Original Research Article

Genetic diversity and relationship between wild and cultivated cowpea [*Vigna unguiculata* (L.) Walp.] as assessed by allozyme markers

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Abstract

In Cameroon, cowpea plays an important role in traditional agroecosystems. Genetic variation in wild and cultivated cowpea in Cameroon has not yet been documented. Allozyme markers because of their codominance and polymorphism are useful tools for studying genetic variation and disparity in plant species. The present study was undertaken to elucidate the relationship between wild and cultivated cowpea from Cameroon. Ten enzyme systems encoding nineteen isozyme loci were used on 62 cowpea germplasm (45 wild and 17 cultivated). A total of thirty-two alleles were found. One allele was only found in cultivated samples (Enp^{98}). Eight alleles were specific only to wild plant (Amp_2^{98}, Amp_{3a}^{103}, Amp_4^{96}, Fdh^{104}, Idh_2^{95}, Pgi_3^{92}, Pgm_2^{95} and Sdh^{95}). Twenty-three alleles were common to both wild and cultivated accessions. Amp_2^{102} ($z = -4.633$, $p < 0.001$) and Fle_3^{96} ($z = -2.858$, $p < 0.010$) were significantly more represented in cultivated compared to wild cowpea forms. The mean number of alleles per locus in wild (1.632 alleles/locus) cowpea were significantly higher ($t = 2.805$, $p < 0.010$) compared to cultivated (1.263 alleles/locus) cowpea. Also, the proportion of polymorphic loci (P = 52.63%) and average Nei's genetic diversity (He = 0.126) were important in wild, compared to the cultivated plants: P = 26.31% and He = 0.063, respectively. The low level of diversity found in domesticated accessions compared to wild can be attributed to a major genetic bottleneck that probably happened during the domestication process. Cluster analysis revealed by UPGMA dendrogram separated the 62 accessions into three clusters. Although an admixture of both wild and cultivated accessions within the same cluster were found, the dendogram, however, highlighted a visible separation between wild and cultivated cowpea. Wild cowpea with many more private alleles indicates an untouched resource available for future breeding.

Keywords: allozyme markers; Cameroon; genetic diversity; landrace; *Vigna unguiculata*; wild

INTRODUCTION

Cowpea (*Vigna unguiculat* (L.) Walp.) is one of the most important tropical grain legumes in Sub-Saharan Africa feeding people and their livestock (Kebede and Bekeko, 2020). Across the continent, it also serves as green manure for the next crop because of its nitrogen fixing characteristics (John et al., 1992). This legume can also serve as cash generating income for many farmers in tropical regions (Manda et al., 2019). Due to its high protein content in leaves (23 to 40% w/w; Dakora and Belane, 2019) and in seeds (22.8 to 28.9% w/w; Weng et al., 2019), cowpea can efficiently substitute meat or fish for people who can't afford because of poverty (Madodé et al., 2011). The world's cowpea production is

estimated to about 6.5 million metric tons annually on about 14.5 million hectares according to Boukar et al. (2018). Africa accounts for the majority (83.4 %) of the world cowpea production (FAOSTAT, 2016). West Africa is the major cowpea producing region in Sub-Saharan Africa with the top-producing countries being Nigeria and Niger, covering cumulatively 80% of the total regional production (Horn and Shimelis, 2020). In Cameroon, the northern and the western regions are the largest contributors to the national production that is estimated to about 11 000 tons from 105 000 hectares planted area (Dudje et al. 2009; Bidima 2012). Bennett and Leitch (1995) ranks cowpea as a diploid plant species (2n = 2x = 22) with an estimated nuclear genome size of approximately 620 Mb. Taxonomic studies of the genus *Vigna* by Pasquet (1999) and Zuluaga et al. (2021) divided cowpea (*Vigna unguiculata*) into ten perennial subspecies and one annual subspecies (ssp. *unguiculata*). These studies also fragmented the ssp. *unguiculata* into var. *unguiculata* for the cultivated forms and var. *spontanea* (Schweinf.) Pasquet for the wild and weedy forms (Pasquet 1999). In Sub-Saharan Africa, var. *spontanea* is quite wide spread. They are found in areas of secondary growth, along roadsides, fields, and field margins (Kouam et al. 2012). These wild forms, when grown close to the cultivated ones, have the potential to interact and can easily interbreed and produce fertile offspring through hybridisation (Kouadio et al., 2007).

Because of the potential impact of climate change and worldwide growth in food demand, detailed knowledge on the genetic status of plant populations is needed for their effective management. In cowpea, similar to many plant crops species, an important proportion of the genetic diversity of the species can be found in unimproved domesticated varieties, known as landraces. These crop landraces are locally adapted and associated with traditional farming systems (Villa et al. 2006). Crop landraces derived from the wild plants during the domestication process. Both plant materials represent a fascinating system for the investigation of the distribution of genetic diversity and relationship that has resulted from the crop evolutionary processes. Wild plants are heavily threatened in their natural habitats because of urbanisation and road construction. High priority should be given to the collection and preservation of this germplasm for the maintenance of genetic variability (Forneck et al., 2003). Wild cowpea and cultivated forms are found in Cameroon. The presence of both types offers to cowpea breeders a valuable gene pool where they can extract genes of interest in their breeding efforts to develop superior cultivars. There is need to increase the understanding of the *Vigna unguiculata* gene pool through gain of information about genetic diversity and population structure needed by cowpea breeders to enhance the future genetic improvement. In this study, we analysed allozyme variation in wild and cultivated germplasm of *Vigna unguiculata* from Cameroon with the following null hypothesis: (1) cultivated cowpea does not differ genetically from wild, (2) there is no genetic relationship between wild and cultivated cowpea.

MATERIAL AND METHODS

Plant material and enzyme electrophoresis

Forty-five wild cowpea (*Vigna unguiculata* ssp. *unguiculata* var. *spontanea*) and seventeen cultivated cowpea (*Vigna unguiculata* ssp. *unguiculata* var. *unguiculata*) accessions of cowpea were sampled across Cameroon based on the availability. The accession names and the geographical coordinates of their collection points are presented in Table 1. For each accession, two to three pods per plant were collected and kept separately. These pods were collected from a single inflorescence peduncle or from two adjacent peduncles. Seeds of these pods were allowed to fully dry and then stored at –20 °C until the laboratory analysis was carried out. A minimum of four seeds from one single pod were analysed for each accession. The analysis consisted of studying allozyme variation using horizontal starch gel electrophoresis. Seeds were soaked in distilled water overnight to initiate germination prior to enzyme expression. Imbibed seeds were then crushed with de-ionized water using porcelain mortar and pestle. Following crushing, enzyme extracts were absorbed onto a 3 mm Whatman filter paper wicks and then applied to a 14% starch gel as described by Second and Trouslot (1980). Ten enzyme systems presented in Table 2 were essayed in citrate/histidine buffer system at a pH of 6.0. The electrode buffer contained 0.41M citric acid trisodium salt, pH 6.0 and the gel buffer comprised 5 mM L-histidine mono HCl 2.5 mM NaCl, pH 6.0. Electrophoresis was carried out at 200 V in the cold room at 4 °C for approximately three hours. Enzyme-specific staining was prepared according to Wendel and Weeden (1989).

Gel scoring and data analysis

As suggested by Pasquet (1999), for each enzyme we numbered as "1" the presumed locus encoding the most anodally migrating bands. Additional loci were numbered sequentially according to their decreasing electrophoretic mobility (marked in subscript in Tables 3 and 4). For each locus, the most common allele was assigned the number 100 (marked in superscript

Table 1. List of accessions analysed in this study and their geographic coordinates

	Accession name	Latitude	Longitude
Wild			
1	SP 1	10 36 N	13 59 E
2	SP 2	10 11 N	14 31 E
3	SP 3	11 08 N	14 18 E
4	SP 4	11 08 N	14 18 E
5	SP 6	No coordinates	
6	SP 7	10 59 N	14 31 E
7	SP 8	10 07 N	14 08 E
8	SP 12	10 53 N	13 47 E
9	SP 13	10 53 N	13 47 E
10	SP 14	10 34 N	13 56 E
11	SP 15	10 41 N	13 36 E
12	SP 19	10 12 N	14 11 E
13	SP 22	10 40 N	14 20 E
14	SP 23	09 34 N	13 31 E
15	SP 28	09 19 N	13 24 E
16	SP 30	10 27 N	14 46 E
17	SP 32	10 07 N	14 08 E
18	SP 41	10 59 N	14 12 E
19	SP 46	11 24 N	14 34 E
20	SP 56	10 28 N	13 41 E
21	SP 57	10 28 N	13 41 E
22	SP 58	10 28 N	13 41 E
23	SP 61	No coordinates	
24	SP 100	09 04 N	12 59 E
25	SP 101	08 49 N	14 11 E
26	SP 103	08 42 N	12 48 E
27	SP 104	08 55 N	13 31 E
28	SP 106	10 04 N	14 08 E
29	SP 107	09 16 N	14 16 E
30	SP 109	11 02 N	14 27 E
31	SP 112	10 57 N	14 39 E
32	SP 113	10 56 N	14 48 E
33	SP 115	10 56 N	14 49 E
34	SP 116	10 46 N	14 39 E
35	SP 117	10 46 N	14 39 E
36	SP 119	10 45 N	14 34 E
37	SP 120	10 45 N	14 34 E
38	SP 123	08 34 N	12 43 E
39	SP 125	10 57 N	14 39 E
40	SP 126	09 45 N	13 34 E
41	SP 127	09 43 N	13 33 E
42	SP 128	10 04 N	14 29 E
43	SP 129	10 04 N	14 29 E
44	SP 132	09 36 N	13 30 E
45	SP 134	09 36 N	13 30 E
Cultivated			
1	CS 152	04 51 N	14 16 E
2	NO 1036	11 47 N	15 06 E
3	OU 23	04 22 N	09 06 E
4	NO 1669	10 54 N	14 12 E
5	OU 174	05 45 N	11 03 E
6	NO 2292	08 29 N	13 19 E
7	CS 53 B	04 39 N	09 53 E
8	NO 2294	10 47 N	13 46 E
9	CS 45	04 52 N	11 15 E
10	OU 176C	05 32 N	10 05 E
11	OU 59A	04 59 N	09 26 E
12	NO 184	10 39 N	13 52 E
13	NO 1479	No coordinates	
14	NO 2527	08 32 N	13 10 E
15	NO 2461	08 38 N	12 38 E
16	OU65	No coordinates	
17	CSB5	No coordinates	

Table 2. List of enzyme systems used

	Enzyme system	Abbreviation	EC Number	Number of loci
1	**Alcohol dehydrogenase**	Adh	1.1.1.1	2
2	**Aminopeptidase**	Amp	3.4.11.1	3
3	**Endopeptidase**	Enp	3.4.--.--	1
4	**Formate dehydrogenase**	Fdh	1.2.1.2	1
5	**Fluorescent esterase**	Fle	3.1.1.--	2
6	**Isocitrate dehydrogenase**	Idh	1.1.1.42	2
7	**Phosphogluconate dehydrogenase**	Pgd	1.1.1.43	2
8	**Phosphoglucoisomerase**	Pgi	5.3.1.9	3
9	**Phosphoglucomutase**	Pgm	5.4.2.2	2
10	**Shikimate dehydrogenase**	Sdh	1.1.1.25	1

in Table 3). Others were measured in millimeters of increased or decreased mobility in relation to this standard and using the same nomenclature as in Pasquet (1999). The genotype of each mother plant was estimated from the progeny array as described Brown and Allard (1970). This was done using MLTR computer program, version 2.2 of Ritland (2002). Genetic diversity indices were calculated with the help of GenAlEx computer program version 6.501 (Peakall and Smouse 2012). The level of gene variability was assessed by calculating allele frequencies, the proportion of polymorphic loci [*P*], the mean number of alleles per loci [*A*], and the total gene diversity [*He*] according to Nei (1973]. Wright's inbreeding coefficient (FIS) (Wright 1922) was used to calculate deviations from Hardy-Weinberg equilibrium for each polymorphic locus [FIS = (He – Ho) / He]. Z-test and t-test were used to compare, respectively, allele frequencies and genetic diversity indices between wild and cultivated cowpea. Jaccard's similarity coefficients between accessions

Table 3. Allele frequencies at variable allozyme loci in wild and cultivated cowpea from Cameroon

Locus	Allele	Frequency		z-test	
		Wild(N = 45)	Cultivated (N = 17)	z	Significance
Adh_1	Adh_1^{100}	1	1	0.000	NS
Adh_2	Adh_2^{100}	1	1	0.000	NS
Amp_2	Amp_2^{98}	0.011	0	0.434	NS
	Amp_2^{100}	0.756	0.118	4.542	***
	Amp_2^{102}	0.233	0.882	–4.633	***
Amp_{3a}	Amp_{3a}^{100}	0.956	1	–0.879	NS
	Amp_{3a}^{103}	0.044	0	0.879	NS
Amp_4	Amp_4^{96}	0.078	0	1.186	NS
	Amp_4^{100}	0.922	1	–1.186	NS
Enp	Enp^{98}	0	0.176	–2.885	**
	Enp^{100}	1	0.824	2.885	**
Fdh	Fdh^{100}	0.611	0.588	0.165	NS
	Fdh^{102}	0.367	0.412	–0.326	NS
	Fdh^{104}	0.022	0	0.616	NS
Fle_1	Fle_1^{100}	1	1	0.000	NS
Fle_3	Fle_3^{96}	0.556	0.941	–2.858	**
	Fle_3^{100}	0.444	0.059	2.858	**
Idh_1	Idh_1^{100}	1	1	0.000	NS
Idh_2	Idh_2^{95}	0.022	0	0.616	NS
	Idh_2^{100}	0.978	1	–0.616	NS
Pgd_1	Pgd_1^{100}	1	1	0.000	NS
Pgd_2	Pgd_2^{100}	1	1	0.000	NS
Pgi_1	Pgi_1^{100}	1	1	0.000	NS
Pgi_2	Pgi_2^{100}	0.333	0.941	–4.271	***
	Pgi_2^{115}	0.667	0.059	4.271	***
Pgi_3	Pgi_3^{92}	0.133	0	1.582	NS
	Pgi_3^{100}	0.867	1	–1.582	NS
Pgm_1	Pgm_1^{100}	1	1	0.000	NS
Pgm_2	Pgm_2^{95}	0.022	0	0.616	NS
	Pgm_2^{100}	0.978	1	–0.616	NS
Sdh	Sdh^{95}	0.022	0	0.616	NS
	Sdh^{100}	0.978	1	–0.616	NS

**: $p < 0.010$; ***: $p < 0.001$; NS: Not significant

were used to construct a dendrogram using the UPGMA (Unweighted Pair Group Method with Arithmetic Mean) method of the NTSYS-pc version 2.1 software programme (Rohlf, 2000).

RESULTS

Allozyme polymorphism and pattern of genetic diversity

Relative mobility of allozymes on the starch gel allowed to observe across the 19 loci, 32 bands in total, representing 32 distinct alleles in both wild and cultivated accessions. Among these, 31 alleles were found within wild germplasm in various frequencies as shown in Table 3. Lower alleles (24) were found within cultivated germplasm and 23 alleles were common in both wild and cultivated accessions (Table 3). Eight alleles were only found within the wild germplasm (Amp_2^{98}, Amp_{3a}^{103}, Amp_4^{96}, Fdh^{104}, Idh_2^{95}, Pgi_3^{92}, Pgm_2^{95} and Sdh^{95}), whereas only one allele (Enp^{98}) was specific to the cultivated accessions. Among the wild accessions, the proportion of polymorphic loci (P = 52.63%) and Nei's genetic diversity (He = 0.126) were higher than in the cultivated accessions: P = 26.32% and He = 0.063, respectively (Table 4). The number of alleles per locus ranges from 1 (Monomorphic locus) to 3 (*Amp*2) with a mean of 1.632 ± 0.155 in wild forms and 1.263 ± 0.102 in cultivated plants. The effective number of alleles per locus ranges from 1 (monomorphic locus) to 1.975 (*Fle*3) with the average of 1.212 ± 0.074 in the wild and

Table 4. Genetic diversity indices in wild and cultivated cowpea from Cameroon

Locus	P		A		Ae		Ho		He		F_{IS}	
	Wild	Culti	Wild	Culti	Wild	Culti	Wild	Culti	Wild	Culti	Wild	Culti
Idh_1	m	m	1	1	1	1	0	0	0	0	m	m
Idh_2	m	m	1	1	1	1	0	0	0	0	m	m
Amp_2	p	p	3	2	1.598	1.263	0.044	0	0.374	0.208	0.882	1
Amp_3	p	m	2	1	1.092	1	0	0	0.084	0	1	m
Amp_4	p	m	2	1	1.168	1	0.022	0	0.144	0	0.847	m
Enp	m	p	1	2	1	1.409	0	0	0	0.29	m	1
Fdh	p	p	3	2	1.967	1.94	0.022	0	0.492	0.485	0.955	1
Fle_1	m	m	1	1	1	1	0	0	0	0	m	m
Fle_3	p	p	2	2	1.975	1.125	0.045	0	0.494	0.111	0.909	1
Idh_1	m	m	1	1	1	1	0	0	0	0	m	m
Idh_2	p	m	2	1	1.045	1	0	0	0.043	0	1	m
Pgi_1	m	m	1	1	1	1	0	0	0	0	m	m
Pgi_2	p	p	2	2	1.799	1.124	0.044	0	0.444	0.11	0.901	1
Pgi_3	p	m	2	1	1.3	1	0.045	0	0.231	0	0.805	m
Pgd_1	m	m	1	1	1	1	0	0	0	0	m	m
Pgd_2	m	m	1	1	1	1	0	0	0	0	m	m
Pgm_1	m	m	1	1	1	1	0	0	0	0	m	m
Pgm_2	p	m	2	1	1.045	1	0	0	0.043	0	1	m
Sdh	p	m	2	1	1.045	1	0	0	0.044	0	1	m
Mean over loci ± SE	p = 52.63%	p = 26.31%	1.632 ± 0.155	1.262 ± 0.103	1.212 ± 0.074	1.098 ± 0.053	0.012 ± 0.008	0 ± 0	0.126 ± 0.039	0.063 ± 0.025	0.929 ± 0.042	1 ± 0
	z = 2.347*		t = 1.908*		t = 1.908*		t = 0.863^{NS}		t = 1.903*		t = 0.794^{NS}	

P = Proportion of polymorphic loci; A = Number of allele per locus; Ae = Effective number of allele per locus; Ho = Observed heterozygosity; He = expected heterozygosity; F_{IS} = Inbreeding coefficient. p = polymorphic; m = monomorphic. *: $p < 0.050$; **: $p < 0.010$; NS: Not significant

1.098 ± 0.053 in cultivated cowpeas. Data from diversity indices indicate that the Cameroonian landrace studied here have a relatively low level of genetic diversity in comparison with the wild counterpart. The inbreeding coefficient (F_{IS}) for all the ten polymorphic loci within wild were higher and positive (F_{IS} = 0.929). The same trend was observed in cultivated accessions (F_{IS} = 1.000, Table 4). These positive inbreeding coefficients suggest that observed levels of heterozygosity was smaller than would have been expected in case of random mating.

Clustering and relationship of sixty-two cowpea genotypes

Cluster analysis of the 62 cowpea accessions by UPGMA method on the basis of the nineteen studied allozyme loci produced a dendogram composed of three main clusters (Figure 1). Most of cowpea landraces (15 out of 17) were grouped in cluster 1, which also included 6 wild accessions (SP1, SP 2, SP 19, SP 61, SP 100 and SP 128). Clusters 2 and 3 were composed of much of wild accessions (Figure 1). Cluster 2 had ten accessions, nine wild and on cultivated (CS 85) while cluster 3 were composed of thirty-one individuals, thirty wild and one cultivated (NO 2471). The presence of cultivated accessions in a cluster composed of most wild plants and vice versa suggests a strong genetic relationship between wild and cultivated cowpea.

DISCUSSION

Allozyme markers have been widely used in the analysis of genetic diversity and relationships in many plant species including cowpea (Vaillancourt et al., 1993; Pasquet, 1999; Kouam et al., 2012), common bean (Santalla et al., 2002; Kouam et al., 2017), rice (Tang et al., 2007) and many other crops species. Maintaining genetic variation is vital to promoting long-term survival and avoiding risk of extinction of plant species. This maintenance appears more challenging for predominantly selfing species like cowpea that generally present low level of genetic variation when compared to obligatory outcrossing species (Hamrick and Godt, 1990). We have analysed 19 allozyme loci in 62 wild and cultivated cowpea accessions from Cameroon. In the wild accessions, diversity indices i.e., the proportion of polymorphic loci, the average number of allele per locus and

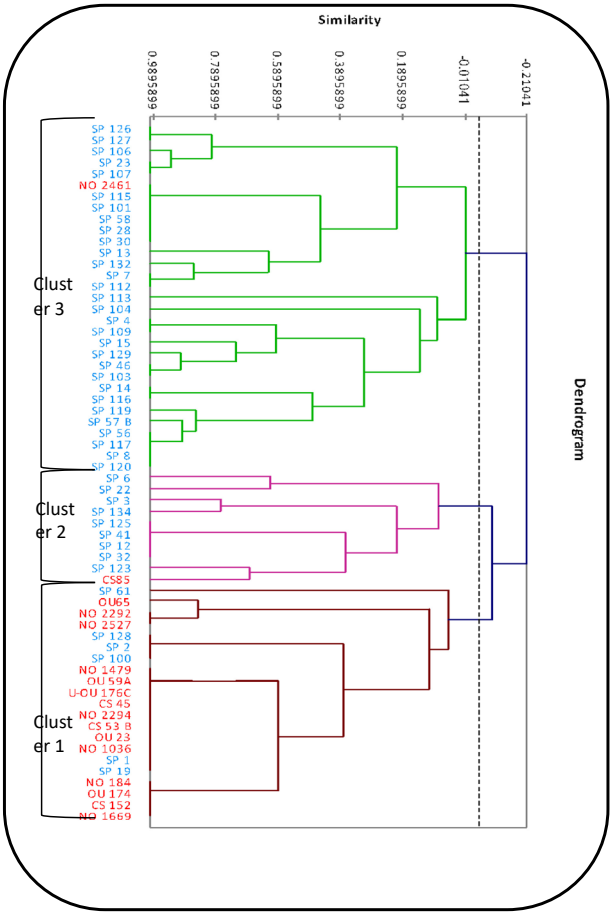

Figure 1. UPGMA dendrogram of cowpea landraces and closely wild relative accessions from Cameroon obtained with studies of 10 enzyme system encoding 19 putative loci

Nei's genetic diversity were much higher than in the cultivated plants. Similarly, previous studies using allozymes reported significantly higher diversity parameters in the wild compared to the cultivated plants (Vaillancourt et al., 1993; Pasquet, 1999). Amplified Fragment Length Polymorphism also showed that wild cowpea is more diverse than its cultivated counterpart (Coulibaly et al., 2002). This result is expected since cultivated plants derived from a small portion of the wild and are projected to represent only a portion of the species genetic variation. Although comparable, these above authors found relatively higher diversity indices in their studies compared to ours results. Analysing 35 enzyme loci on 114 accessions, Pasquet (1999) found in wild sample P = 80%, A = 2.88, He = 0.199 and in cultivated plants P = 23%, A = 1.26, He = 0.084. Vaillancourt et al. (1993) reported with 155 accessions and 26 isozyme loci P = 73%, A = 2.42 and He = 0.168 in wild cowpea and P = 23%, A = 1.23 and He = 0.029 in cultivated plants. This difference observed is explained by the fact that their studies included numerous samples from various countries and origin in sub-Saharan Africa, representing much larger gene pool likely to capture many alleles and much diversity compared to the present study, where accessions are only from Cameroon.

Positive and significant inbreeding coefficients were found in both wild and cultivated cowpeas, indicating significantly less heterozygotes. This deficiency in heterozygotes or excess in homozygotes have two main causes: (1) restricted neighborhood causing much more mating between plant relatives as reported Keller and Waller (2002) or (2) positive assortative mating with preferential mating between individuals of similar genotypesas stated Lstiburek et al. (2005). Many other researchers on highly selfing crops reported positive and significant inbreeding coefficients and attributed it to the structure of populations that favors crossing between relatives: Suvi et al. (2019) in *Oryza sativa*, Kouam et al. (2017) in *Phaseolus vulgaris* and Bi et al. (2003) in *Phaseolus lunatus*. Floral anatomy in *Vigna unguiculata* shows close proximity of its reproductive structures that are stigma and anthers (Lush, 1979). This disposition is known to promote self-pollination and production of more homozygous individuals, leading to high inbreeding coefficients in cowpea populations. Multivariate analysis of the 62 accessions through clustering revealed in general three major clusters, one cluster with cultivated accessions and two clusters of wild accessions although some exceptions were found: the overlapping of some wild accessions with cultivated plants and the presence of some cultivated accessions in a cluster dominated by wild accessions. However, the close association of some var. *unguiculata* and var. *spontanea* accessions intensely supports the involvement of the latter in cowpea domestication as demonstrated Feleke et al. (2006) studies, screening PCR-RFLP profiles in wild and cultivated cowpea. Six wild accessions were found among cultivated accessions in cluster 1. This result likely provides evidence of introgression in cowpea. NO2461 was the only cultivated accession found in cluster 3 dominated by wild accessions. This accession was far distant from other landraces analyzed in this study. It belongs to the Textilis groups known to be the most primitive cowpea cultivated plant and is expected to be more close to wild cowpea var. *spontanea* as also reported Pasquet (1996).

CONCLUSION

The genetic diversity of wild and cultivated cowpea accession originating from Cameroon was investigated in this study. It demonstrates the usefulness of allozyme markers to provide information on genetic diversity and relationship among wild and cultivated *Vigna unguiculata*.

Several alleles were found, with significantly more alleles identified within the wild cowpea germplasm. Our results show that wild cowpea germplasms from Cameroon have more different isoenzymes and is a source of important traits available for cowpea breeding.

CONFLICT OF INTEREST

The authors declared no conflicts of interest with respect to research, authorship and publication of this article.

ETHICAL COMPLIANCE

The authors have followed the ethical standards in conducting the research and preparing the manuscript.

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