geographical origin and that the molecular tests give us evidence of conserved variability in the Niger Tiger Nuts ecotypes.

Keywords: Tiger Nuts (*Cyperus esculentus* L.), Ecotypes, Genetic Diversity, SSR Markers, Regions, Niger

1. Introduction

Variability in individual phenotypes is a feature of the living world [1]. Some of these variations are expressed at the phenotypic level (morphology, physiology, behaviour, etc.). Others are hidden and their detection requires the use of biochemical and molecular biology techniques adapted for this purpose (variability of proteins or DNA sequences). This variation is called polymorphism and can be measured using genetic markers [1-3]. Organisms can modify their development, physiology and evolutionary history according to the environmental conditions in which they find themselves. These responses are specific to the traits under consideration and to environmental resources and represent characteristics that vary between genotypes, populations and species [4]. This ability for a given genotype to express different phenotypes in different environments is called phenotypic plasticity [5].

Yellow Tiger Nuts (*Cyperus esculentus*) is a plastic species, and this plasticity can therefore have consequences for the intra-specific determination of specimens [4]. The effect of the environment can profoundly change the appearance of the plant. Plasticity can also easily lead to a wide variety of morphological forms resulting from interactions between the environment and the different genotypes [4]. Genetic markers provide information on the genotype of an individual and are

not modified by the environment [6, 7]. They can be used throughout an experiment and are observable at any stage of plant development and on any organ (the genetic information of the plant is contained in its entirety in all cells). They can give results after a single collection of vegetative material from the field [8]. The agro-morphological characterization of Niger Tiger Nuts ecotypes has allowed them to be distinguished in terms of their morphological variability and agronomic potential [9]. The present study aims to carry out a molecular characterization to understand the organization of the structure and genetic diversity of Niger Tiger Nuts using codominant and polymorphic nuclear markers of the microsatellite type. These microsatellites are highly polymorphic by variation in the number of repeats and therefore very informative. Moreover, they are easily detectable by the polymerase chain reaction (PCR) technique and reproducible [10].

2. Material and Methods

2.1. Plant Material

The plant material consists of 150 Tiger Nuts samples (15 samples per ecotype) grouped into 10 ecotypes (Table 1). The ecotypes studied were collected in November 2019. Table 1 lists the ecotypes, their types, collection sites and provenance.

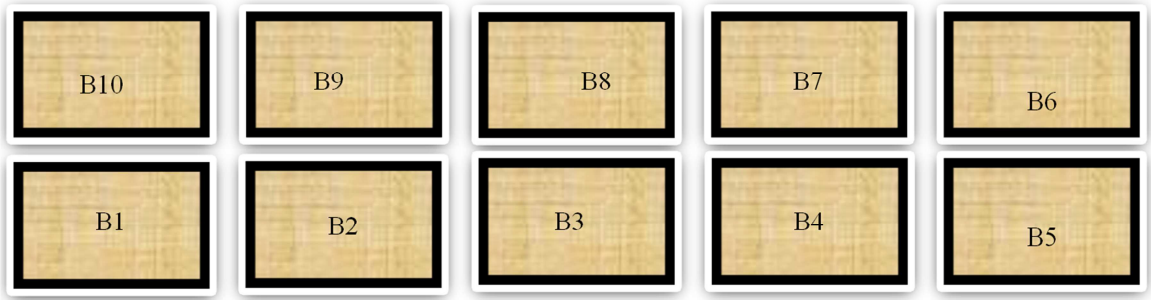
Table 1. *Different Tiger Nuts ecotypes selected for molecular analysis.*

Sample numbers	Type	Collection site	Origin
E1	Yellow Tiger Nuts	FA/Essay	Maradi
E2	Yellow Tiger Nuts	FA/Essai	Maradi
E3	Yellow Tiger Nuts	FA/Essai	Dosso
E4	Black Tiger Nuts	FA/Essai	Maradi
E5	Northern Tiger Nuts	FA/Essai	Dosso
E7	Wild Tiger Nuts	Rijia Samna	Dosso
E11	Yellow Tiger Nuts	Dan Tsoutsou	Maradi
E15	Northern Tiger Nuts	Dan Toudou	Maradi
E16	Northern Tiger Nuts	Rijia Samna	Dosso
E18	Northern Tiger Nuts	Guidan Moussa	Maradi

FA/Essay: material produced in trials at the Faculty of Agronomy of Niamey, E: ecotype

2.2. Experimental Set-up

The trial was set up in ten (10) test trays. Tubers of the 10 Tiger Nuts ecotypes were germinated at a rate of 30 bunches per ecotype and per tray. (Figure 1).



B1: E11, B2: E4, B3: E2, B4: E18, B5: E16, B6: E5, B7: E15, B8: E1, B9: E3, B10: E7

Figure 1. *Trial design for biomass production.*

2.3. Collection of Extraction Samples

The samples are young leaves from two-week-old plants (Figure 2). The young leaves of each plant were cut with clean scissors, then put into numbered envelopes and stapled. Each batch of ecotypes was placed separately in plastic with sillicagel and incubated at 37°C to allow the leaves to dry out while retaining their DNA.

Figure 2. *Germinating yellow Tiger Nuts seedlings (arrows) and seedlings at collection.*

2.4. DNA Extraction of the Studied Ecotypes

DNA extraction was done on the leaves of two-week-old seedlings using the "MATAB" protocol (Doyle and Doyle 1990).

2.5. Parameters Studied

2.5.1. Descriptive Parameters of Genetic Diversity

The following genetic diversity parameters were calculated: polymorphism rate (P) at the 95% threshold, allelic diversity (A), observed (Ho) and expected (He) heterozygosity rate, and subpopulation panmixity deviation (Fis) under Hardy-Weinberg assumptions.

2.5.2. Deviation from Panmixity

This gap is measured within a sub-population by [11]. IS fixation index [12] and is calculated by the following formula:

$$F_{IS} = 1 - \frac{Ho}{He} \text{where,}$$

Ho is the proportion of heterozygous individuals observed on average in a sub-population,

He is the theoretical heterozygosity expected in a panmixed population.

(i). Allelic Diversity

Allelic diversity (A) was calculated as the ratio of the total number of alleles to the number of loci surveyed.

A = (Total number of alleles) / (Number of loci surveyed)

(ii). Polymorphism Rate

The polymorphism rate (P) is an estimate of the number of polymorphic loci in relation to the total number of loci studied. It is considered here that a locus is polymorphic when the most frequently occurring allele has a frequency of less than 95%.

P= (Number of polymorphic loci) / (Number of loci studied)

(iii). Genetic Diversity Structure

Genetic differentiation (Fst) between genetic groups and populations was calculated using the model of [13].

2.6. Statistical Analysis of Data

2.6.1. Genetic Diversity

All parameters for assessing the diversity of Yellow Tiger Nuts were calculated using GenAlex 6.5 software [14]. Data analysis was carried out at the country, regional, ecotype and type levels.

2.6.2. Relationship Between Niger Tiger Nuts

To identify the genetic relationships between the different Tiger Nuts ecotypes, a genetic distance matrix, based on simple matching distance, was calculated using the Darwin V.5.158 software [15]. Another distance matrix was calculated using the adegenet 2.1.1 implementation package in R.3.5.1 to confirm the observed genetic relationships. Two types of analysis and graphical representations of these distance matrices were performed: a principal component analysis (PCA) and a dendrogram constructed using the nearest neighbours (NJ) method [15]. In addition, another principal coordinate discriminant analysis (PCDA) was performed using the R.3.5.1 package adegenet to confirm the genetic structuring of the collection of Niger Tiger Nuts ecotypes.

2.6.3. Bayesian Approach

In order to determine the number of genetically homogeneous groups or types, an assignment method was used following a Bayesian approach. For this purpose, the software STRUCTURE 2.3.4 [16, 17] was used.

3. Results

3.1. Genetic Polymorphism

Genetic diversity parameters were calculated with 15 microsatellite (SSR) loci (Table 2) for the 150 samples of the Yellow Tiger Nuts ecotypes. Three loci were found to be monomorphic: StvCyR_45a, StvCyR_483a and StvCyR_604a. The three monomorphic loci were removed from the analyses. The dataset thus consists of 143 individuals with the 12 markers retained as polymorphic at the 95% threshold. In total, 51 alleles were detected for all 143 samples with an average of 4.25 alleles per locus. The number of alleles obtained for each locus analysed varied from 2 alleles for the markers StvCyR_181a and StvCyR_551a to 8 alleles for the marker StvCyR_577a (Table 2).

Table 2. *Characteristics of the microsatellite markers used.*

Locus	Types	Fluorophore	Forward Primer Séquence (5'-3')	Reverse Primer Sequence (5'-3')	Type of repeat
StvCyR_45a	Nuc	Yelow	AAGGGAAGGTTCAAAGCTAAATGC	AATGAAATTTGAAACTCAGGCTCG	(CT) 12
StvCyR_64a	Nuc	Red	GTACAAACCACAAACCGTAGACCC	ACTCTCCTCCTCCATCGTAAGCTC	(GA) 5
StvCyR_104a	Nuc	Blue	GCTAGCATGTAACCCCTGTCTCTTAG	GTCTGTCTCCCTCCCTCTTCTCTC	(AT) 6
StvCyR_116c	Nuc	Yelow	TTTATTGGATTTTTGTGGGGACTG	AACACTGTAAGAGGCTGCTATGGG	(AG) 5
StvCyR_181a	Nuc	Blue	TCAATAGAAGAATCCCACTCAGCC	GTGGAGGTAAAGATCAGCAACCAG	(GATA) 5
StvCyR_197a	Nuc	Red	TCGTGAAACATGGATACAATCAGG	GACCTGACCTGACCCAAAACC	(AT) 7
StvCyR_476a	Nuc	Blue	CATGATAGTGTAGCCAAACGCAAC	AAAGGATAGTTTTGATTCACCGGC	(TC) 5
StvCyR_483a	Nuc	Green	TGTGTTGTGTGGAAGAGAGGAGAG	AACAAACCTCAGAACTCAACTGCC	(GA) 7
StvCyR_551a	Nuc	Blue	GAAATTTGGAGGTGATTCGATATG	AGTTTATGAGGAAGCAGAGAAACATC	(AT) 6
StvCyR_590a	Nuc	Green	TCTGAGGGACTAAATGCTAATGTTTTAC	TCAGAAAAGATAACGCGTAGCAG	(CTT) 5

Locus	Types	Fluorophore	Forward Primer Séquence (5'-3')	Reverse Primer Sequence (5'-3')	Type of repeat
StvCyR_604a	Nuc	Yelow	AAAACCCTTGAAGAAACCAAAACC	ACCTAGAGAGTCAGCTTCAGCACC	(CT) 3
StvCyR_327ska	Nuc	Green	CATTAGACTTCGCTCTCATCTCTGG	AGGGGAGGAGTGGGATTTATAGAG	(AGGGG) 3
StvCyR_494ska	Nuc	Red	GACTATCCGTGAAGATATGTTTGGC	CAGTTGGCTACCTTGACCCTTG	(AACTT) 4
StvCyR_577a	Nuc	Yelow	TTTGGTTACTTTGGTTTCAAGATAGAAG	ACCACAAATTCATGAGGTCTTCAG	(TC) 4
StvCyR_93ska	Nuc	Green	GCAGATATGCCTCTTCAGAGTTCAG	CAGGAGGACATTGTTGTAAGAGGG	(AATGG) 4

3.2. Analysis of Genetic Diversity at the Country Level

The highest number of alleles is 8 and was observed in the StvCyR_577a marker. The observed heterozygosity is on average 0.17 (Table 3). It varies from 0 (StvCyR_104a, StvCyR_116c and StvCyR_476a) to 0.77 (StvCyR_93ska). The average genetic diversity or expected heterozygosity (He) is 0.28 and varies from 0.03 (StvCyR_181a and StvCyR_327ska) to 0.60 (StvCyR_93ska). The majority of loci show a highly significant (P<0.001) heterozygote deficit. The average heterozygote deficit is significant, with a Fis of 0.54. The heterozygote deficit being very important, the Hardy-Weinberg equilibrium is not respected at the 0.05 threshold, meaning an excess of homozygotes. On the other hand, the StvCyR_590a and StvCyR_93ska loci show a very significant excess of heterozygotes (P<0.001) with Fis of -0.17 and -0.27 respectively.

Table 3. *Genetic diversity of 143 Tiger Nuts samples at 12 SSR loci.*

Locus	N	RA	I	Ho	He	Fis
StvCyR_64a	142,00	3,00	0,50	0,08	0,30	0,74***
StvCyR_104a	143,00	4,00	0,37	0,00	0,16	1,00***
StvCyR_116c	141,00	4,00	0,19	0,00	0,07	1,00***
StvCyR_181a	143,00	2,00	0,09	0,02	0,03	0,39***
StvCyR_197a	143,00	4,00	0,79	0,01	0,50	0,97***
StvCyR_476a	143,00	4,00	0,33	0,00	0,13	1,00***
StvCyR_551a	142,00	2,00	0,34	0,16	0,19	0,17***
StvCyR_327ska	143,00	3,00	0,10	0,02	0,03	0,39***
StvCyR_494ska	142,00	5,00	0,92	0,27	0,50	0,45***
StvCyR_577a	135,00	8,00	0,62	0,05	0,26	0,80***
StvCyR_590a	133,00	7,00	1,17	0,67	0,57	-0,17***
StvCyR_93ska	94,00	5,00	1,10	0,77	0,60	-0,27***
Average	137,00	4,25	0,54	0,17	0,28	0,54***
Minimum	94,00	2,00	0,09	0,00	0,03	
Maximum	143,00	8,00	1,17	0,77	0,60	

RA = Allelic richness, H = Expected heterozygosity, Ho = Observed heterozygosity, Fis = Fixation index, I = Shannon diversity index. Percentage of polymorphism: 100%.

3.3. Genetic Diversity at the Regional Level

The allelic richness of the Maradi region is higher than that observed in the Dosso region (Table 4). On the other hand, the values of their indices (I) are low and roughly equal. The observed heterozygosity for the Dosso region is low (0.238) compared to that observed in the Maradi region (0.248). The expected average heterozygosity or genetic diversity is 0.313. The mean heterozygote deficit is highly significant, with a mean Fis of 0.415 (Fis, 1000 permutation test, P<0.001) for both regions (Table 4). As the heterozygote deficit is very large for all individuals studied, the Hardy-Weinberg equilibrium is not respected. All loci were found to be 100% polymorphic at the 95% level in both Tiger Nut production regions in Niger.

Table 4. *Genetic diversity at regional level.*

Area	N	RA	I	Ho	He	Fis	%P
Dosso	55,857	3,357	0,575	0,238	0,313	0,424***	100
Maradi	77,786	4,000	0,611	0,249	0,313	0,405***	100
Avarage	66,821	3,679	0,593	0,243	0,313	0,415***	100

RA = Allelic richness, H = Expected heterozygosity, Ho = Observed heterozygosity, Fis = Fixation index,
I = Shannon diversity index.%P: percentage of polymorphism, *=Fis significant at 0.05, N = number of samples

3.4. Genetic Diversity at Ecotype Level

The average allelic richness per locus of the ecotypes is similar. The positive mean value of the fixation index indicates a very high heterozygote deficit, but lower than that observed in the wild-type ecotypes E5 (0.515) and E7 (0.400). An excess of heterozygotes was observed in the ecotypes E18 E3 and E11 (Table 5).

Table 5. *Genetic diversity at ecotype level.*

Ecotypes	N	RA	I	Ho	He	Fis	%P
E1	9,071	1,857	0,366	0,207	0,229	0,323*	71,43
E15	13,714	1,786	0,334	0,271	0,212	0,060*	57,14
E16	13,786	2,286	0,471	0,333	0,286	0,213*	85,71
E18	13,786	1,929	0,420	0,267	0,261	-0,028	50,00
E2	13,000	2,071	0,398	0,270	0,242	0,083*	64,29
E3	13,214	1,429	0,261	0,321	0,180	-0,772	35,71
E11	14,000	2,214	0,402	0,279	0,220	-0,226	42,86
E4	14,214	2,143	0,369	0,177	0,203	0,278*	78,57
E5	14,429	2,000	0,392	0,145	0,237	0,515*	71,43
E7	14,429	2,214	0,427	0,183	0,248	0,400*	78,57
Avarage	13,364	1,993	0,384	0,245	0,232	0,160*	63,57

E1, E15, E16, E18 = Large stumps; E2, E3, E4, E11= Small stumps; E5, E7= Wild stumps, RA = Allelic richness
I = Shannon Diversity Index.%P: percentage of polymorphism, *=Fis significant at 0.05, N = number of samples.

3.5. Genetic Differentiation Between Regions and Diversity Within Regions

The analysis of molecular variance (AMOVA) carried out at the level of the Yellow Tiger Nuts producing regions showed that 6% of the total variability is due to the difference between the two regions and 94% of the variability is due to intra-region differentiation (Table 6). Furthermore, the genetic differentiation Fst between the two regions is highly significant (P < 0.001).

Table 6. *Two-level analysis of molecular variance (AMOVA) for Tiger Nut production area in Niger.*

Source	df	SS	Variance	Percentage of variance (%)
Between Area	1	20.164	0.130	6
Within Area	284	617.424	2.174	94
Total	285	637.588	2.304	100

df: degree of freedom, SS: sum of squares. Fst: 0.056**

3.6. Genetic Differentiation Between Ecotypes and Intra-Ecotype Diversity

The analysis of molecular variance (AMOVA) (Table 7) shows that 27% of the variability for Yellow Tiger Nuts ecotypes is due to differentiation between them and 73% of this variability is due to differentiation within ecotypes (Table 8).

Table 7. *(2-level AMOVA for the 10 Tiger Nuts ecotypes studied.*

Source	df	SS	Variance	Percentage of variance (%)
Between ecotypes	9	175.013	0.622	27%
Within ecotypes	276	460.324	1.668	73%
Total	285	635.337	2.290	100%

df: Degree of freedom, SS: Sum of squares.

Table 8. *Pairwise genetic differentiation of Tiger Nuts ecotypes.*

Ecotype	E1	E15	E16	E18	E2	E3	E11	E4	E5	E7
E1	0.000	**	**	**	**	**	**	**	**	**
E15	0.174	0.000	ns	ns	**	**	**	**	**	**
E16	0.176	ns	0.000	ns	**	**	**	**	**	**
E18	0.188	0.024	0.030	0.000	**	**	**	**	**	**
E2	0.324	0.324	0.291	0.337	0.000	ns	ns	**	**	**
E3	0.361	0.349	0.312	0.367	0.019	0.000	ns	**	**	**
E11	0.350	0.314	0.281	0.338	0.113	0.102	0.000	**	**	**
E4	0.363	0.289	0.246	0.322	0.151	0.147	0.104	0.000	**	**
E5	0.389	0.394	0.354	0.398	0.240	0.259	0.225	0.121	0.000	ns
E7	0.399	0.410	0.369	0.415	0.292	0.313	0.256	0.199	0.036	0.000

E: ecotype

3.7. Genetic Diversity in Tiger Nut Types

The genetic diversity between and within individuals of the different types of Tiger Nuts encountered in Niger shows roughly equal values for all parameters studied. However, the allelic richness of the large (3.143) and small (3.357) Tiger Nuts is greater than that of the wild (2.643) Tiger Nuts. The diversity is much greater for the large (He=0.268, I=1.480) and wild (He=0.253, I=0.450) Tiger Nuts compared to that of the small (He=0.242; I=0.468) Tiger Nuts. Individuals belonging to all different types of pike show significant Fis indicating non-compliance with the Hardy-Weinberg equilibrium (Table 9).

Table 9. *Genetic diversity at the type level of Yellow Tiger Nut.*

Types	N	RA	I	Ho	He	Fis	%P
GrS	50,357	3,143	0,480	0,263	0,268	0,337*	85,71%
PetS	54,429	3,357	0,468	0,262	0,242	0,291*	85,71%
PetSS	28,857	2,643	0,450	0,163	0,253	0,503*	100,00%
Avarage	44,548	3,048	0,466	0,229	0,254	0,384*	90,48%

GrS =Grand Tiger Nuts; PetS =Pet Tiger Nuts; PetSS =Pet Tiger Nuts, N =? RA = Allelic richness, He = Expected heterozygosity, Ho = Observed heterozygosity, Fis = Fixation index, I = Shannon diversity index.%P: percentage of polymorphism, *=Fis significant at 0.05, N = number of samples.

3.8. Genetic Differentiation Between Types and Intra-Type Diversity of Yellow Tiger Nuts

Analysis at the level of types (large, small, wild) showed that 26% of the total variability is due to the difference between types in different regions. 5% from differentiation between individuals of different types. 69% of the variability is due to differentiation between individuals within the same type (Table 10). The Fst genetic differences between types are all highly significant ($P < 0.001$) (Table 11).

Table 10. *3-level AMOVA for the three Tiger Nut types studied.*

Source	df	SS	Variance	Percentage of variance
Between types	2	132.590	0.698	26
Between individual	140	294.662	0.129	5
Within individual	143	264.000	1.846	69
Total	285	691.252	2.673	100

df: Degree of freedom, SS: Sum of squares.

Table 11. *Fst genetic differentiation by pair of Niger Tiger Nut types.*

	GrS	PetS	PetSS
GrS	0.000	**	**
PetS	0.250	0.000	**
PetSS	0.332	0.192	0.000

GrS =Great Tiger Nuts; PetS=Little Tiger Nuts..., PetSS =Wild Tiger Nuts.

3.9. Structure

3.9.1. Genetic Relationships and Classification of Tiger Nuts Ecotypes

A proportion of the variability (27%) resulting from differentiation between the ecotypes studied was observed in the analysis of molecular variance. Will this differentiation allow these ecotypes to be divided into groups? The dendrogram (Figure 3) presents a structuring of the individuals in the entire collection at the country level. The general typology of the tree, grouping all the individuals, allows them to be divided into three (3) groups. The ecotypes of each provenance are mixed in the different groups defined according to the Neighbors Joining method, except for the ecotypes of the wild Yellow Tiger Nuts which are widely separated towards their collection site (Rijia Samna) (figure 4). These groups are divided into three genetic clusters defined according to Tiger Nuts type (Northern Tiger Nuts, Lesser Tiger Nuts and Lesser Wild Tiger Nuts).

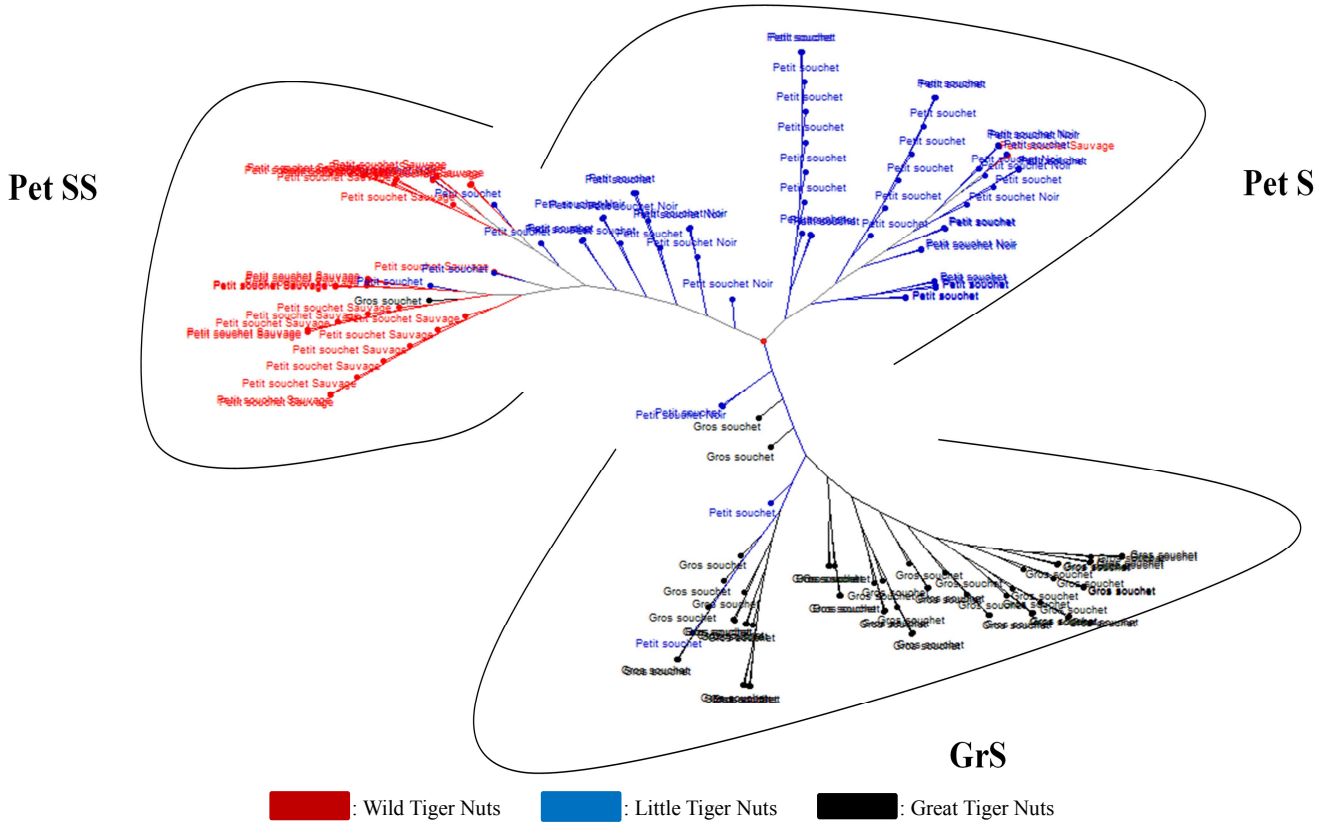


Figure 3. *Dendrogram showing the relationships between the ecotypes of Niger.*

To compare this analysis with the results of the principal coordinate discriminant analyses, the different individuals of each type of Tiger Nut in Figure 3 were coloured according to their belonging to the three populations (GrS, Pet S and Pet S). The projection of these samples in the plane formed by axes 1 and 2 of the principal component analysis (PCA) (Figure 4a and 4b) and the principal coordinate discriminant analysis (PCDA) (Figure 4c) allows the three groups to be clearly identified (Figure 4). Furthermore, it can be seen that these three genetic groups group together the three types of Tiger Tiger Nuts, i.e. Big Tiger Nuts (GrS), Small Tiger Nuts (Pet S) and Small Wild Tiger Nuts (Pet S S).

Figure 4. *(a, b): Principal Component Analysis (PCA) of Cyperus esculentus ecotypes by Tiger Nuts types. (c): Principal Coordinate Discriminant Analysis (PCDA) of Cyperus esculentus ecotypes by Tiger Nuts types.*

3.9.2. Grouping Based on the Assumption of Group Membership

The classification of Tiger Nuts ecotypes according to the group or population model led to the existence of three subgroups for K=3 (Figure 5a). Of the 143 samples with distinct genotypes, 134 had a group membership of 80% or more and could therefore be assigned to one of the three subgroups (Figure 5b). The remaining ecotypes that had subgroup membership coefficients of less than 80% were considered "unclassified" or "admixed" and were not assigned to a subgroup. Most of them shared alleles of the subgroups Pet SS (small wild Tiger Nuts); Pet S (small Tiger Nuts) and GrS (large Tiger Nuts) in proportions ranging from 0.007 to 0.75%.

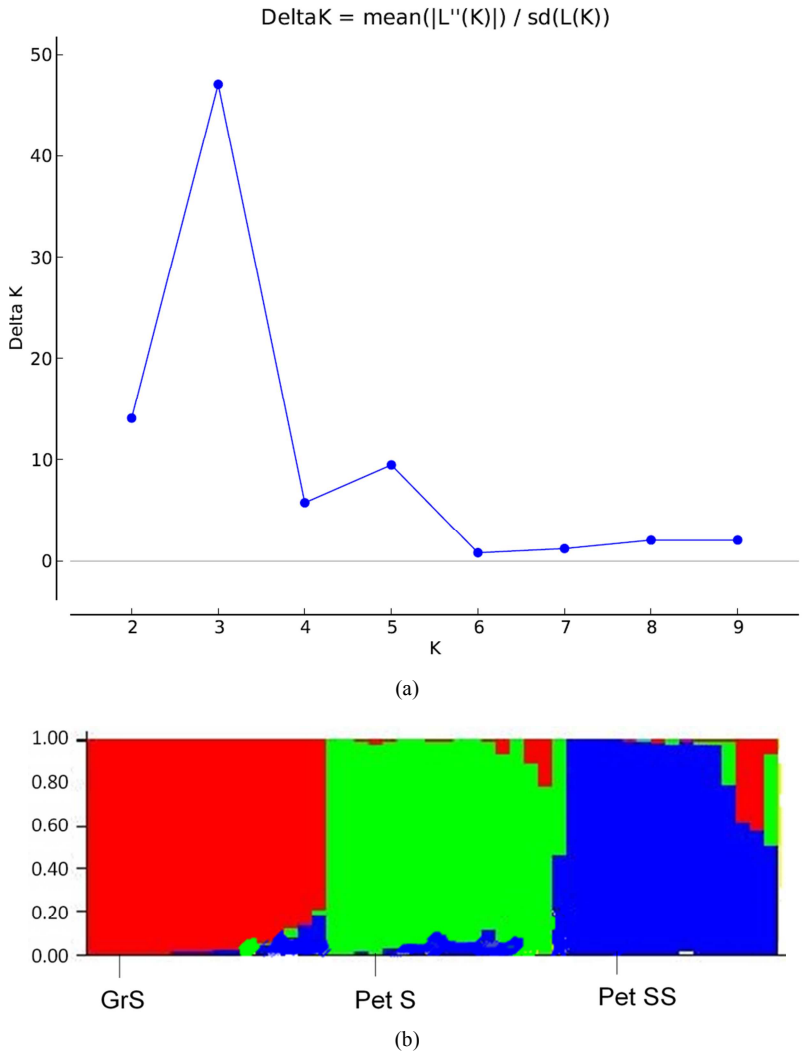


Figure 5. *a) Admixture model representation of the number of groups of nutsedge ecotypes according to the type of Niger nutsedge collection as a function of Delta K (K groups k ranging from 1 to 10 (according to the method of [18]), Delta k = f(K); b): Graphical representation of the estimation of the coefficients of membership of the three groups.*

4. Discussion

The 143 samples studied are all genetically different from each other at the 12 loci considered. This variability has been demonstrated in several studies carried out in Spain [19-20]. This could be explained by the fact that, in addition to sexual multiplication, Tiger Nuts multiplies mainly by vegetative means [4]. and is very plastic [21, 22].

The number of alleles obtained for each loci analysed varies from 2 alleles for the markers StvCyR_181a and StvCyR_551a to 8 alleles for the marker StvCyR_577a. Allelic variation observed at the microsatellite loci varied considerably between groups. The total number of alleles detected for the 143 samples is 51 with an average of 4.25 per loci. A previous diversity analysis of cultivated *Cyperus esculentus* species reported 7 alleles at 4 polymorphic loci [4]. Allelic variation is similar in tuber crops such as yam with 4 alleles per loci [23], sweet potato with 4.6 alleles per locus [24].

The average genetic diversities obtained at country, producing region, ecotype and type level of the Tiger Nut (He= 0.28, 0.313, 0.232, 0.254) are statically similar and also similar to that observed (0.237) by (Horak and al., 1987) [25]. The mean Fis values for all populations are positive and high, which is synonymous with an excess of homozygotes that may result from inbreeding of the individuals studied. Indeed, negative Fis values were observed at the StvCyR_590a, StvCyR_93ska, E18, E3 and E11 ecotypes. These excesses of heterozygotes could be due to either a negative or random association or to random selection [26] cited by (Bedjaoui, 2019) [27]. These same authors assert that vegetative propagation allows the two alleles of each locus to independently accumulate mutations and thus diverge within individuals and consequently lead to negative Fis values [27]. The allelic richness of the large (3.143) and small (3.357) strains is greater than that of the wild-type strains (2.643). This difference can be explained by sample size. Indeed, sample size has an influence on allelic richness [28]. Molecular analysis of variance at the ecotype level shows Fst values ranging from 0.019 to 0.399 and reflects low to high genetic differentiation at the population level. Based on the Fst values, 73% of the variability is due to intra-population differences at the ecotype level and 27% between populations. These results corroborate those of (Tayyar and al., 2003) [29]. who stated that populations of the yellow Tiger Nuts *Cyperus esculentus* had the highest genetic diversity compared to populations of the neighbouring species Cyperus rodondus.

The dendrogram obtained from the simple distance matching on the different ecotypes studied clearly shows a genetic differentiation between the populations of Yellow Tiger Nuts, Small Tiger Nuts and Wild Tiger Nuts. Similar observations are found in the PCA and DACP performed on the Yellow Tiger Nuts ecotypes. These three types are grouped into three clusters (I, II, III). The average genetic diversity obtained in these three types (GrS, Pet S, Pet S S) of Tiger Nuts is statistically similar. But the analysis of the genetic differentiation parameter Fst by pairs at the 95% threshold shows some dissimilarities between the ecotypes of the three groups. Indeed, when considering the genetic groups in pairs, the dissimilarities between the large (GrS) and small (Pet S S) wild-type strains (Fst = 0.332) are quite high. The dissimilarities between the small strains (Pet S) and the small wild strains (Pet S S) are low (Fst =0.192).

5. Conclusion

The molecular approach was based on microsatellite-type molecular markers, which are polymorphic and easy to use. Their genome-wide distribution, their extremely high polymorphism and their codominant character (homozygous and heterozygous can be distinguished) make them excellent genetic markers. These molecular markers, combined with other analysis techniques such as Bayesian assignments, are valuable tools for identifying structuring in populations of species such as yellow Tiger Nuts. These markers revealed a relatively high diversity within the Niger Tiger Nut ecotypes. The study showed that the genetic structure of Niger Tiger Nuts is not linked to a given geographical origin and that molecular tests provide strong evidence of conserved variability in Niger Tiger Nuts ecotypes. Rather, the different groups obtained are defined on the basis of certain agromorphological traits. The heterogeneity of the populations obtained could underline the circulation of Tiger Nuts seeds across the country. However, further studies are needed to assess the consequences of this seed flow and its importance for the adaptation and production of yellow Tiger Nuts in Niger.

References

[1] Boukary. H. 2014. Caractérisation agro-morphologique et moléculaire des écotypes locaux d'oignon (*Allium cepa* L.) du Niger, Thèse de doctorat de l'Université Abdou Moumouni, Niamey. 111pp.

[2] Rabiou Abdou., 2014. Caractérisation de la diversité génétique de cultivars d'oignon (*Alliumcepa L.*) du Niger en vue de leur conservation in situ et de leur amélioration. Thèse de Doctorat. Université de Liège-Gembloux Agro-Bio Tech. 151 p.

[3] Idi-Saidou. S., 2014. Ressources génétiques du fonio [Digitaria exilis (kippist) stapf] du Niger: évaluations agro morphologique et génétique. Thèse de doctorat unique ès-Sciences Naturelles de l'Université Abdou Moumouni de Niamey. Spécialité: Génétique de populations et amélioration des plantes/Biostatistique. 137p.

[4] Dodet M., 2006. Diversité génétique et phénologie de *Cyperus esculentus* L. (Cyperaceae) pour une gestion intégrée de l'espace dans les cultures de Haute Lande. Thèse de doctorat, Université de Bourgogne. France. 226p.

[5] Weiner J., 2004. Allocation, plasticity and allometry in plants. Persp. Plant Ecol. Evol. Syst. 207-215. 9p.

[6] Kazan K., Manners J., Cameron D., 1993. Genetic variation in agronomically important species of Stylosanthes detemined using random amplified polymorphic DNA markers. Theor. Appl. Genet. 882-888.

[7] Chebbil. H., Pascual Villalobos- M. J., Cenis J. L., Correal. E. 1995. Caractérisation morphologique et moléculaire des espèces ligneuses du genre Medicago. 191-206. 16p.

[8] Douhovnikoff. V., Dodd R. S., 2003. Intra-clonal variation and a similarity threshold for identification of clones: application to Salix exigua using AFLP molecular markers. Theor. Appl Genet. 1307-1315. 9p.

[9] Bori., 2020. Caractérisation socio-économique, agromorphologique, physico-chimique et moléculaire du Souchet (*Cyperus esculentus* L.) du Niger. These de Doctorat. Université Abdou Moumouni de Niamey (Niger), 160p.

[10] Bousba. R., Djekoun A., Duraa S., Ykhlef. N. 2013. Caractérisation moléculaire et association marqueur ssr phénotype pour la tolérance au stress hydrique chez le blé dur (Triticum durum desf). European Scientific Journal. Edition vol. 9, No. 12: 1857-7881. 16p.

[11] Wright. S., 1978. Evolution and the genetics of populations. Variability within and among natural populations. USA: University of Chicago Press.

[12] Kondombo., 2010. Diversités agro-morphologique et génétique de variétés locales de sorgho [Sorghum bicolor (L.) Moench] du Burkina Faso. Eléments pour la valorisation des ressources génétiques locales. Thèse de Doctorat. L'université De Ouagadougou. 158p.

[13] Weir B., Cockerham C., 1984. Estimating F-statistics for the analysis of population structure. Evolution. 1358-1370. 14p.

[14] Peakall. R., Smouse. P., 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research-an update. Bioinformatics 2537-2539.

[15] Perrier. X., Jacquemoud-Collet J., 2006. Darwin software. http://darwin.cirad.fr/darwin

[16] Pritchard. J., Falush. D., Stephens. M., 2002. Inference of population structure in recently admixed populations. American Journal of Human Genetics. 177-179.

[17] Falush. D. Stephens. M. Pritchard. J., 2003. Inference of population structure using multilocus genotype data: linked loci and correlated allele frequencies. Genetics. 1567–1587. 11p.

[18] Evanno G., Regnaut S., Goudet J., 2005. Detecting the number of clusters of individuals using the software structure: a simulation study. Mol. Ecol. 2611–2620.

[19] Pascual. B., Maroto. J., 1993. Estudios de tipificacion y seleccion de Ifneas clonales cultivadas de *Cyperus esculentus* L. Actas II Congreso Iborico de Ciencias hortfcolas. 1015-1020. 7p.

[20] Abad. P., Pascual. B., Maroto J., Lopez-Galarza. S., Vicente. M., Alagarda. J., 1998. RAPD analysis of cultivated and wild yellow Tiger Nuts (*Cyperus esculentus* L.). Weed Sciences. 46: 318–321. 5p.

[21] Barret. S., 1982. Genetic variation in weeds. in R. Cha rudattan and H. L. Walker.eds. Biological Control of Weeds with Plant Pathogens. New York: J. Wiley. 25p.

[22] Holt. J. S., 1994. Genetic variation in life history traits in yellow Tiger Nuts (*Cyperus esculentus*) from California. Weed Sciences. 378–384.

[23] Adoukonou-Sagbadja. H., Antoine A., Paulin S., Rollande A., Geoffroy K., Corneille A., Clément A. 2014. Variabilité génétique des accessions d'igname introduites au Bénin à partir des îles du Sud-Pacifique. J. Appl. Biosci. 14p.

[24] Favoretto. P., Elizabeth Ann V., Paulo César T., 2011. Molecular characterization of potato cultivars using SSR markers. Horticultura Brasileira. 542-547. 6p.

[25] Horak. Michael J., Jodie S. Holt and Norman C. Ellstrand., 1987. Genetic Variation in Yellow Nutsedge (*Cyperus esculentus*). Weed Science, Vol. 35, No. 4. pp. 506-512.

[26] Hartl. D., Clark. A., 1997. Principles of population genetics. 3rd ed. Sinauer. Associates.

[27] Bedjaoui H., 2019. Etude de la diversité génétique de quelques accessions de palmier Dattier (Phoenix dactylifera L.) en Algérie moyennant les marqueurs de l'ADN de type SSR. Mémoire de Thèse. Université Mohamed Khider Biskra Faculté des sciences exactes et sciences de la nature et de la vie Département des Sciences Agronomiques. 184p.

[28] Foulley. J. Ollivier. L., 2006. Estimating allelic richness and its diversity. Livest. Sci. 101: 150-158.

[29] Tayyar. Rana I., Jamie H. T., Nguyen Jodie., Holt. S., 2003. Genetic and morphological analysis of two novel Tiger Nuts biotypes from California. Weed Science. 731-739p. 10p.