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Genetic diversity of *Cleome gynandra* germplasm in Burkina Faso using SSRs molecular markers

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***Cleome gynandra* L. is an important traditional leafy vegetable in Burkina Faso diets. Because of its high nutritional value, it makes a good dietary supplement, providing an important nutritional and medicinal requirement. The aim of the present study was to assess the genetic diversity and intra- and interpopulation relationships of the *C. gynandra* collection using SSR molecular markers specific to *C. gynandra*. Nine microsatellite primers were used to assess the genetic diversity of 33 *C. gynandra* accessions. A total of 50 alleles were counted (an average of 5.56 alleles per marker). Polymorphism information content (PIC) ranged from 0.82 to 0.43 for markers CG01 and CG033, respectively, with an average of 0.60, showing the high genetic diversity of the *C. gynandra* collection studied. In fact, six markers are highly informative (PIC > 0.5). Inter-morphotype differentiation showed an STF of 0.17 was recorded between the green morphotype and the dark purple morphotype. In effect, the two morphotypes are different. The organization and structuring of diversity resulted in three distinct genetic groups, irrespective of morphotype color, and accession origin.**

Key words: Molecular markers, SSRs, *Cleome gynandra*, Burkina Faso.

INTRODUCTION

Cleome gynandra plays an important role in human and animal nutrition. It has been used in several regions of the world as an emergency crop allowing populations to survive periods of hunger and famine. *C. gynandra* is a leafy vegetable that is widely consumed and valued by people in Burkina Faso and other parts of Africa (Ouédraogo et al., 2013; Steve et al., 2017). The leaves and tender stems are used to prepare various dishes such as rice sauce, salads, couscous, etc. The leaves can be boiled, shaped into pellets, and sun or air dried and reused later (Faber et al., 2010). Leaf pellets and powder can be stored for up to a year (Steve et al., 2017). They are soaked in water before being reused in cooking. The seeds can be used as a mustard substitute (Steve et al., 2017).

In Burkina Faso, *C. gynandra* was once used in

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emergency, food situations such as periods of famine, floods, and drought (Millogo-Rasolodimby, 2001). In recent years, the leaves and young stems of *C. gynandra* are used with other leaves to prepare *Babenda,* a dish highly prized by Burkinabé people especially during festive ceremonies (Tarnagda et al., 2019). The tender leaves and twigs of *C. gynandra* are also used to prepare leafy sauces, consumed with rice, and to prepare couscous mixed with cereal flour or peanut paste (Konkobo-Yaméogo et al., 2002).

Agromorphological evaluation revealed significant differences in the response of *C. gynandra* accessions to agroclimatic variability that could be reflected in differences in genotypes (Sakandé et al., 2022). Phenotypic characterization provides a crude estimate of the average functional variants of genes in a given individual or population. However, most phenotypes do not always reflect true genetic variation, as they are influenced by the environment that interacts with the genotype (Lallemand, 2004).

Thus, to better understand the genetic differences between accessions, it would be necessary to assess their level of variability using molecular markers.

Molecular characterization based on DNA markers is nowadays the most reliable analysis in the genetic characterization of a species. Therefore, genetic measurement of diversity through molecular analysis is one of the best options to more accurately distinguish between genotypes (Govindaraj et al., 2015; Bhandari et al., 2017).

Most of the genotypic characterization of *C. gynandra* has been done using dominant markers such as RAPDs (K'Opondo et al., 2009), AFLPs (Omondi et al., 2017), and more recently SSRs microsatellite markers (Omondi et al., 2017; Thovhogi et al., 2021).

In Burkina Faso, the study of the genetic diversity of *C. gynandra* was conducted using ISSRs molecular markers (Kiébré et al., 2017c). This study showed a moderate genetic diversity within the collection that can be explained by the fact that ISSRs markers are dominant markers that are non-specific with a low level of polymorphism compared to SSRs markers that are codominant.

The present study therefore aims to assess the genetic diversity and inter- and intra-population relationships of the *C. gynandra* collection using *C. gynandra*-specific SSRs molecular markers. The specific objectives are: (i) to assess the level of diversity of the collection, (ii) to determine the intra and interpopulation diversity of the collection, and (iii) to determine the structuring of the genetic diversity of the *C. gynandra* collection in Burkina Faso.

MATERIALS AND METHODS

Plant

The plant material consists of 33 *C. gynandra* accessions collected in 2019 from gardeners in Burkina's three climatic zones and characterized agromorphologically (Sakandé et al., 2022). The 33 accessions are composed of three morphotypes including 17 accessions of green morphotypes, 8 accessions of light purple morphotypes and 8 accessions of dark purple morphotypes. These accessions are distributed in the three climatic zones of Burkina Faso, namely 25 accessions in the Sudano-Sahelian climate, 5 accessions in the Sudanian climate and 3 accessions in the Sahelian climate (Table 1).

Markers used

Nine microsatellite primers were used for molecular characterization. These are the only *C. gynandra*-specific SSRs markers used so far for molecular diversity studies (Omondi et al., 2017; Thovhogi et al., 2021). The use of co-dominant markers specific to *C. gynandra* will allow us to better understand the molecular diversity of the *C. gynandra* collection in Burkina Faso (Table 2).

Extraction of genomic DNA

The extraction of genomic DNA was done at the molecular biology unit of the "Genetics and Plant Improvement" research team of the Biosciences Laboratory of the Joseph KI-ZERBO University. The extraction method used is the one developed by Agbangla et al. (2002).

For each accession, 1 mg of fresh 14-day-old leaves were collected and finely ground in 750 µl of Tris-EDTA-Sorbitol (TES) buffer solution using a mortar and pestle. The grinding of each sample were centrifuged at 10,000 RPM for 15 min at 4°C. At the end of centrifugation, the supernatant was removed and 750 µl of (cethylmethyl ammonium bromide CTAB) buffer preheated to 65°C was added to the pellet. The tubes were shaken to completely resume the pellet in the lysis buffer. Samples were then incubated at 65°C in a water bath for 2 h 30 min. The contents of the tubes were homogenized by inverting the tubes every 15 min.

At the end of the incubation, the samples were cooled for few minutes at room temperature. A volume of 750 µl of the mixture of chloroform and isoamyl alcohol (CIAA) in the proportions 24:1 was added. The samples were again centrifuged at 10,000 RPM for 10 min at 4°C without breaking. The supernatant was collected in new tubes and 750 µl of isopropanol (stored at -20°C) was added to each sample to precipitate the DNA. The samples were shaken by inverting until the DNA ball was observed. They were centrifuged again at 10,000 RPM at 4°C. The supernatant was removed and the samples were dried at room temperature. The DNA pellet was then taken up in 150 µl of ultrapure water and stored in a freezer at -20°C.

The DNA was recovered in ultrapure water; the blank was represented by 150 µl of ultrapure water deposited with a micropipette, in each of the 33 and then the quantification plate.

PCR Amplification

PCR reactions were performed in a final volume of 25 µl containing 1 µl of 3' primer (forward primer), 1 µl of 5' primer (reverse primer), 17.5 µl of ultra pure water, 2.5 µl of PCR premix consisting (of 1U Taq polymerase, 250 µM Tris-HCL, 10 mM KCl, 1.5 mM $MgCl_2$) and 3 µl of genomic DNA of concentration 5 ng/µl from the accession.

PCR amplification was performed according to a program consisting of an initial denaturation phase at 94°C (4 min), followed by a series of 60 cycles with denaturation at 94°C (45 s), hybridization at the temperature of each primer (1 min), elongation at 72°C (1 min 30 s), and final elongation at 72°C for 4 min. The

Table 1. Accessions classification used, their morphotypes, and climatic origin.

No.	Genotypes	Morphotypes	Climate zones
1	OUA9	Green	Sudan-Sahel
2	OUA10	Green	Sudan-Sahel
3	OUA1	Green	Sudan-Sahel
4	OUA3	Green	Sudan-Sahel
5	OUA2	Green	Sudan-Sahel
6	BOB3	Green	Sudanese
7	KOU	Green	Sudan-Sahel
8	KOM1	Green	Sudan-Sahel
9	KOM2	Dark purple	Sudan-Sahel
10	OUA6	Green	Sudan-Sahel
11	GAN	Green	Sudan-Sahel
12	BOB2	Green	Sudanese
13	REO2	Light violet	Sudan-Sahel
14	MAN	Light violet	Sudan-Sahel
15	TEN	Light violet	Sudan-Sahel
16	DED2	Dark purple	Sudan-Sahel
17	ZOU	Light violet	Sudan-Sahel
18	DED3	Dark purple	Sudan-Sahel
19	OUA7	Green	Sudan-Sahel
20	GOU	Light violet	Sudan-Sahel
21	BOB4	Light violet	Sudanese
22	DED1	Dark purple	Sudan-Sahel
23	KAY2	Green	Sahelian
24	OUA5	Green	Sudan-Sahel
25	OHG	Dark purple	Sahelian
26	BOB1	Dark purple	Sudanese
27	DED4	Dark purple	Sudan-Sahel
28	FAD	Light violet	Sudan-Sahel
29	MOG	Green	Sudan-Sahel
30	BOND	Green	Sudanese
31	ZOR	Green	Sudan-Sahel
32	KOM3	Violet foncé	Sudan-Sahel
33	KAY1	Light violet	Sahelian

amplification products were then subjected to electrophoresis at 100 V on a 2% agarose gel in which 15 µl of 5% Ethidium Bromide (BET) was added as a fluorescent developer. The migration time was 1 h 30 min in 0.5x Tris Borate EDTA buffer (TBE). Deposition was performed in the presence of a molecular weight marker consisting of two microsatellites of different sizes ranging from 50 to 1500 bp.

At the end of the migration, the revelation of the amplified products was done under ultra violet light of a transilluminator. The migration gel was then photographed with a Canon PowerShot A620, 7, 1 Megapixel camera.

Reading of electrophoretic profiles

For each pair of primers (or locus), the reading was done on the electrophoretic profiles associated with it. As the SSRs are codominant markers and *C. gynandra* is diploid, it is essential to differentiate between homozygous individuals with only one band (1 allele) and heterozygous individuals with two bands (2 alleles). By continuing to follow the level of alleles with reference to the molecular weight markers, we established the genotype of each individual of the 33 accessions studied and for all loci.

Statistical analysis of molecular data

The bands revealed by the markers were used for the coding and analysis of the molecular data. Genetic diversity among *Cleome gynandra* accessions was analyzed at two levels, namely intra-population variability and inter-population variability. For this purpose, three software packages were used, namely, GenAlEx 6, 501 and Darwin 6.0.4.

Genetic parameters were calculated using GenAlEx software in order to assess the level of diversity of the entire collection. Genetic diversity structuring was determined using DARwin V6.0 software. It was used to generate the dissimilarity matrix between accessions using the "simple matching" procedure and the construction of

Table 2. Characteristics of SSR markers used.

Locus	Primers	Repeats	Ta en C	Size (bp)
CG01	R: CGTCAGTAGCATTTGGTTCG F: TTCCAATACAAAGGGTGACAAC	(AG) 20	57	215
CG017	R :AATGGATTTGGTTCATGTGG F:TTTGAAGTGGCAACAGCGTA	(AACCCTA) 5	60	205
CG018	R:CCTTCTTCATTCCCAAACGA F:CGAAATGCTTCACTTGCTCA	(AACCCT) 6	60	276
CG022	R:CGCTTCCATGGACTGGTAAT F:ATGGGCTTTCCGTTTTTCAT	(CAACAC) 7	60	227
CG024	R:ATGGCGTATGGGTTGAAGAT F:GGATGCAATTGTACAGCTCG	(TTGTGACCT) 4	60	254
CG027	R:ATTGGAGGCAAACGAATGAG F :ATATTTGTGTGGGGTGGCTG	(GAATGCTT) 3	60	200
CG028	R:ATCAATTCTCCTGCGCAAAC F:ACCTTCGTTTTTGTTGTCGG	(TAGAATTT) 3	63	270
CG032	R:TGGACAGATTTTCTGGTGGA F:GGGCCTGCAAAAACAAATAA	(AGACC) 7	60	221
CG033	R:CTCAACGTTCCACCTCCAAC F:CCTTAACGATCACGCATTCA	(ATATA) 8	60	184

Source: Omondi et al. (2017) and Thovhogi et al. (2021).

dendrograms from this dissimilarity matrix using the Neighbor-Joining method.

Genetic diversity of the collection studied

The genetic diversity parameters used to describe the molecular diversity of *C. gynandra* were: allele polymorphism rate (P), total number of alleles (Na), average number of alleles per locus (N), number of effective alleles (Ne), Shannon diversity index (I), expected heterozygosity (He), and polymorphism information content (PIC).

Polymorphism or polymorphism rate (P) refers to the number of polymorphic loci (npj) divided by the total number of loci (n total). Its formula is P = npj/total nt.

The total number of alleles (Na) or allelic richness represents the total number of alleles obtained in a population. It is obtained according to the relation Na = n-1 (n is the number of all alleles provided by all primers).

The average number of alleles per locus (N) is the sum of alleles found for a locus divided by the total number of loci.

The number of effective alleles is the number of alleles that can be present in a population, Ne = 1/(1-h) = 1/Σpi2; where pi: the frequency of allele i at the locus considered and h = heterozygosity.

The Shannon diversity index gives an idea of the specific diversity of an environment. It was calculated according to the formula of Martynov et al. (2003) I = -ΣPi ln (Pi).

Expected heterozygosity (He) or Nei gene diversity index (D): The expected heterozygosity rate (He) can be calculated from the allele frequencies determined for each locus according to the formula: He = 1/N[n/n-1(1-Σpi2)]; where N is the number of loci, n: the number of accessions, pi: the frequency of allele i at the locus under consideration.

The polymorphism information content (PIC) is calculated according to the algorithm: PIC = 1- Σfi², with fi, the frequency of each allele.

Interpopulation genetic diversity

To assess diversity between populations, two subpopulations were defined according to climatic zones, and morphotypes (green, light purple and dark purple).

Two parameters were estimated in the description of genetic diversity between the defined subpopulations. These are the index of genetic differentiation between populations (Fst) and the minimum distance of (Nei) between pairs of genetic groups.

The index of genetic differentiation between populations (Fst): its value is between 0 and 1. It is 0 in case of strong genetic similarity between subpopulations and 1 in case of fixation of different alleles in subpopulations. Its formula is Fst = 1- HS/HT; where HS is the average expected heterozygosity of the subpopulations and HT is

Table 3. Level of genetic diversity of *Cleome gynandra* using nine SSR markers.

Locus	Na	Ne	I	Ho	He	PIC	P (%)
CG001	10	5.42	1.94	0.58	0.82	0.82	100
CG0018	5	2.32	1.09	0.15	0.57	0.57	100
CG0017	6	3.48	1.45	0.18	0.71	0.71	100
CG0032	6	2.42	1.11	0.33	0.59	0.59	100
CG0033	6	1.76	0.96	0.39	0.43	0.43	100
CG0027	2	1.83	0.65	0.27	0.45	0.45	100
CG0024	6	2.98	1.32	0.45	0.66	0.66	100
CG0022	4	1.84	0.88	0.03	0.46	0.46	100
CG0028	5	3.48	1.38	0.00	0.71	0.71	100
Mean	5.56	2.84	1.20	0.27	0.60	0.60	100

Na: Number of different alleles per locus in the population; Ne: number of effective alleles; I: Shannon diversity index; Ho: observed heterozygosity; He: expected heterozygosity, PIC: Polymorphism Information Content, P: polymorphic loci rate

the expected heterozygosity of the total population; the minimum distance of Nei between pairs of genetic groups: its value also varies from 0, for identical populations, to 1 for totally different populations.

RESULTS

Level of diversity of SSR markers tested

All nine primers tested were polymorphic. Allele sizes ranged from 50 to 300 bp. Electrophoresis revealed a total of 50 alleles. Each of the primers revealed 100% polymorphism (Table 3). The number of alleles ranged from 2 for primer CG027 (with 1.83 effective alleles) to 10 for primer CG01 (with 5.42 effective alleles) for an average allelic richness of 5.56 alleles per locus.

The polymorphism information content (PIC) varied from 0.43 for the CG0033 primer to 0.82 for the CG001 primer with an average of 0.60. The most discriminating marker was primer CG001 and the least discriminating marker was primer CG0033. Thus, six of the most polymorphic markers (CG01, CG018, CG017, CG 032, CG024, CG028) used have a PIC greater than 0.5 and thus are very highly informative. However, ICP values of 0.43, 0.45 and 0.46 for markers CG033, CG027, CG022, respectively gave reliable and actionable information in characterization studies. Figure 1 shows the migration profile of the GC01 primer.

Genetic diversity of accessions according to climatic zones

The genetic diversity parameters of *C. gynandra* according to their origin showed the greatest diversity for accessions from the Sudanian climate (Table 4). There was an effective allele number of 5.22 with a polymorphism rate of 100%, an expected heterozygosity of 0.59, a Shannon diversity index of 1.167 and a potential polymorphism information of 0.59. It is followed by the Sudano-Sahelian climate with an effective allele number of 2.56, a Shannon diversity index of 0.71, with a polymorphism of 88.89% and a PIC of 0.42.

The genotypes from the Sahelian climate recorded the lowest values with 2.00 effective alleles, a polymorphism rate of 88.89%, a Shannon diversity index of 0.57 and a polymorphism information potential of 0.38, respectively.

Genetic diversity of accessions according to morphotypes

Compared to the three morphotypes (Table 5), diversity was the highest in the green morphotype, followed by the light purple and dark purple morphotypes, respectively. The green morphotype recorded a number of effective alleles of 2.7, with a 100% polymorphism rate, an expected heterozygosity of 0.51, a Shannon diversity index of 1.106, and a potential in polymorphism information of 0.56. The dark purple morphotype recorded the low genetic parameters with an effective allele number of 2.215 and a Shannon index of 0.85. The light purple morphotype recorded the intermediate genetic parameters compared to the other two with a number of effective alleles of 2.31 and a Shannon index of 0.91.

Inter-population differentiation

Differentiation between climatic zones

Significant differentiations were observed between the Sahelian and Sudanese climates (Fst =0.14); between the Sudanese and Sahelian climates with an Fst of 0.13. The lowest differentiation was recorded as 0.09 between the Sudanese and Sahelian climates (Table 6). The

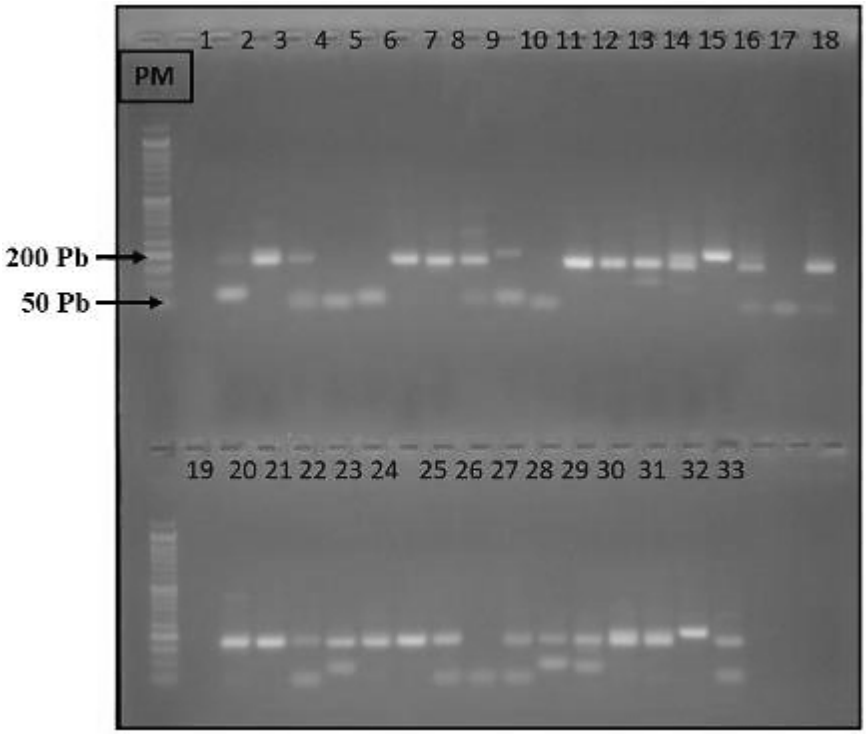

Figure 1. Migration profile obtained with the GC01 marker of 33 genotypes of *Cleome gynandra*.

Table 4. Diversity according to climate zones.

Climate zone	Na	Ne	I	He	PIC	%P
Sudanian climate	5.22	2.78	1.17	0.59	0.59	100
Sudano-Sahelian climate	2.56	2.07	0.71	0.43	0.43	88.89
Sahelian climate	2.00	1.75	0.58	0.38	0.38	88.89

Na: Number of different alleles per locus in the population; Ne: number of effective alleles; I: Shannon diversity index; Ho: observed heterozygosity; He: expected heterozygosity, PIC: Polymorphism Information Content, P: polymorphic loci rate.

Table 5. Diversity according to morphotypes.

Morphotypes	Na	Ne	I	PIC	He	P (%)
Green	4.667	2.78	1.106	0.56	0.56	100
Light violet	3.444	2.31	0.91	0.462	0.51	100
Dark violet	3.222	2.22	0.85	0.48	0.48	100

Na: Number of different alleles per locus in the population; Ne: number of effective alleles; I: Shannon diversity index; Ho: observed heterozygosity; He: expected heterozygosity, PIC: Polymorphism Information Content, P: polymorphic loci rate

largest minimum Nei distance of 0.31 was observed between the Sahelian and Sudan-Sahelian climates and the smallest minimum Nei distance (0.15) was observed between the Sahelian and Sudan-Sahelian climates.

Inter-morphotype differentiation

Inter-population genetic diversity parameters between pairs of groups showed the significance between the

Table 6. Genetic differentiation between climatic zones.

Climate zone	Minimum distance from Nei			Climate zone	Differentiation index Fst		
	Sou	S-sah	Sah		Sou	S-sah	Sah
Sou	0			Sou	0		
S-sah	0.153	0		S-sah	0.09	0	
Sah	0.31	0.15	0	Sah	0.14*	0.13*	0

Sou: Sudanese; S-Sah: Sudanese-Sahelian; Sah: Sahelian; *: significant difference.

Table 7. Differentiation according to morphotypes.

Morphotype	Minimum distance from Nei			Morphotype	Differentiation index Fst		
	Green	Light violet	Dark Violet		Green	Light violet	Dark Violet
Green	0			Green	0		
Light violet	0.22	0		Light violet	0.07	0	
Dark Violet	0.34	0.22	0	Dark Violet	0.17*	0.09	0

*: Significant difference.

green and dark purple morphotypes (Table 7). For example, the genetic differentiation index Fst varied by 0.17 between green and dark purple morphotypes. It was 0.09 between light and dark purple morphotype. The lowest genetic differentiation index of FST was observed between light and dark purple morphotypes. The largest minimum Nei distance was observed between the green and dark purple morphotype as 0.34.

Genetic structure of C. gynandra accessions

The Neighbor-Joining tree representation of the dissimilarity matrix gave a distribution of accessions in three genetic groups A, B and C (Figure 2). Genetic group A, which is very composite, contained the largest number of accessions. It consists of 14 accessions. These accessions come from three climatic zones of the country: two (2) from the Sudanian climate, one (1) from the Sahelian climate and 11 accessions from the Sudano-Sahelian climate. It contains green, light purple and dark purple morphotypes. Group B contains 12 accessions. The Sahelian and Sudanian climates composed of two (2) accessions each. The Sudano-Sahelian climate contained eight (8) accessions. As for group C, it is the most homogeneous with seven accessions. They all come from the Sudano-Sahelian climate. This group is essentially composed of green morphotypes.

Structuring of the constituted groups

The different genetic performances of the three genetic groups of *C. gynandra* in Burkina Faso presented in Table 8 show that: genetic group A recorded average values for the other parameters of genetic diversity; genetic group B with 12 alleles counted, this group has the best allelic frequencies, the highest observed heterozygosity (0.296) and the highest expected heterozygosity (0.59) and allelic richness (2.67) values; genetic group C has the lowest allelic frequencies, an allelic richness of Na = 3.11, an effective allele number of Ne= 2,111 with He = 0.458 and a polymorphism of 100%.

DISCUSSION

The nine microsatellite markers used to assess the molecular polymorphism of the 33 *C. gynandra* accessions from Burkina Faso were all found to be 100% polymorphic at the 95% threshold. The high polymorphism could explain the adaptability of *C. gynandra* populations to a wide range of environmental conditions. It would be maintained by recurrent gene transfers between populations. According to Ould Ahmed et al. (2010), a very high polymorphism rate indicates a high level of polymorphism within accessions and the efficiency of the markers used for the analysis of the genetic diversity of the populations studied.

The average polymorphism information content (PIC) value of 0.60 in the present study is greater than 0.5. According to Bostein et al. (1980), markers with PIC above 0.5 are considered highly informative. Thus, six of the most polymorphic markers (CG01, CG018, CG017, CG 032, CG024, CG028) are therefore very highly informative (ICP greater than 0.5). However, the ICP values of 0.43, 0.45 and 0.46 observed, respectively for

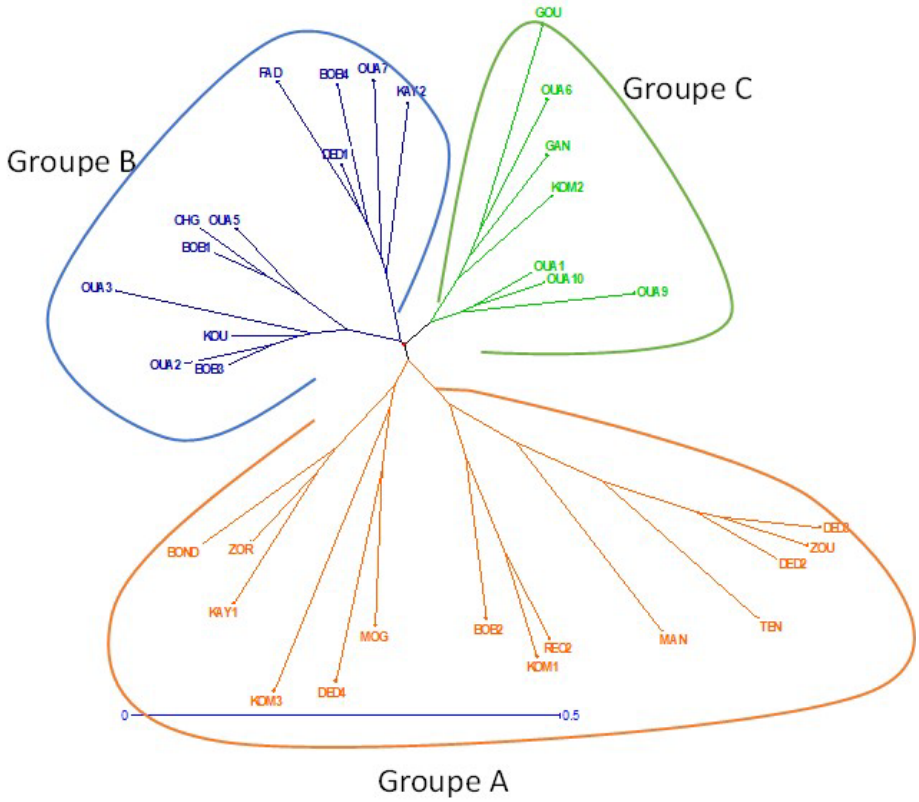

Figure 2. Dendrogram of 33 *C. gynandra* genotypes with SSRs markers**:** group A in red, group B in blue, and group C in green.

Table 8. Diversity parameters of genetic groups.

Group	Na	Ne	I	Ho	He	PIC	%P
Group A	4.33	2.29	0.92	0.27	0.47	0.47	88.89
Group B	3.67	2.673	1.05	0.29	0.590	0.59	100.
GroupeC	3.11	2.111	0.81	0.21	0.43	0.46	100

Na: Number of different alleles per locus in the population; Ne: number of effective alleles; I: Shannon diversity index; Ho: observed heterozygosity; He: expected heterozygosity, PIC: Polymorphism Information Content, P: polymorphic loci rate.

markers CG033, CG027, CG022, although low would give reliable and exploitable information in characterization studies. The average PIC value (PIC = 0.60) is comparable to the work of Omondi et al. (2017) on *C. gynandra* accessions from East Africa (0.60). It is lower than the value recorded on the results of *C. gynandra* accessions from East Africa and Tanzania (0.73) (Thovhogi et al., 2021). It is higher than the values found in accessions from Burkina Faso (Kiébré et al., 2017c) with ISSRs dominant markers.

These differences would be justified by the territorial extent of this collection and the very diverse origin of these accessions. Indeed, it is a sub-regional collection from several East African countries while the collection area of our collection is more restricted.

As for the difference with the results of Kiébré et al. (2017c) with ten of the dominant ISSR markers on 100 accessions from Burkina Faso, it would be related to the nature of the ISSR markers used in his study which are non-specific and dominant. The dominant markers reveal low genetic diversity compared to the codominant markers.

According to Singh et al. (2013), PIC is dependent on many factors such as the species' breeding pattern, collection area, sample size, number of samples, type of markers used, and sensitivity of the DNA fragment separation method.

A total of 50 alleles were detected with the number of

alleles ranging from 2 to 10 per locus (an average of 5.56 alleles per locus). Allelic richness is a useful criterion in conservation practice since marker-assisted maximization of allelic richness is effective in conserving a maximum number of both neutral and non-neutral alleles (Bataillon et al., 1996).

The different diversity parameters estimated in the *C. gynandra* collection showed a variation in diversity according to morphotypes and climatic zones. Thus, the high values of heterozygosity, Shannon diversity index and polymorphism in the Sudano-Sahelian zone indicate a higher diversity of *C. gynandra* germplasm in the Sudano-Sahelian zone compared to the other two zones.

Analysis of the distribution of genetic diversity across morphotypes reveals significant genetic differentiation between green and dark purple morphotypes. Furthermore, variation in genetic parameters such as heterozygosity, Shannon diversity index and polymorphism values observed between individuals of the three morphotypes, further illustrate the existence of genetic diversity.

A high average heterozygosity value was observed in the green and dark purple morphotypes of *C. gynandra,* meaning that green morphotype accessions are more diverse. Furthermore, the low heterozygosity observed in light and dark purple morphotype accessions could be attributed to the number of accessions of these morphotypes used in the study. Both populations showed differences in loci with regard to the number of private alleles observed. This tree-like distribution of the dendrogram can also be explained by the existence of a broad common genetic base between the different populations and accessions, despite the phenotypic distance (color of morphotypes).

Conclusion

The nine microsatellite markers used to assess the molecular diversity of the 33 *C. gynandra* accessions from Burkina Faso were all polymorphic. Molecular markers CG01, CG018, CG017, CG 032, CG024, and CG028 with PIC above 0.5 were the most polymorphic. The genetic parameters of our study were all higher than those obtained in previous studies on the genetic diversity of *C. gynandra* in Burkina Faso. It thus showed the necessity of using codominant and specific markers in genetic diversity studies.

Differentiation indices according to morphotypes showed that green morphotypes and dark purple morphotypes are genetically different.

A structuring into three genetic groups is independent of morphotype color. Group C contains the elite accessions which come from the North Sudanian phytogeographic sector. The accessions of this group could be used for the selection of high yielding varieties in fresh biomass. This study will make it possible to organize the conservation of this collection which abounds in an important genetic diversity on the one hand, and to feed the domestication and selection program of these resources on the other hand.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

REFERENCES

Agbangla C, Tostain S, Dansi A, Daïnou O (2002). Evaluation de la diversité génétique par RAPD d'un échantillon de *Dioscorea alata* d'une région du Bénin, la sous-préfecture de Savè. *J. Rech. Sci. Uni. Lomé* (Togo) 6(1):197-202.

Bataillon TM, David JL, Schoen DJ (1996). Neutral genetic markers and conservation genetics: simulated germplasm collections. Genetics 144:409-417.

Bhandari HR, Bhanu AN, Srivastava K, Singh MN, Shreya HA (2017). Assessment of Genetic Diversity in Crop Plants.An Overview. Advances in Plants and Agriculture Research 7.279-286.

Faber M, Oelofse A, van Jaarsveld PJ, Wenhold FAM, Van Rensburg WSJ (2010). African Leafy vegetables consumed by households in the Limpopo and KwaZulu-natal provinces in south Africa. South African Journal of Clinical Nutrition 23:30-38.

Govindaraj M, Vetriventhan M, Srinivasan M (2015). Importance of genetic diversity assessment in crop plants and its recent advances: An overview of its analytical perspectives. Genetics Research pp. 431-487.

K'Opondo FBO, Rheenen HAV Muasya RM (2009). Assessment of genetic variation of selected spiderplant (*Cleome gynandra L*.) morphotypes from western Kenya. African Journal of Biotechnology 8:4325-4332.

Kiébré Z, Nanema KR, Bationo/Kando P, Sawadogo B, Sawadogo M, Zongo JD (2017c). Genetic Diversity of Spider Plant (*Cleome gynandra* L.) of Burkina Faso Using ISSRs Markers. International Journal of Current Research in Biosciences and Plant Biology 4:44-51.

Konkobo -Yaméogo C, Karimou AR, Kaboré S Diasso K, Diawara B, Ouedraogo JB (2002). *Les pratiques alimentaires à Ouagadougou, Burkina Faso: Céréales, légumineuses, tubercules et légumes*. CNRST, CIRAD 148 p.

Lallemand J (2004). Evaluation de la diversité génétique d'espèces cultivées. HDR, Université de Poitiers 450 p.

Millogo-Rasolodimby J (2001). *L'Homme, le climat et les ressources alimentaires végétales en périodes de crise de subsistance au cours du 20e siècle au Burkina faso.* Thèse Docteur, Université de Ouagadougou 249 p.

Omondi EO, Debener T, Linde M, Abukutsa-Onyango M, Dinssa FF, Winkelmann T (2017). Biology, nuclear DNA content and genetic diversity in spider plant (*Cleome gynandra*) germplasm from various African countries. Plant Breeding 136:578-589.

Ouédraogo IW, Tranchant C, Bonzi-Coulibaly YL (2013). Evaluation of mineral contents in *Cleome gynandra* leaves and stalks from Burkina Faso. Journal of the Cameroon Academy of Sciences 11(1):49-54.

Ould Ahmed M, Ben Salem F, Bedhiaf S, M'naouer D (2010). Analyse moléculaire de la diversité génétique des dromadaires (*Camelus dromedarius*) en Tunisie. Biotechnology, Agronomy, Society and Environment 14(3):399-408.

Sakandé B, Traore AK, Kiebre Z, Sawadogo B, Bationo Kando P (2022). Multi-local evaluations of agromorphological performances of *Cleome gynandra L*. in Burkina Faso. International Journal of Innovation and Applied Studies 36(1):9-20.

Singh N, Choudhury DR, Singh AK, Kumar S, Srinivasan K (2013). Comparison of SSR and SNP Markers in Estimation of Genetic Diversity and Population Structure of Indian Rice Varieties. PLoS ONE 8(12):1-14. doi: 10.1371/journal.pone.0084136

Steve C, Ben M, Percy C (2017). Indigenous green leafy vegetables,

namibia's natural power foods: How you can grow and use them. 2p.
Tarnagda B, Cissé H, Nzambe JU M, Ouattara-Sourabié PB, Itsiembou Y, Guira F, Zongo C, Traoré Y, Savadogo A (2019). Technologie de production du « *babenda* » un aliment à base de céréale et de légumes feuilles au Burkina Faso. American Journal of Innovative Research and Applied Sciences pp. 11658-11668
Thovhogi F, Eastonce T, Gwata ET, Godwin RA, Mchau RAG, Sieyaam S Safodien SS, Koopman T (2021). Molecular Characterization of Spider Plant (*Cleome gynandra*). Accessions Using SSR Markers. Agronomy 11:1-13.