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Genetic Diversity of Spider Plant (*Cleome gynandra* L.) of Burkina Faso Using ISSRs Markers

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Abstract

Genetic diversity is the basis of plant breeding. This study aims to determine the level and the organization of the genetic diversity within spider plant genetic resources in Burkina Faso, using ten (10) polymorphic markers Inter Simple Sequence Repeat (ISSR). A total of 40 alleles were identified with an average of 4 alleles per locus and 2.919 effective alleles. The expected heterozygosity and the Shannon's diversity index were respectively 0.279 and 1.695. The analysis of the structuration of the genetic diversity allowed identifying three significantly different groups. These results revealed a relatively low diversity. Although the variations between the cultivated and spontaneous accessions are weak, they indicate a beginning of organization of genetic diversity according to the selection.

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Introduction

In the context of climate change, the crops diversity, particularly genetic diversity, are threatened (Montaigne, 2011). It is thus necessary to formulate preservation strategies of this diversity. However, preservation and effective valorization of genetic resources, require, as a preliminary, a description as exhaustive as possible of their genetic diversity (Kremer, 1994). Indeed, genetic diversity became a primordial tool to define the objectives, the methods and the priorities in the breeding and conservation programs (Stochwell et al., 2003).

Although the collections description using phenotypic markers is essential, however, the phenotypic markers are often influenced by the environmental factors. They are not thus enough to carry out complete evaluation of diversity (Konaté, 2007). Indeed, some variations are expressed at the phenotypic level while others remain "hidden" and their observation requires the use of adapted techniques (Hubert-Vincent, 2007). Thus, molecular markers prove to be essential for an exhaustive description of the species genetic diversity. The molecular markers offer the opportunity to avoid the insufficiencies related to the morphological observations by identifying variations directly on DNA molecule (Mingeot and Watillon, 2005).

Very few diversity studies, using the molecular markers, are carried out on spider plant (*Cleome gynandra* L.). One of the rare molecular diversity studies was carried out by K' Opondo et al. (2009) using Random Amplified Polymorphic DNA (RAPD) markers for the characterization of western Kenya spider plant accessions. The study revealed a variability within accessions of Kenya, an organization of the accessions in three groups and a significant differentiation between the studied morphotypes. A research activity on spider plant genetic diversity using molecular markers is not yet carried out in Burkina Faso. This study thus aims to determine the level and the organization of spider plant genetic diversity in Burkina Faso, using Inter Simple Sequence Repeat (ISSR) markers.

Materials and methods

Plant Materiel and molecular markers

The plant material consisted of 100 accessions of spider plant, collected in the three climatical zones of Burkina Faso. Twenty-eight (28) accessions were collected in the Soudanian zone, characterized by an annual pluviometry higher than 1100 mm and temperatures ranging between 20 and 25°C. Fifty four (54) accessions were collected in the Soudano-sahelian zone. It is the vastest zone, characterized by an annual pluviometry ranging between 600 and 900 mm and the temperatures varying from 20 to 30°C. Eighteen accessions (18) were collected in the sahelian zone which characterized by an annual pluviometry lower than 600 mm and temperatures higher than 30°C.

Seventeen (17) ISSR markers were used in this study (Table 1). These markers were already successfully used by El-domyati et al. (2011) for the genetic diversity study of *Cleome droserifolia* (Forssk.) Delile, a species close to *Cleome gynandra* L. The microsatellite markers are, in general, transferable; thus could be used for diversity study of several close species.

Table 1. Characteristics of ISSR markers used.

N°	Primers	Nucleotides repeats	Sequences 5'-3'	Tm (°C)
1	814	$(CT)_8TG$	CTCTCTCTCTCTCTCTTG	53.7
2	844A	$(CT)_8AC$	CTCTCTCTCTCTCTCTAC	53.7
3	844B	$(CT)_8GC$	CTCTCTCTCTCTCTCTGC	56
4	17898A	$(CA)_6AC$	CACACACACACAAC	53.7
5	17898B	$(CA)_6GT$	CACACACACACAGT	42
6	17899A	$(CA)_6AG$	CACACACACACAAG	42
7	17899B	$(CA)_6GG$	CACACACACACAGG	44
8	HB8	$(GA)_6GG$	GAGAGAGAGAGAGG	44
9	HB9	$(GT)_6GG$	GTGTGTGTGTGTGG	44
10	HB10	$(GA)_6CC$	GAGAGAGAGAGACC	44
11	HB11	$(GT)_6CC$	GTGTGTGTGTGTCC	44
12	HB12	$(CAC)_3GC$	CACCACCACGC	38
13	HB13	$(GAG)_3GC$	GAGGAGGAGGC	38
14	HB14	$(CTC)_3GC$	CTCCTCCTCGC	38
15	HB15	$(GTG)_3GC$	GTGGTGGTGGC	38
16	UBC-820	$(GT)_8C$	GTGTGTGTGTGTGTGTC	52.8
17	UBC-827	$(AC)_8G$	ACACACACACACACACG	52.8

DNA extraction

Extraction of Genomic DNA from young leaves was performed using Mixel Alkyl Triméthyl Ammonium Bromide (MATAB) method, previously developed by Agbangla et al. (2002). DNA extraction process successful was checked by electrophoresis migration on 0.8% agarose gel. Genomic ADN is then included in Tris-EDTA (TE) and preserved in a fridge at -20°C.

PCR conditions

For each DNA sample, amplification was performed in 20 µl reaction volume containing 2 µl buffer 10X (10 mM Tris-HCL pH 9, 50 mM of KCl, 1.5 mM of $MgCl_2$), 0.4 µl dNTPs (10 mM), 0.4 µl *Taq* polymerase (2U), 3

µl DNA (5 ng/ µl), 4 µl primer (10 µM) and 10.2 µl ultrapure water.

After homogenization, amplification was programmed with the thermocycler to fulfill 40 cycles after an initial denaturation cycle for 4 min at 94°C. Each cycle consisted of a denaturation step at 94°C for 1 min, an annealing step at Tm (°C) for 2 min, and an extension step at 72°C for 2 min, following by a final extension for 7 min at 72°C.

Detection of PCR products

After amplification, the PCR products were detected using agarose gel electrophoresis (3% in 1 × TBE buffer), then stained with ethidium bromide (0.5 ug/ml). The bands were visualized under UV light of transilluminator (DI-01-220) and photographed using a camera brand Canon Power Shot A620, 10 Mega Pixels. The PCR products were separated for 2 h at 90 V in TBE 0.5X. Each band was considered a single locus. Data were scored as (1) for the presence and (0) for the absence of a given DNA band. The size of DNA bands in base pairs was estimated using 1kb DNA standard ladder (Invitrogen, Carlsbad, CA, USA).

Data analysis

Clear and unambiguous bands were considered for study. Polymorphism of locus (P), total number of alleles (A^t), number of alleles per locus (A), number of effective alleles (A_e), Shannon's diversity index, calculated according to Martynov et al. (2003), expected heterozygosity (He) or Nei Genetic diversity index, polymorphism information content (PIC) and minimum distance of Nei between pairs of genetic groups were estimated using GenALEx 6.501. Genetic diversity structuring was carried out using the software DARwin V6.0. It was also used to calculate genetic differentiation between genetic groups based on F_{st} and generate dissimilarity matrix between accessions according *«simple matching»* method. A dendrogram was then constructed based on the dissimilarity matrix data using Neighbor-Joining method in order to identify genetic relatedness among the accessions.

Results

Diversity of spider plant of Burkina Faso

Ten (10) markers among the 17 were polymorphic. The polymorphic markers were thus used for the calculation of the genetic parameters (Table 2). At all, 40 alleles were identified among which 38 (95%) were polymorphic. The number of alleles per locus varied from 2, for markers HB13 and UBC827, to 6 for marker 814.0 with an average of 4 alleles per marker tested. As for the number of effective alleles (Ae), it varied from 1.07 (HB13) to 4.63 (814.0) with an average of 2.919 whereas the expected heterozygosity lied between 0.058 (for markers HB13) and 0.368 (for marker 1789A) with an average of 0.279.

Table 2. Genetic diversity of spider plant revealed by markers used.

Primers	A^t	A_e	He	I	PIC	P (%)
17898B	4	2 .61	0 .197	1 .298	0 .195	100
17898A	5	4 .04	0 .368	2 .726	0 .365	100
814.0	6	4 .63	0 .325	2 .934	0 .322	100
844A	5	3 .83	0 .335	2 .532	0 .332	100
844B	5	3 .64	0 .248	1 .840	0 .245	100
17899A	3	2 .47	0 .337	1 .424	0 .334	100
17899B	5	3 .68	0 .321	2 .483	0 .318	100
HB13	2	1 .07	0 .058	2 .230	0 .058	50
HB14	3	2 .06	0 .228	1 .074	0 .226	100
UBC827	2	1 .16	0 .12	0 .408	0 .122	50
Moyenne	4	2 .919	0 .279	1 .695	0 .276	95

A^t: Total number of alleles, A_e: number of effective d'alleles, He: heterozygosity expected, PIC: Polymorphism Information Content, I: Shannon's diversity index, P: Rate of polymorphic loci

The alleles size varied from 200 pb (markers HB13 and UBC827) to 1900 pb (markers 814.0). Shannon's diversity Index (I) extended from 0.408 for marker UBC827 to 2.934 for marker 814.0. The polymorphism information content (PIC) varied from 0.058 (HB13) to 0.365 (1789A) with an average of 0.276. The rate of

polymorphic loci (P) lied between 50 (HB13 and UBC827) and 100 % for the others markers with an average of 95 %. The Fig. 1 is a profile of migration obtained with the marker 17898B for 8 individuals.

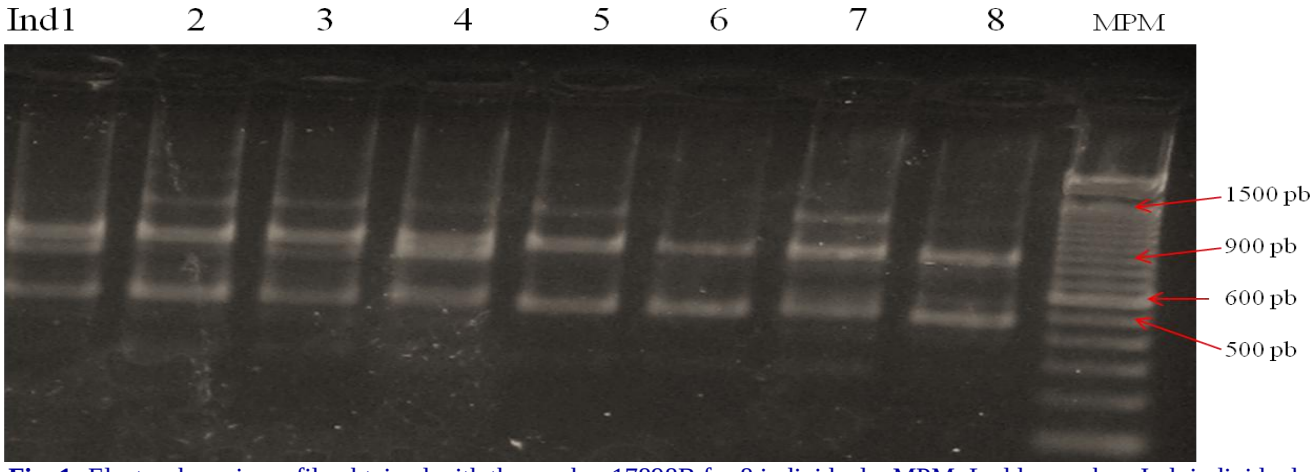

Fig. 1: Electrophoresis profile obtained with the marker 17898B for 8 individuals. MPM: Ladder marker; Ind: individual.

Considering climatical zones, the genetic parameters were in general higher in Soudanian zone (Table 3) that in the others, with a number of effective alleles of 2.985, a polymorphism of 0.680, an expected heterozygosity of 0.3, a Shannon's diversity index of 1.742 and a polymorphism information content of 0.289. This zone is followed by Soudano-sahelian zone. The Sahelian zone had the weakest genetic parameters with a number of effective alleles of 2.809, a polymorphism of 0.705, an expected heterozygosity of 0.262, an Shannon's diversity index of 1.524 and a polymorphism information content of 0.248.

Considering "cultivated" and "spontaneous" accessions, a higher diversity was observed within the accessions collected in the vegetable gardens that within those collected in the spontaneous populations (Table 4).

Referring to the three morphotypes (green, violet and slightly violet) genetic diversity was higher within the green morphotype than the others morphotypes. In fact, this morphotype had a number of effective alleles of 2.964, a polymorphism of 0.710, an expected heterozygosity of 0.294, a Shannon's diversity index of 1.737 and a polymorphism information content of 0.287. The violet morphotype presented the weakest genetic parameters in general (Table 5).

Table 3. Genetic diversity of collection according to climatical zones.

Climatical zones	A_e	P (0.95)	He	I	PIC
Sahelian	2 .809	0 .705	0 .262	1 .524	0 .248
Soudano-sahelian	2 .881	0 .717	0 .270	1 .624	0 .265
Soudanian	2 .985	0 .680	0 .300	1 .742	0 .289

A_e: number of effective d'alleles, P (95 %): Rate of polymorphic loci, He: expected heterozygosity, I: Shannon's diversity index, PIC: Polymorphism Information Content.

Table 4. Genetic diversity of collection according to "cultivated" and "spontaneous" accessions.

Factor	A_e	P (0.95)	He	I	PIC
Cultivated accessions	2 .937	0 .718	0 .290	1 .713	0 .281
Spontaneous accessions	2 .902	0 .696	0 .273	1 .643	0 .268

A_e: number of effective d'alleles, P (95 %): Rate of polymorphic loci, He: expected heterozygosity, I: Shannon's diversity index, PIC: Polymorphism Information Content.

Table 5. Genetic diversity according to the morphotypes.

Morphotypes	A_e	P (0.95)	He	I	PIC
Green	2 .964	0 .710	0 .294	1 .737	0 .287
Purple	2 .895	0 .702	0 .280	1 .599	0 .264
Slightly violet	2 .859	0 .701	0 .265	1 .594	0 .259

A_e: number of effective d'alleles, P (95 %): Rate of polymorphic loci, He: expected heterozygosity, I: Shannon's diversity index, PIC: Polymorphism Information Content.

Differentiation between populations

Nei minimum distance and genetic differentiation index (Fst) showed a weak differentiation between the three climatical zones. The longest Nei minimum distance (0.028) and the greatest Fst (0.0251) were observed between the Sahelian and Soudanian zones whereas the low values (0.013 and 0.0021 respectively) are obtained between the Sahelian and Soudano-sahelian zones. A Nei minimum distance of 0.017 and genetic differentiation index of 0.0159 were observed between Soudanian and Soudano-sahelian zones. As for the "cultivated" and "spontaneous" accessions, the Nei minimum distance between them was 0.013 while the Fst was 0.023. Although the influence of the factors is weak, the factor "climatical zones" exerts a more significant effect on genetic differentiation between the subpopulations than the others. It is followed by the factor "cultivated or spontaneous". The factor "morphotype" exerts the least influence on genetic differentiation between the populations.

Organization of genetic diversity of the collection

Based on the genetic diversity within the accessions, three genetic groups were identified (A, B and C). The groups A and B were respectively made up of 32 accessions and 17 accessions. The group C which contains the greatest number of accessions (51) comprises 3 sub-groups C1, C2, C3. The sub-group C1 contains 14 accessions, the sub-group C2 consists of 15 accessions and the sub-group C3 gathers 22 accessions. All the climatical zones were represented in the three groups. It is the same for the three morphotypes which were found in all the genetic groups. The majority of the accessions collected in the vegetable gardens are in the groups A and C whereas the group B contains mainly accessions taken in the spontaneous populations.

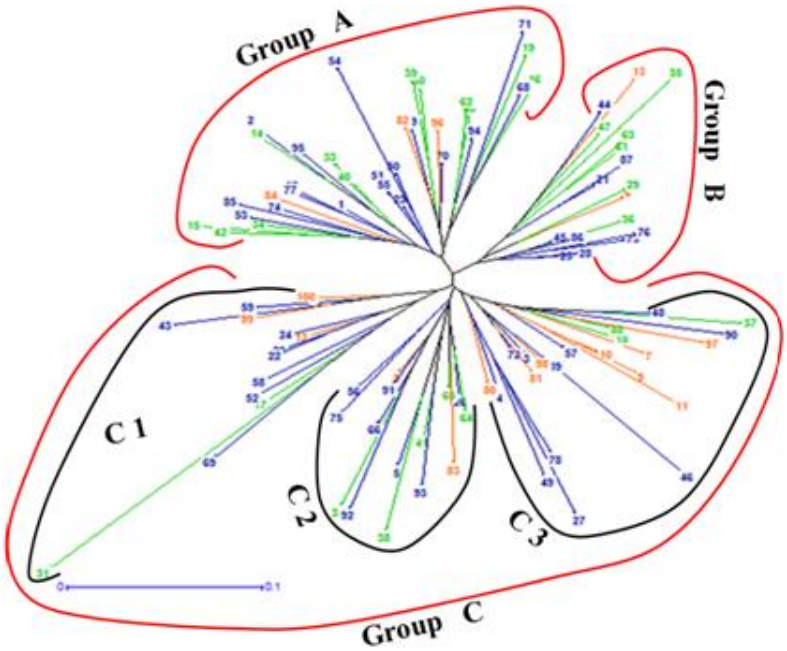

Fig. 2: Radial representation of the dendrogram of the spider plant accessions, constructed from the dissimilarity matrix according to the Neighbor-Joining method. **Legend:** Sahelian zone (red), Soudano-sahelian zone (Blue); Soudanian zone (green).

Description of genetic groups

The genetic parameters of the three genetic groups are consigned in Table 6. The group C which has a number of effective alleles of 2.903, a polymorphism of 0.716, an expected heterozygosity of 0.277, a Shannon's diversity index of 1.659 and polymorphism information content of 0.271 records the highest genetic parameters. The Group A with a number of effective alleles of 2.679, a polymorphism of 0.688, expected heterozygosity of 0.228, a Shannon's diversity index of 1.399 and a polymorphism information content of 0.221 has the weakest genetic parameters. The group B has genetic parameters ranging between those of the two others groups.

Differentiation between genetic groups

The genetic distances between the three genetic groups showed significant differentiation between them (Table 7). The longest Nei minimum distance (0.091) and the greatest Fst (0.209) were observed between the groups A and B while the low values were obtained between the groups A and C.

Table 6. Characteristics of genetic groups.

Genetic groups	A_e	P (0.95)	He	I	PIC
Group A	2 .679	0 .688	0 .228	1 .399	0 .221
Group B	2 .667	0 .698	0 .211	1 .212	0 .199
Group C	2 .903	0 .716	0 .277	1 .659	0 .271

A_e: number of effective d'alleles, P (95 %): Rate of polymorphic loci, He: expected heterozygosity, I: Shannon's diversity index, PIC: Polymorphism Information Content.

Table 7. Differentiation between genetic groups.

Genetic groups	Minimum distance of Nei			Differentiation index (Fst)		
	Group A	Group B	Group C	Group A	Group B	Group C
Group A	0			0		
Group B	0.091	0		0.209*	0	
Group C	0.066	0.078	0	0.141*	0.154*	0

*: significant difference.

Discussion

The used markers revealed a genetic diversity within the spider plant collection of Burkina Faso. The rate of polymorphism of the alleles (95 %) was higher than the rate (67 %) obtained by El-Domyati et al. (2011) with the same markers. This high polymorphism testifies to the high level of polymorphism of the accessions and indicates an effectiveness of the used markers (Ould Ahmed et al., 2010).

The polymorphism information content (PIC) which varied from 0.058 to 0.365 with an average of 0.276 is similar to the PIC (0.33) obtained by El-Domyati et al. (2011). The PIC which varied from 0 (for monomorphic locus) to 1 (for polymorphic locus) reveals the degree of polymorphism of the tested markers. Thus, the markers 17898A and 17899A were most polymorphic with PIC values of 0.365 and 0.334, respectively.

The allelic richness which lied between 2 and 6 per marker with an average of 4, is relatively significant. The alleles size which fluctuated between 200 and 1900 pb is very close to the alleles size obtained by Handaji et al. (2012) which varied from 150 pb to 1900 pb. These results are similar to those observed by Zietkiewicz et al. (1994), whose reported that ISSR technique generates amplified DNA fragments which size generally ranging between 200 and 2500 pb. The Shannon's diversity index which lied between 0.408 and 2.934 with an average of 1.695 is relatively high. The high value of the Shannon's diversity index indicated a significant variability within the studied accessions and a great discriminatory capacity of the tested markers.

In return, the number of effective alleles obtained by this author (5.62) is higher than that obtained in this study (2.919). The Néi's diversity index which lies between 0.120 and 0.368 with an average of 0.279 is lower than that observed by K' Opondo et al. (2009) on the spider plant accessions of Kenya (0.3 - 0.6).

Higher genetic diversity within the accessions collected in the vegetable gardens compared to those collected in the spontaneous populations could be explained by the introduction into the vegetable gardens of varieties from

other areas or country. The exchanges of seeds revealed by the ethnobotanical survey contribute to increase the genetic diversity of the vegetable gardens (Kiébré et al., 2015).

Although the differences of the allelic frequencies between sub-populations are weak, they suggest a higher diversity in the Soudanian zone compared to the two other climatical zones. This difference could be due to the different climatic factors between the zones which condition the species adaptation. Indeed, of the different evolutionary factors can involve a differentiation between sub-populations (Ould Ahmed et al., 2010).

The used molecular markers showed a weak genetic differentiation between the cultivated and spontaneous populations. This weak differentiation between these populations could be explained by the fact that certain farmets directly collect their seeds in the spontaneous populations.

The structuring of the accessions in three genetic groups is similar to that observed by K' Opondo et al. (2009) for the spider plant morphotypes from Kenya. The greatest genetic differentiation, observed between Soudanian and Sahelian zones could be explained by the geographical distance between the two zones which limits gene flows between the populations. The similarities observed between the accessions of Sahelian and Soudano-sahelian zones could be allotted to the exchanges of seeds enter the farmers of these zones taking into consideration their geographical proximity.

The influence of the factors "climatical zones", and "cultivated or spontaneous" on the level and the organization of genetic diversity is very weak. The genetic differentiation index between the climatical zones (Fst = 0.0251) indicates that only 2,51 % of total variability are allotted to the "climatic zones" factor. The genetic differentiation index between the cultivated and spontaneous accessions (Fst = 0.0233) indicates that only 2.33 % of total variability are due to the "cultivated or spontaneous" factor. These results determine a very weak structuring of diversity according to the climatical zones and "cultivated or spontaneous" factor.

The great similarity between the sub-populations shows that they have a broad common genetic basis although their geographical origins are sometimes distant. The low genetic divergence between the cultivated and spontaneous populations shows that the selection did not yet influence significantly the diversity of the cultivated accessions. Indeed, the culture of spider plant is at its beginnings in Burkina Faso.

These results are different from those obtained by K' Opondo et al. (2009) which showed a clear differentiation between morphotypes from Kenya. This difference could be explained by the morphotypes used by these authors which from selection, thus with relatively fixed characteristics, whereas the collection used in this study consists of farmer's varieties and accessions directly collected in the spontaneous populations.

Conclusion

The study revealed a genetic diversity within the spider plant collection of Burkina Faso. Ten among seventeen markers tested were polymorphic. These results showed that the molecular markers used were appropriate for the assessment of the genetic diversity of spider plant of Burkina Faso. A structuring of diversity in three genetic groups was established. Although the variations between the sub-populations are weak, the study indicates a beginning of organization of genetic diversity according to the selection. This structuring will be specified gradually with the development of the culture of spider plant in Burkina Faso.

Conflict of interest statement

Authors declare that they have no conflict of interest.

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