

Journal of Experimental Agriculture International

32(2): 1-9, 2019; Article no.JEAI.46816 ISSN: 2457-0591 (Past name: American Journal of Experimental Agriculture, Past ISSN: 2231-0606)

# Molecular Characterization of *Corchorus olitorius* L. of Burkina Faso

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# Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

#### Article Information

DOI: 10.9734/JEAI/2019/v32i230099 <u>Editor(s):</u> (1) Dr. Marco Aurelio Cristancho, Professor, National Center for Coffee Research, CENICAFÉ, Colombia. <u>Reviewers:</u> (1) Suoyi Han, Industrial Crops Research Institute, China. (2) Dr. R. Mahalakshmi, India. Complete Peer review History: <u>http://www.sdiarticle3.com/review-history/46816</u>

Original Research Article

Received 17 November 2018 Accepted 25 January 2019 Published 14 March 2019

# ABSTRACT

*Corchorus olitorius* is more and more cultivated in Burkina Faso because of its socio - economic interest. Hence the objective of this study which will contribute to improve the knowledge of the genetic diversity of the species in Burkina Faso. To this end, seventeen SSR markers were used to characterize ninety-six (96) accessions of the four phytogeographic sectors of Burkina Faso. These accessions consist of 16 accessions of the variety *C. olitorius var insicifolius* and 80 accessions of the variety *C. olitorius var olitorius*. The results of the analysis of the diversity of microsatellite markers tested, they were seized sixteen (16) of the seventeen (17) SSRs are polymorphic with a rate of polymorphism of 92.19 % and number of 53 alleles with a mean of three (3) alleles per locus. As for the Shannon diversity index, with an average of 1.05, it is between 0.53 for the HK-27 marker and 1.90 for the HK-19 marker. The polymorphism information content (PIC) potential ranged from 0.11 for the HK-12 marker to 0.49 for the HK-6 marker with an average of 0.32. A structure of diversity into three groups regardless of phytogeographic areas or botanical variety has always been.

Keywords: Corchorus olitorius; molecular; genetic diversity; Burkina Faso.

# **1. INTRODUCTION**

*Corchorus olitorius* L is a leafy vegetable grown in Burkina Faso because of its socio-economic interest. Indeed, besides of its role in food, the marketing of leaves of *Corchorus olitorius* is a source of income for the population. Also, in traditional medicine, all parts of the plant are used to treat some diseases such as dysentery, diabetes, typhoid fever, constipation, toothache [1].

As a result, various research projects have been undertaken to lay the foundation for species improvement. Thus, the agromorphological characterization of accessions in Burkina Faso has shown the existence of a large diversity indicating possibilities for improvement of the species [2,3].

However, the phenotypic markers are difficult for the assessment of diversity, given that they are often influenced by environmental factors [4,5]. As a result, molecular markers become indispensable in assessing diversity. Thus, the analysis of the genetic diversity of C. *olitorius* has been carried out by many researchers [6,7,8] in several African countries. However, no molecular analysis of the diversity of C *olitorius* accessions has yet been made in Burkina Faso. This study aims to determine the level and organization of genetic diversity using SSR markers.

#### 2. MATERIALS AND METHODS

#### 2.1 Material

#### 2.1.1 Plant material

The plant material consists of 96 accessions of *Corchorus olitorius* from the germplasm of the genetics and plant breeding team of the Biosciences laboratory. These accessions consist of 16 accessions of the variety *C. olitorius* var insicifolius and 80 accessions of the variety *C. olitorius var olitorius*. These accessions come from the four phytogeographical sectors of Burkina Faso.

#### 2.1.2 Markers used

Seventeen SSR markers were used (Table 1) for this study. These markers were developed by Vizon Sci. Inc., in Canada from an elite variety of *C. olitorius*, O-4 from the gene bank of the Jute Research Institute of Bangladesh (IRJB). The primers size comprises between 20 to 25 nucleotides in length, 40 to 60 % of GC and 3' non-complementary nucleotides. The hybridization temperatures of the primers are between 55°C and 60°C and have already been used by Huq et al. [8] for the evaluation of the diversity of *Corchorus olitorius* and *Corchorus capsularis*.

#### 2.2.3 DNA extraction

For each accession, the fresh leaves (seven days' old) were harvested, crushed with a suitable pestle and parafilm paper and immaculate on the FTA card. The fingerprints were stored in silica gel and transported to the laboratory. After drying these fingerprints at room temperature laboratory, in the disks approximately 1 mm in diameter are punched on these FTA cards. Each disc is then washed twice with 200 µl of 70 % ethanol and then rinsed twice with the same amount of Tris EDTA (TE) buffer. The disc is then dried at room temperature and directly transferred to the PCR tube for amplification.

#### 2.2.4 PCR amplification

The PCR reactions were carried out in a final volume of 25  $\mu$ l containing 1  $\mu$ l of the 3 'primer (forward primer), 1  $\mu$ l of the 5' primer (reverse primer), 18  $\mu$ l of ultrapure water, 5  $\mu$ l of premix PCR composed of 1U Taq polymerase, 250  $\mu$ M Tris-HCL, 10 mM KCl, 1.5 mM MgCl<sub>2</sub> and a disk from the FTA map containing the genomic DNA of the accession to amplify varying concentration 0.25 to 0.50 ng/ $\mu$ l.

The reaction mixture was then placed in a thermal cycler for PCR amplification. This amplification was carried out according to a program composed of an initial denaturation phase at 95 °C. for 5 minutes, followed by a series of 35 cycles. Each cycle is composed of a denaturation phase at 95 °C for 30 s, hybridization at the temperature (°C) of each primer for 40 s and an extension at 72 °C for 30 s. At the end of the 35 PCR cycles, a final extension at 72 °C for 5 min was performed, followed by cooling to 4 °C until deposit on the agarose gel.

Name	Sequence 3'- 5' (forward primer)	Sequence 5'- 3' (reverse primer)	Repeated reasons	Tm (°C)
HK-2	GTTTATCCAACCAATACCAACCA	TGCCTCGTTGCTGGACATTGCA	(CT) 11(GT) 14	59.6
HK-4	CAAAAGTAGTGAAGAACATGAGCA	GCCAAATTCTGATATACGCCTGA	(GT) 28	58.3
HK-5	AGTGACTTATAGTCTAATTAGTGA	ACAGATAGGATGTTAACGGGA	(GT) 24	55
HK-6	CTATCTCCCATTGTACCTGCA	GGCAGATTGTGTGAGACTATCA	(GT) 10	58
HK-9	TTACATTATATATGTCCAGCCA	AGTGGCTACTGGTTCCTACA	(TC) 21(T) 33	54.5
HK-10	GAACATCAAGACTGAGTAAGACCTA	TTGAGGATTTTCATATGCATGCA	(TG) 16	57.5
HK-12	CGCTCGCCTAAGTGAAGGCA	ATAAAATACAAGGGACACTTAGCA	(CA) 19	58.7
HK-15	GAGAGGAATGATGCTGAGATTCA	GACACCCTCCGCCTATCTCA	(GA) 15	60
HK-18	GCTGTTGTCTCTCTATTGGTGA	TTCCACGCTCCTTGTTGCCA	(AG) 16	58.9
HK-19	TATGAAGGTGAACTACTTGTCACA	AGCTTCCATTTCGAACATTCCA	(CA) 21(AG) 10	57
HK-20	GTAAAGCACAGGATTAGTCCCA	GGAAAGTGAACCTCTAGTAGATGA	(CT) 30	58.9
HK-22	CTGTTTGTCAATCTCTTTTGAGTCA	GTCCAAAACATCGTGCAGTGTGA	(GA) 25	59.3
HK-23	GGCCCTTCTAATTAACCTCCA	AGTTTTGTTTCCAGATATTGCTCA	(GA) 17	56.6
HK-27	TTGTGTGCAAACACGAGTGCA	GGTAGCCATGTTTACTTCCTGA	(CA) 26	58.1
HK-29	CTGAATGAAAGATTGCTTTTAATCC	CATGCATCATTTGCATTGCATGCA	(GA) 39	57.9
HK-30	GAGTGATTAGAGGGCAGCCA	TGCAACAAAGTATCCAAATCGAC	(CA) 21	58.3
HK-38	ACCAAGTATGATCTGACCTCT	AGCTAAAAACAACACAAAAATATCTTGC	(CTÁT) 16	56.9

#### Table 1. Characteristics of SSR markers tested in Corchorus olitorius

# 2.2.5 Electrophoretic migration and reading of the bands

The amplification products were then subjected to agarose gel electrophoresis at a concentration of 2 % prepared with 1 X TBE solution. The deposits were made in the presence of a molecular weight marker of varying size. 50 to 500 bp and the migration was made at 100 V for 1 h 30 min in 0.5x Tris Borate EDTA buffer (TBE). At the end of the migration, a solution of 5 % Ethidium Bromide (EB) was used as developer. Tape playback was done using a DI-01-220 transilluminator with a 10 mega pixel camera. These bands were identified on the basis of their position on the gel. A binary coding was used for this, 1 was noted in case of presence and 0 in case of absence of band for each individual and for each primer tested.

# 2.3 Molecular Data Analysis

Genetic diversity in accessions of C. olitorius has been analyzed at two levels: intra-population variability and inter-population variability. In this case, the GenALEx version 6.501 software was used to evaluate the genetic parameters of the entire collection and diversity according to phytogeographic and varietal type factors. To describe the genetic diversity between defined subpopulations. the index of genetic differentiation between populations (Fst) was determined using the FSTAT V2.9.3.2 software and the minimum (Nei) distance between pairs of groups was determined from the GenALEX software. A structuring of the genetic diversity was carried out using the software DARwin V6.0. This software was first used to generate the dissimilarity matrix between the accessions according to the "simple matching" procedure. Then, from this matrix of dissimilarities. dendrograms were constructed according to the Neighbor-Joining method.

# 3. RESULTS

# 3.1 Genetic Diversity of Accessions of *Corchorus olitorius* L.

The results of the analysis of the diversity level of the microsatellite markers tested, (Table 2) show that sixteen (16) of the seventeen (17) SSRs are polymorphic against one (HK-10) which has been monomorphic. Polymorphic markers revealed a total of 53 alleles with an average of three (3) alleles per locus. The number of alleles ranged from 2 for primers HK-27, HK-29, HK-30 and HK-38 to 4 for the markers HK-5, HK-6, HK-9, HK-12, HK-15, HK-18, HK-19, HK-20, HK-22. The effective number of alleles (Ae) ranges from 2.52 for the HK-27 marker to 5.87 for the HK-19 marker and the expected unbiased heterozygosity rate of 0.08 for the HK-9 marker to 0.36 for the HK-30 marker.

As far as Shannon diversity index, with an average of 1.05, it is included 0.53 for the KH-27 marker and 1.90 for the HK-19 marker. The polymorphism information content (PIC) potential ranged from 0.11 for the HK-12 marker to 0.49 for the HK-6 marker with an average of 0.32. Regarding the polymorphic loci (P) level, all the differnent primers had a polymorphism level of 100 % except for three (3) primers, HK-5, HK-9, HK-19 for a 75 % rate. and 50 % for primer HK-27.

The study revealed the existence of genetic diversity within the studied accessions. Estimated genetic distances between individuals in the total population ranged from 0.019 to 0.52. A polymeric information content (PIC) of 0.32 was obtained. An average of 3 alleles per locus and an effective number of alleles of 4.21 were observed. The expected heterozygosity and the Shannon diversity index were 0.279 and 1.69, respectively.

# 3.2 Genetic Diversity of the Collection According to the Phytogeographic Sectors

The analysis according to the phytogeographical sectors shows that the values of the genetic parameters *are* generally higher in the Southern-Sahelian sector, intermediate in the North-Sudanian sector and weak in the South Sudanese sector (Table 3). Thus, an effective number of alleles of 4.32, a Shannon diversity index of 1.11, an expected heterozygosity of 0.21, a polymorphism information potential of 0.21 and a polymorphism rate of 85.19 % were observed in the Southern-Sahelian sector. On the other hand, in the South-Sudanian sector, the values of these genetic parameters were respectively 3.83; 0.59; 0.12; 0.11 and 40.74 %.

Four (4) private alleles were identified in this sector against one (01) in North Sudanian.

N°	Markers	A <sup>t</sup>	A <sup>e</sup>	H <sup>e</sup>		PIC	Р
1	HK-2	3	3.80	0.20	1.03	0.43	100.00
2	HK-4	3	4.19	0.28	1.34	0.31	100.00
3	HK-5	4	4.50	0.10	0.79	0.34	75.00
4	HK-6	4	5.35	0.24	1.53	0.49	100.00
5	HK-9	4	4.36	0.08	0.61	0.33	75.00
6	HK-12	4	4.49	0.11	0.80	0.11	100.00
7	HK-15	4	5.24	0.23	1.54	0.25	100.00
8	HK-18	4	4.43	0.09	0.65	0.34	100.00
9	HK-19	4	5.87	0.31	1.90	0.35	75.00
10	HK-20	4	5.34	0.22	1.43	0.25	100.00
11	HK-22	4	4.62	0.13	0.93	0.36	100.00
12	HK-23	3	4.08	0.21	0.99	0.26	100.00
13	HK-27	2	2.52	0.17	0.53	0.39	50.00
14	HK-29	2	2.56	0.22	0.74	0.23	100.00
15	HK-30	2	3.12	0.36	1.09	0.41	100.00
16	HK-38	2	2.80	0.29	0.92	0.31	100.00
Means		3.31	4.21	0.20	1.05	0.32	92.19

Table 2. Level of genetic diversity of the 16 primers tested in Corchorus olitorius

A<sup>t</sup>: total number of alleles, A<sup>e</sup>: effective number of alleles, H<sup>e</sup> expected heterozygosity, PIC: Polymorphism Information Content, I: Shannon diversity index, P: polymorphic loci rate

# 3.3 Genetic Diversity of the Collection According to the Botanical Varieties

Considering the botanical variety factor, a higher diversity was observed in the accessions of the botanical variety *C. olitorius var olitorius* than in the accessions of *C. olitorius var insicifolius* (Table 4). Thus, forty-nine (49) or 92.45 % of the total bands were counted in *C. olitorius var olitorius* against thirty-eight (38) or 71.70 % of the bands in *C. olitorius var insicifolius*. With respect to the private number of alleles, eleven (11) private alleles were counted in *C. olitorius var olitorius* and no private allele in *C. olitorius var insicifolius*.

#### 3.4 Inter-populations Differentiation

The minimal distance of Nei and the index of genetic differentiation (Fst) showed a weak differentiation not only between the accessions of the three phytogeographical sectors but also between the two botanical varieties. With regard to the agro-climatic factor (Table 5), the greatest Nei minimum distance of 0.006 and 0.0109

differentiation index (Fst) were observed between the South Sudanese sector and the South Sahelian sector. In contrast, the shortest Nei minimum distance of 0.001 and differentiation index (Fst) of 0.0016 were observed between the accessions of the North-Sudanese and South-Sudanese sectors. As for the botanical variety factor, the minimum distance of Nei was 0.002 and the differentiation index Fst was 0.0064 (Table 6).

#### 3.5 Organization of Genetic Diversity

The genetic structuring of accessions established according to the "Neighbor-Joining" method gives a distribution of accessions in three genetic groups 1, 2 and 3. These three groups consist of 8.37 and 51 accessions, respectively. This structuring was made without any link with either the phytogeographic factor or the varietal type factor. Indeed, all phytogeographic sectors are represented in all three groups. The same is true for the two botanical varieties found in all genetic groups.

Table 3. Distribution of genetic diversity by phytogeographic sector in Corchorus olitorius

N°	Phytogeographic sector	Α	A	A <sup>p</sup>	A <sup>e</sup>	I	He	H0	Pic	P(%)
1	Southern-soudaniaen	28	0	0	3.83	0.59	0.11	0.12	0.11	40.74
2	Northd-soudaniean	44	03	01	4.09	0.93	0.17	0.17	0.17	75.93
3	Southern Sahelian	48	04	04	4.32	1.11	0.21	0.21	0.21	85.19

A<sup>t</sup>: total number of alleles; A<sup>T</sup>: rare number of alleles; A<sup>P</sup>: private number of alleles; A<sup>E</sup>: effective number of alleles, I: Shannon diversity index; He: expected heterozygosity; H0: observed heterozygosity; PIC: Polymorphism Information Content; P (95 %): polymorphism at the 95 % threshold.

N°	Botanical varieties	A <sup>t</sup>	A	A <sup>p</sup>	A <sup>e</sup>		He	Pic	P(%)
1	V1	49	7	11	4.23	1.05	0.173	0.33	0.87
2	V2	38	00	00	3.97	0.80	0.153	0.28	0.63

#### Table 4. Distribution of genetic diversity according to the botanical varieties

 $A^{t}$ : total number of alleles;  $A^{r}$ : rare number of alleles;  $A^{p}$ : private number of alleles;  $A^{e}$ : effective number of alleles, I: Shannon diversity index; He: expected heterozygosity; H0: observed heterozygosity; PIC: Polymorphism Information Content; P (95%): polymorphism at the 95% threshold.

Phytogeographic	Nei	minimum di	istance	Differentiation index Fst			
sectors	S-sou	N-sou	S-sah	S-sou	N-sou	S-sah	
S-sou	0			0			
N-sou	0.001	0		0.0016 <sup>ns</sup>	0		
S-sah	0.006	0.003	0	0.0109 <sup>ns</sup>	0.0109 <sup>ns</sup>	0	
N-sou: North Su	Idanese; S-sah:	South Sahelia	n; S-sou: Sout	h Sudanese; ns	s: not significal	nt	

# Table 6. Inter-varieties genetic differentiation

N° Botanical vari	Botanical varieties	Nei mii	nimum distance	Differentiation index Fs		
		V1	V2	V1	V2	
1	V1	0		0		
2	V2	0.002	0	0.0064 <sup>ns</sup>	0	

V1: Corchorus olitorius var olitoriu; V2: Corchorus olitorius var incisifolius; ns: non significatif

# 3.6 Description of Genetic Groups

#### 4. DISCUSSION

The genetic parameters of the three genetic groups are shown in Table 7. Group 2 consists of thirty-seven (37) accessions with a total of 48 alleles; an effective number of alleles of 4.729; a number of private alleles of 6, a Shannon diversity index of 1.297; an expected heterozygosity of 0.263, a polymorphic information potential of 0.448 and a polymorphism rate of 83.33 % has the highest genetic parameters. Group 1 consisting of eight (8) accessions has the lowest genetic parameters with a total number of alleles of 22 including a single private allele, an effective number of alleles of 3.896, a Shannon diversity index of 0.559, an expected heterozygosity of 0.125; a polymorphism information potential of 0.224 and a polymorphism rate of 33.33 %. Group 3 consisting of 51 accessions is characterized by individuals with average genetic parameters.

The existence of a very large intergroup diversity was observed. The largest Nei minimum distance of 0.066 was observed between groups 1 and 2 and the lowest Nei minimum distance of 0.033 between groups 1 and 3. About the differentiation index (Fst), the largest A value of 0.218 was observed between groups 1 and 3 and the lowest value of 0.169 between groups 1 and 2 (Table 8).

The 92.19 % polymorphism rate observed indicates a high level of polymorphism of the SSR markers used. Similar levels of polymorphism (92.45 %, 91.11 % and 92.20 %) have also been reported by many researchers [6,7,9] with the same markers. These results therefore reinforce the idea of the effectiveness of SSR primers for the discrimination of individuals within a species, even with a narrow genetic base.

Polymorphism information content (PIC) that indicates not only the number of alleles detected but also the relative frequency of these alleles is an important means of estimating genetic diversity [9]. The low value of the average PIC (0.32) is very close to the results obtained by Zhang et al. [10] of 0.30 out of 30 accessions of C. olitorius of various origin. This low PIC value may be due to a low level of diversity within the species. Genetic diversity studies using AFLP markers [11,9], SSRs [8,10] have also revealed low diversity within the species. These results reflect a narrow genetic base within the species. The average number of alleles per locus (3.31) observed in the present study is small relative to the average number of alleles (6.33) obtained by Hug et al. [8] on 16 germplasm accessions from the Bangladesh Research Institute using 27 SSR

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markers. This difference could be related to the number of markers used and the much smaller geographical origin of the accessions used in this study. Indeed, this study only considers accessions from Burkina Faso, while that of [8] was performed with accessions of various origins and Africa). According to many (India researchers [8,5,9], genetic diversity is highly dependent on the size of the sample, the collection area, and the number and type of microsatellite markers used. The matrices of genetic distances between individuals revealed by the dissimilarity matrix that vary from 0.02 to 0.52 are relatively larger compared to those obtained (0.04-0.49) by Mir et al. [7]. This reflects a higher genetic diversity within accessions in Burkina Faso. This greater diversity may be due to the nature and size of the plant material in this

study, 96 accessions versus 47 C. olitorius accessions used by Mir et al. [7]. In addition, this characterization takes into account the two botanical varieties within the C. olitorius species; which could be the source of this greater genetic diversity. For this, an inter-variety crossing could allow an improvement of the genetic diversity in the species. Although the Nei minimal distance and the genetic differentiation index (Fst) values indicate a small difference between the two botanical varieties, the identification of private alleles within the variety C. olitorius var olitorius suggests a level higher diversity within this variety than C. olitorius var insicifolus. Thus, the existence of private alleles in C. olitorius var olitorius could be linked to a mutation phenomenon. Indeed, according to Mbaye [12], Corchorus olitorius var insicifolius derives from

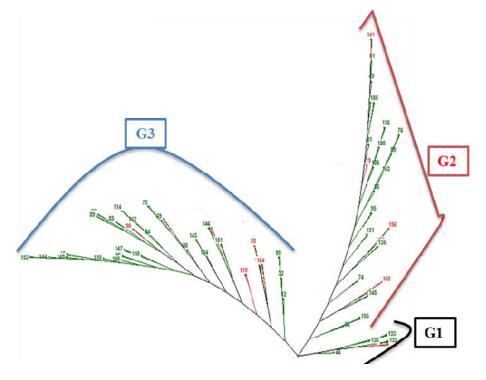


Fig. 1. Dendrogram of the 96 accessions of *C. olitorius* constructed from the matrix of dissimilarities according to the Neighbor-Joining method Legend: green (variety *C. olitorius var olitorius*); red (variety *C. oltorius var incisifolius*)

Table 7. Distribution of genetic diversity by three genetic groups

Groups	Nomber	A <sup>t</sup>	A	A <sup>p</sup>	A <sup>e</sup>		Н	He	Pic	P(%)
Group 1	8	22	00	01	3.896	0.559	0.110	0.125	0.244	33.33
Group 2	37	48	02	06	4.729	1.297	0.255	0.263	0.448	83.33
Group 3	51	43	14	0	3.754	0.643	0.106	0.108	0.237	75.93

A<sup>t</sup>: total number of alleles; A<sup>r</sup>: rare number of alleles; A<sup>p</sup>: private number of alleles; A<sup>e</sup>: effective number of alleles, I: Shannon diversity index; He: expected heterozygosity; H0: observed heterozygosity; PIC: Polymorphism Information Content; P (95 %): polymorphism at the 95 % threshold.

Genetic	Nei	minimum dista	ance	Differentiation index Fst			
groups	Groupe 1	Groupe 2	Groupe 3	Groupe 1	Groupe 2	Groupe 3	
Group 1	0			0			
Group 2	0.066	0		0.169**	0		
Group 3	0.033	0.045	0	0.218 <sup>**</sup>	0.192**	0	

Table 8. Genetic differentiation inter genetics groups

*C. olitorius var. olitorius* by spontaneous and factorial mutation that led to a change in the structure of certain genes. Besides of that, the low level of diversity of *C. olitorius var insicifolius* compared to *C. olitorius var. olitorius* suggests its relatively recent origin.

The structuring of accessions into three genetic groups is similar to that obtained by Banerjee et al. [5] with 140 accessions, despite the high number of markers and the more diverse geographical origins of the accessions used by the latter. As a result, Burkina Faso collection is therefore more diverse and therefore constitutes an important source of useful genes for the genetic improvement of Corchorus olitorus. This higher diversity may be due to the fact that the species is still in protoculture in Burkina Faso. Indeed, according to many researchers [13,14], domestication causes rare allele losses and increased homogenization in crop plants, unlike spontaneous accessions that have significant genetic diversity.

The low values of the Nei minimum distance and the genetic differentiation index (Fst) between the genotypes of the three phytogeographic sectors reflect a small difference in accessions from one sector to another. This is confirmed by the structuring of accessions into three genetic groups regardless of their geographical origin. These results would eventually translate into a considerable flow of genes between the different collection areas. In addition, the high number of private alleles in genotypes of accessions in the sub-Sahelian sector shows a greater genetic diversity in this area. This suggests that this sector could be considered as the center of origin of the plant in Burkina Faso. In addition, the presence of private alleles in the population of this zone and in group 2 indicates that individuals from these two populations have significant genetic potential for future improvement work.

# **5. CONCLUSION**

The results of the study show a low genetic diversity within these accessions. This diversity is independent of the phytogeographic sector and

the varietal type. The genetic group (G2) has the highest genetic parameters, individuals can be retained as elite parents in future work of improvement of the species. Although the variations between the two botanical varieties are small, the study suggests a recent origin of *C. olitorius var insicifolius* compared to *C. olitorius var. olitorius*.

#### ACKNOWLEDGEMENTS

These research projects were made possible thanks to the support of the International Foundation for Science (IFS), Sweden. This structure was of a financial support through the scholarship granted to KIEBRE Mariam the principal author of this paper. We also thank the team of Genetics and Plant Breeding of University Ouaga I Pr JKZ through the technical platform available to us.

#### **COMPETING INTERESTS**

Authors have declared that no competing interests exist.

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Peer-review history: The peer review history for this paper can be accessed here: http://www.sdiarticle3.com/review-history/46816