Original Research Paper

MOLECULAR CHARACTERIZATION OF *Corchorus olitorius* L. of BURKINA FASO

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/The experiment 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 (16) of the seventeen (17) SSRs are polymorphic with a rate of polymorphism of 92 % 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 KH-27 marker and 1.90 for the HK-19 marker. The polymorphism information (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.(incomplete)

Key words: *Corchorus olitorius*, molecular, genetic diversity, Burkina Faso.

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 (Ta-bi *et al*., 2016;).

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 (Kiébré *et al*., 2016, Kiébré *et al.*, 2017).

However, the phenotypic markers are essential for the assessment of diversity, given that they are often influenced by environmental factors, which makes it difficult to assess this diversity (Palit *et al*., 1996, Banerjee *et al*., 2012). 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 Akter *et al*. (2008), Mir *et al*. (2008) and Huq *et al*. (2009) 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.

- **Materials and methods**
- ✓ **Material:**
- ✓ **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.

- ✓ **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 comprise 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. (2009) for the evaluation of the diversity of *Corchorus olitorius* and *Corchorus capsularis*

Table 1: Characteristics of SSR Markers Tested in *Corchorus olitorius*

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	TTACATTATATAATGTCCAGCCA	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	(CTAT) 16	56.9

- ✓ **DNA extraction**

For each accession, the fresh leaves (age) 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 in the laboratory, 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

- ✓ **PCR amplification**

The PCR reactions were carried out in a final volume of 25 µl containing 1 µl of the 3 'primer (forward primer), 1 µl of the 5' primer (reverse primer), 18 µl of ultrapure water, 5 µl of premix PCR composed of 1U Taq polymerase, 250 µM Tris-HCL, 10 mM KCl, 1.5 mM MgCl2 and a disk from the FTA map containing the genomic DNA of the accession to amplify varying concentration 0.25 to 0.50 ng / µl.

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.

- ✓ **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 (BET) 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.

- ✓ **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.

- **Results**

- ✓ **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 marker. 9 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 (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 primers tested 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 potential of 0.276 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.

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

N°	Markers	A^t	A^e	He	I	PIC	P
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
MOYENNE		3.31	4.21	0.20	1.05	0.32	92.19

At: total number of alleles, Ae: effective number of alleles, He expected heterozygosity, PIC: Polymorphism Information Content, I: Shannon diversity index, P: polymorphic loci rate

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 sub-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 0.93, an expected heterozygosity of 0.21, a polymorphism information potential of 0.21 and a polymorphism rate of 85, 19 % were observed in the sub-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.

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 3). 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*.

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

N°	Phytogeographic sector	A^t	A^r	A^p	A^e	I	H	Uh	Pic	P(%)
1	**Southern-soudaniaen**	28	0	0	3.83	0.59	0.11	0.12	0.11	40.74
2	**Northd-soudanian**	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

Ae: effective number of alleles, P (95 %): polymorphism at the 95 % threshold, He expected heterozygosity, PIC: Polymorphism Information Content, I: Shannon diversity index. The Sahelian sector was excluded for this analysis.

Table 4: Distribution of genetic diversity according to the botanical varieties

N°	Botanical varieties	A^t	A^r	A^p	A^e	I	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

Ae: effective number of alleles, P (95 %): polymorphism at the 95 % threshold, He expected heterozygosity, PIC: Polymorphism Information Content, I: Shannon diversity index. The Sahelian zone was excluded for this analysis.

✓ **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 0.073 and 0.271 differentiation index (Fst) were observed between the North Sudanese sector and the Sahelian sector. In contrast, the shortest Nei minimum distance of 0.003 and differentiation index (Fst) of 0.017 were observed between the accessions of the North-Sudanian and South-Sudanian sectors. As for the botanical variety factor, the minimum distance of Nei was 0.002 and the differentiation index Fst was 0.0064 (Table 5).

Table 5: Genetic Differentiation Between Phytogeographic Sectors

Phytogeographic sectors	Nei minimum distance			Differentiation index Fst		
	S-sou	N-sou	S-sah	S-sou	N-sou	S-sah
S-sou	0			0		
N-sou	0.001	0		0.0016^{ns}	0	
S-sah	0.006	0.003	0	0.0109^{ns}	0.0109^{ns}	0

N-sou: North Sudanese; S-sah: South Sahelian; S-sou: South Sudanese; ns: not significant

Table 6: Inter-varieties genetic differentiation

N°	Botanical varieties	Nei minimum distance		Differentiation index Fst	
		V1	V2	V1	V2
1	V1	0		0	
2	V2	0.002	0	0.0064^{ns}	0

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

✓ **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 respectively of 8; 37 and 51 accessions. 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.

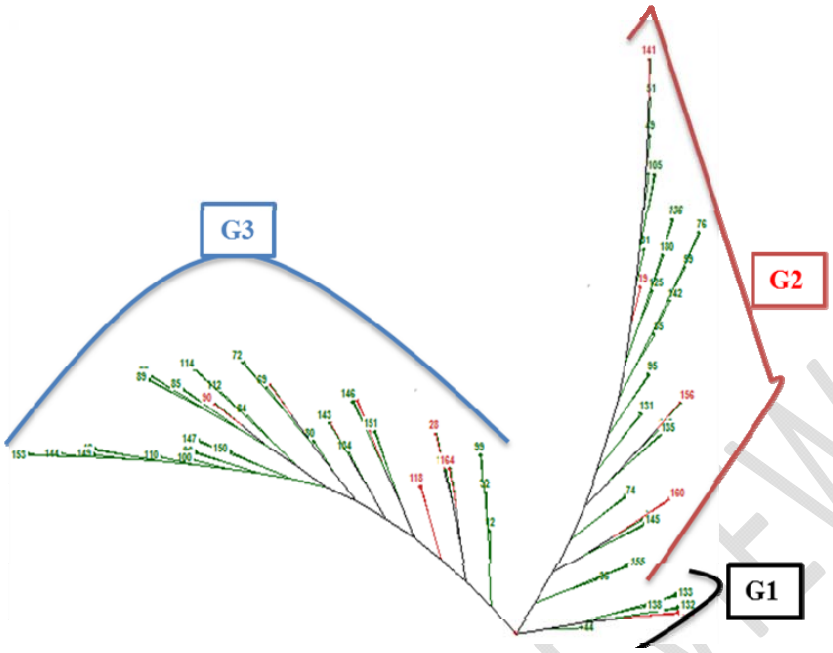

Figure 1: Dendrogram of the 96 accessions of C. olitorius constructed from the matrix of dissimilarities according to the Neighbor-Joining method Legend: green (variety *C. olitorius var olitorius*); red (variety *C.oltorius var incisifolius*)

✓ **Description of genetic groups**

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, a 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.

Table 7: Distribution of Genetic Diversity by Three Genetic Groups

Groups	Nomber	A^t	A^r	A^p	A^e	I	H	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

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: Genetic Differentiation inter Genetics Groups

Genetic	Nei minimum Distance			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	
Group3	0.033	0.045	0	0.218**	0.192**	0

Discussion

The (delete) 92% 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 Akter *et al*. (2008); Mir et al. (2008) and Ghosh *et al*. (2014) 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 (Ghosh *et al*., 2014). The low value of the average PIC (0.31) is very close to the results obtained by Zhang *et al*. (2015) 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 (Benor *et al*., 2011, Ghosh *et al*., 2014), SSRs (Huq *et al*., 2009, Zhang *et al*., 2015) 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 Huq *et al*., (2009) on 16 germplasm accessions from the Bangladesh Research Institute using 27 SSR 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 Huq *et al*. (2009) was performed with accessions of various origins (India and Africa). According to Huq *et al*., (2009), Banerjee *et al*., (2012) and Ghosh *et al*., (2014), 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*. (2008). 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*. (2008). 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 (2002), *Corchorus olitorius var insicifolius* derives from *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(delete) Banerjee *et al*. (2012) 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 Yuan et al. (2010) and Benor *et al*. (2012), 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.

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) group 2 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.*

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