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Genetic diversity and population structure of *Vigna unguiculata ssp. unguiculata var. spontanea* in Sudan

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ABSTRACT:

Enzyme electrophoresis was used to estimate the genetic diversity and population structure of thirteen *Vigna unguiculata ssp. unguiculata var. spontanea* populations in Sudan. Plant genotypes were homozygous at most loci and at several populations. Nine of the twenty-one allozyme loci analysed (42.9%) showed detectable polymorphism, but only 11.4% of loci were polymorphic within local populations. Gene diversity at the species level and at the population level was low (Hes = 0.084; Hep = 0.049, respectively). Analysis of fixation indices, calculated for all polymorphic loci in each population showed a substantial deficit of heterozygotes relative to Hardy – Weinberg expectations. This deficit is partly associated with inbreeding due to self and consanguineous mating. High inbreeding and strong genetic differentiation coefficients were found. Allele frequency data revealed a low degree of within population genetic diversity *(Hs* = 0.049) and a high degree of genetic heterogeneity among populations *(Gst = 0.409*). The indirect estimates of gene flow were calculated based on the level of genetic differentiation between populations and frequencies of private alleles. These were 0.274 and 0.043 respectively. Genetic and geographic distances were positively correlated although not significant, indicating that very little genetic variation is explained by difference in geographic pattern. This may be a result of inbreeding and genetic drift through a few founders coupled with limited pollen flow.

Keywords:
Enzyme, Genetic diversity, Population structure, *Vigna unguiculata*.

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INTRODUCTION

Taxonomic studies of the genus *Vigna* divided cowpea (*Vigna unguiculata L. Walp*.) into ten perennial subspecies and one annual subspecies (ssp. *unguiculata*) (Pasquet 1993, 1997, 1999). These studies split the ssp. u*nguiculata* into var. *unguiculata* for the cultivated forms and var. *spontanea* (Schweinf.) Pasquet for the wild and weedy forms (Pasquet 1999). All member of cowpea is a diploid plant species (2n = 2x = 22) (Pasquet, 1999). In the Africa continent, *var. spontanea* is quite wide spread and easily interbreed with the cultivated forms (*var. unguiculata*) and produce fertile offsprings through hybridisation (Kouadjo 2007). Annual cowpea is primarily a self-pollinated plant species (Fery 1985); however, cross -pollination does exist and is mediated by bees of the genus *Xylocopa* and *Megachilidae* family (Tignegre et al. unpublished). These pollinators are expected to do many more within flower patch flights than between flowers patch flights (Levin and Kerster 1974, Pasquet et al. 2008). The crop plays a critical role in the live of millions of people in the world, especially in Sub-saharan Africa where it feed people, their livestock and the next crop (Singh et al. 1997, Quin 1997).

Enzyme electrophoresis has been widely used for the characterization of the genetic variability, the evaluation of the genetic structure as well as the determination of the genetic relationship within and among plant populations (Hamrick and Godt 1990). The genetic structure is one aspect of the genetic organisation of a species, often cited to play a key role in the speciation process (Wright 1965). The level of allozyme variation provides a basis on which to build sound programs for the conservation of the genetic diversity of rare and endangered species (Hamrick et al. 1991). In addition, allozyme diversity can be used as a yardstick to measure the effectiveness of *in situ* and *ex situ* conservation programs (Hamrick et al. 1991, Hamrick and Godt 1996, Barrett and Kohn 1991). Wild relatives of cultivated crops receive special attention because of their poor representation in gene banks and their high value as large stores of genetic variation (Frankel 1974, Brown 1978, Marshall 1990). Despite the importance of knowledge on genetic variation for providing information for conservation purposes, detailed studies of genetic variation are not available for cowpea populations. Pattern of geographical variation in allele frequencies within a species may provide valuable information on past and current evolutionary forces affecting the evolution of the species such as gene flow, genetic drift, and natural selection (Wright 1978, Loveless and Hamrick 1984, Slatkin and Barton 1989).

At the accession level, the genetic variation of wild cowpea have been evaluated using allozyme (Pasquet 1993, 1999, Panella and Gept 1992, Vaillancourt et al. 1993), Random amplified polymorphic DNA (Ba et al. 2004), Amplified fragment length polymorphism (Coulibaly et al. 2002) and Restriction fragment length polymorphism (Feleke et al. 2006). This genetic diversity is yet to be studied at the population level. Detailed analyses at the population level may produce new insights and give a better understanding of the distribution of the genetic variation in cowpea populations. For the study of thirteen wild cowpea (*Vigna unguiculata ssp. unguiculata var. spontanea*) populations in different localities in Sudan, the following objectives were established: (1) To estimate the level of genetic variation at the population and species level. (2) To estimate the level of inbreeding, differentiation and detect the structure of cowpea populations. (3) To estimate the level of gene flow among populations

MATERIAL AND METHODS

Sampling procedure and enzyme electrophoresis

Seeds of *Vigna unguiculalata ssp. unguiculata var. spontanea* were collected from thirteen natural populations in Sudan in October 2003. Eight to twelve plants were collected in each population. One to three pods were sampled for each plant and the seeds present in each pod were kept in separate envelopes and store at – 20^{o}C until analyses were carried out. For each plant, only one pod was used and up to four seeds were analysed for the study. Seeds were soaked in distilled - de-ionized water and left overnight at room temperature to initiate germination, prior to enzyme expression. Germinating seeds were then crushed with mortar and pestle and homogenized with distilled – de-ionized water. Enzyme extracts were absorbed onto chromatography wicks and applied into a 14% starch gels. Horizontal gel electrophoresis was then done at a constant voltage of 200 V at 4^{o}C for approximately three hours using a continuous histidine-citrate buffer system (Second and Trouslot 1980). Gels were stained for ten enzyme systems to resolve twenty one allozyme loci: amino peptidase (AMP, three loci), endopeptidase (ENP, one locus), formate

dehydrogenase (FDH, one locus), fluorescent esterase (FLE, two loci), isocitrate dehydrogenase (IDH, two loci), malate dehydrogenase (MDH, four loci)), 6-phosphogluconate dehydrogenase (PGD, two loci), phosphoglucose isomerase (PGI, three loci), Phosphoglucomutase (PGM, two loci), shikimate dehydrogenase (SDH, one locus). Stain recipes were taken from Wendel and Weeden (1989).

Data analysis

Putative loci were designated sequentially, with the most anodally migrating isozyme designated "1", the next "2", and so on. The most common allele at each locus was arbitrary assigned the value 100, slower and faster bands on the zymmogram relative to this common one, which represent other alleles, were given lower and higher values corresponding to their relative migration using the same nomenclature as in Pasquet (1999). The genotype of each mother plant was estimated from the progeny array following the method of Brown and Allard (1970) and using the MLTR computer program, version 2.2 (Ritland 2002).

We calculated using Popgene software version 1.31 (Yeh et al. 1999) standard measures of genetic diversity for each population. This includes the mean number of alleles per locus (*A*), the percentage of polymorphic loci (P), and mean observed (*Ho*) and expected heterozygosity (*He*) at the species and within-population levels. Wright's fixation index (*F*) (Wright 1922) was used to calculate deviations from Hardy-Weinberg equilibrium for each polymorphic locus within populations [F = (He – Ho) / He]. Chi-square tests were used to test for significant deviations in the fixation indices from the expected value that is F = 0 (Li and Horvitz 1953). Chi-square tests were also used to test for heterogeneity in allele frequencies among populations (Workman and Niswander, 1970). Total genetic heterozygosity (H_T), heterozygosity within populations (H_S), genetic diversity among populations (D_{ST}), and the proportion of genetic diversity found among populations (G_{ST}) were calculated following the equations of Nei (1973, 1977) and using GenAlex computer program (Peakall and Smouse, 2006). The genetic structure within and among populations was also evaluated with F-statistics (F_{IT}, F_{IS} and F_{ST}) following Weir and Cockerham (1984) and using Fstat computer program (Goudet 1995). These F-statistic values were tested for difference from zero using permutation tests (Goudet 1995).

The F_{IT} and F_{IS} coefficients measure deficiencies of homozygotes or heterozygotes relative to the panmictic expectations within the overall sample and within populations respectively. The F_{ST} coefficient estimates relative population differentiation. Standard errors were estimated by jack-knifing using the Fstat program (Goudet 1995). Nei's (1972) genetic distances (D) were calculated for each pair-wise combination of population and values were used to construct a dendrogram using the UPGMA (unweighted pair group method with arithmetic mean) method. Indirect estimates of gene flow were calculated based on Wright's (1951) equation: $Nm = 1/4(1/F_{ST} - 1)$, where *Nm* is the average number of migrants exchanged per generation between populations. A second estimate of *Nm,* using the distribution of ''private alleles'' (alleles found in only one population), was calculated using GenAlex computer program (Peakall and Smouse, 2006) following the procedure of Barton and Slatkin (1986).

RESULTS

Patterns of genetic diversity

Of the twenty-one isozyme loci essayed, nine (42.9%) were polymorphic across the range of *V. unguiculata.* The remaining twelve loci (*Fle-1, Idh-2, Mdh-1, Mdh-2, Mdh-3, Mdh-4, Pgd-1, Pgd-2, Pgi-1, Pgi-2, Pgm-1* and *Sdh*) were monomorphic, displaying unique allele each, in all populations. An average of 11.4% of the loci was polymorphic within population, with individual population values ranging from 0 to 19.1% (**Table 1**). We found thirty-four distinct alleles overall across the twenty-one loci. Six of the thirty-four alleles were unique to a single population: Pgm_2^{104} and Idh_1^{090} in SDN03; Pgi_3^{096} in SDN09; Fle_3^{098} in SDN17; Pgm_2^{096} in SDN26 and Enp^{096} in SDN27. The mean number of alleles per locus was 1.62 at the species level. Across populations it ranges from 1 to 1.19 with a mean at 1.128. (Table1). Gene diversity or expected heterozygosity for the species was somewhat low (0.084); in other words, only 8.4% of individuals are genetically expected to be heterozygous at a given locus under random mating conditions. At the population level, expected heterozygosity ranges from 0 (SDN09) to 0.074 (SDN22) with the average at 0.049. Observed levels of heterozygosity in populations (Table 1), however, were almost always lower than Hardy-Weinberg expectations, averaging 0.014.

Table 1. Population coordinates, Proportion of polymorphic loci (P), number of allele per locus (A) Observed heterozygosity (Ho), Expected heterozygosity (He) and Fixation index (F) for thirteen populations of wild *V. unguiculata* in Sudan

Population	Latitude	Longitude	P	A (SE)	Ho (SE)	He (SE)	F
SDN03	12 17 N	34 10 E	0.143	1.143 (0.080)	0.000 (0.000)	0.053 (0.031)	1.000 (0.000)***
SDN06	11 56 N	34 18 E	0.095	1.095 (0.095)	0.010 (0.013)	0.039 (0.040)	0.800 (0.062)***
SDN09	11 47 N	34 27 E	0.000	1.000 (0.000)	0.000 (0.000)	0.000 (0.000)	m
SDN12	11 45 N	34 22 E	0.191	1.191 (0.127)	0.048 (0.044)	0.068 (0.048)	0.303 (0.123)^{NS}
SDN16	11 33 N	34 11 E	0.048	1.048 (0.069)	0.010 (0.013)	0.009 (0.012)	- 0.111 (0.010)^{NS}
SDN17	12 48 N	30 06 E	0.143	1.191 (0.137)	0.000 (0.000)	0.051 (0.037)	1.000(0.000)***
SDN19	12 29 N	29 47 E	0.191	1.191 (0.127)	0.024 (0.025)	0.073 (0.046)	0.614 (0.124)***
SDN22	11 54 N	29 40 E	0.191	1.191 (0.108)	0.027 (0.022)	0.074 (0.043)	0.679 (0.082)***
SDN25	11 42 N	29 45 E	0.048	1.048 (0.069)	0.010 (0.013)	0.020 (0.029)	0.524 (0.022)*
SDN26	11 15 N	29 40 E	0.048	1.048 (0.069)	0.000 (0.000)	0.023 (0.033)	1.000 (0.000)**
SDN27	11 02 N	29 41 E	0.143	1.191 (0.137)	0.020 (0.018)	0.053 (0.040)	0.664 (0.117)**
SDN30	11 24 N	29 37 E	0.143	1.143 (0.084)	0.005 (0.006)	0.043 (0.028)	0.926 (0.028)***
SDN33	11 13 N	29 25 E	0.095	1.191 (0.150)	0.024 (0.018)	0.045 (0.035)	0.454 (0.025)*
Average	Pop. level		**0.114**	**1.128 (0.096)**	**0.014 (0.013)**	**0.049 (0.033)**	**0.662 (0.025)*** **
	Specie level		**0.429**	**1.619 (0.062)**	**0.013 (0.002)**	**0.084 (0.011)**	

***** :significance at the 0.1% nominal level; ** :significance at the 1% nominal level; * :significance at the 5% nominal level**
^{NS}: Not significant. ^{m}: Monomorphic.

Population structure and gene flow

Between populations, allele frequencies were significantly different at eight of the nine polymorphic loci as shown by Chi square test (Table 2). The estimates of genetic structure using Nei's genetic diversity estimates are shown in **Table 2.** The average of total heterozygosity (H_T) and within population genetic diversity (H_S) were 0.083 and 0.049, respectively. The inter-population genetic diversity (D_{ST}) and the coefficient of genetic differentiation among populations (G_{ST}) varied from 0.000 (*Enp*) to 0.172 (Fdh) and from -0.028 (Enp) to 1.000 (Pgi-*3*), with a mean of 0.034 and 0.409, respectively. The result indicates that about 41% of the genetic variation in our sample can be attributed to variation among populations. The inbreeding coefficients (F_{IS} and F_{IT}) for eight of the nine polymorphic loci were significantly greater than zero (Table 2). The mean estimates of these coefficients were significant (F_{IS} = 0.684 P < 0.001; F_{IT} = 0.835 P < 0.001), reflecting that observed levels of heterozygosity within populations and the entire study area were smaller than would have been expected in case of random sexual reproduction. High F_{ST} values for most of the polymorphic loci (Table 2) indicate significant genetic differentiation at the population level (F_{ST} = 0.477, P < 0.001). The majority of test for pair-wise genetic differentiation among populations (60 out of 78) were statistically greater than zero; the highest value (0.904) found between SDN 09 and SDN16 (**Table 3**). Genetic distance (D) values between pair of populations are also shown in Table 3. They ranged from 0.002 to 0.128 with a mean at

Table 2. Chi square tests, Nei's gene diversity, F-Statistics and gene flow estimates at nine polymorphic loci for thirteen populations of wild *V. unguiculata* in Sudan

Gene	$\times^2$	Hs	Ht	Dst	Gst	Fit	Fis	Fst	Nm^W	Nm^S
Amp2	56.840***	0.120	0.122	0.002	0.014	0.502***	0.430**	0.127^{NS}	1.719	
Amp3	62.720***	0.285	0.401	0.116	0.290	0.846***	0.748***	0.389**	0.393	
Amp4	54.691***	0.079	0.170	0.091	0.535	0.728**	0.339*	0.587***	0.176	
Enp	0.001^{NS}	0.011	0.011	0.000	-0.028	-0.006^{NS}	-0.070^{NS}	0.066^{NS}	3.521	
Fdh	123.162***	0.287	0.460	0.172	0.375	0.814***	0.657***	0.459**	0.295	
Fle3	179.373***	0.133	0.265	0.132	0.499	0.916***	0.804***	0.573***	0.186	
Idh1	95.452***	0.044	0.060	0.016	0.266	1.000***	1.000***	0.381**	0.406	
Pgi3	92.789***	0.000	0.142	0.142	1.000	1.000***	1.000***	1.000***	0.000	
Pgm2	211.556***	0.074	0.118	0.044	0.375	1.000***	1.000***	0.474***	0.278	
Overall		0.049	0.083	0.034	0.409	0.835***	0.684***	0.477***	0.274	0.043

***** :significance at the 0.1% nominal level; ** :significance at the 1% nominal level; * :significance at the 5% nominal level; ^{NS}: Not significant.**

0.047. These distances did not show significant effect on geography as the correlation between genetic and geographic distance was weak (r = 0.10, P > 0.050). A UPGMA dendrogram illustrating genetic relationships among populations were constructed (**Figure 1**). Indirect estimates of gene flow indicate low levels of pollen migration among *V. unguiculata* populations. The application of Wright's (1951) model gave an estimate of Nm^W = 0.274. The low value of *Nm* reflects the high value of F_{ST}. An independent estimate of gene flow (Barton and Slatkin 1986) based on the frequencies of private alleles gave an even lower estimate of gene flow (Nm^S = 0.043). Both estimates of *Nm* being lower than unity suggest strong population differentiation.

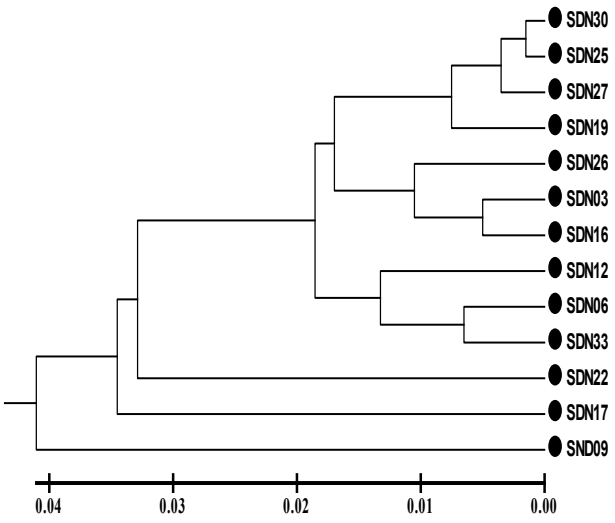

Fig 1: An UPGMA clustering tree based on Nei's (1972) genetic distance calculated for 21 allozyme loci in 13 populations of wild *V. unguiculata*

DISCUSSION

Genetic diversity is essential to avoid risk of extinction and promote the long-term survival of plant species. The loss of genetic variation is thought to decrease both the short-term and the long -term adaptability of populations in variable and changing environments (Hamrick 1994, Young et al. 1996). Populations of selfing species and animal -pollinated species with mixed mating systems (i.e., partially selfed, partially outcrossed) have lower levels of genetic diversity than obligatory outcrossing species (Hamrick and Godt, 1990). Lush (1979) describe cowpea as a highly selfing plant species. Although selfing would leads to individual homozygosity, this study showed that wild *vigna unguiculata* exhibit substantial allozyme variation and agrees with the report of Allard et al. (1968) indicating that selfing species are not necessarily nil in allelic variants. This study showed that *V. unguiculata* var. *spontanea* maintains lower diversity at the population than at species level as generally observed in plants (Hamrick and Godt, 1990). The mean estimates of genetic diversity parameters (*P*, *A* and *He*) within populations of *V. unguiculata* were low (11.4%, 1.13 and 0.049 respectively) to the means for selfing species (20.0%, 1.31, and 0.074) (Hamrick and Godt, 1990). *V. unguiculata*, however, had at the species

Table 3. Nei's standard genetic distance (lower diagonal) and pairwise FST values (upper diagonal) between 13 wild *V. unguiculata* populations

	SDN03	SDN06	SDN09	SDN12	SDN16	SDN17	SDN19	SDN22	SDN25	SDN26	SDN27	SDN30	SDN33
SDN03		0.233**	0.563**	0.357**	0.076^{NS}	0.547*	0.356**	0.455**	0.058^{NS}	0.236**	0.270**	0.149*	0.314***
SDN06	0.027		0.706*	0.105*	0.250^{NS}	0.359^{NS}	0.309^{NS}	0.472**	0.250^{NS}	0.385*	0.279*	0.255**	0.113^{NS}
SDN09	0.062	0.065		0.670**	0.909**	0.759*	0.663**	0.678**	0.808**	0.813**	0.670**	0.639**	0.669**
SDN12	0.049	0.018	0.092		0.407*	0.330^{NS}	0.280*	0.313**	0.360*	0.464*	0.267*	0.331**	0.297**
SDN16	0.010	0.015	0.050	0.038		0.614^{NS}	0.411*	0.493*	-0.029^{NS}	0.428^{NS}	0.324*	0.100^{NS}	0.311*
SDN17	0.087	0.042	0.128	0.047	0.074		0.323^{NS}	0.443*	0.588*	0.628*	0.457*	0.549*	0.466*
SDN19	0.050	0.044	0.106	0.048	0.045	0.049		0.234*	0.278*	0.496**	0.035^{NS}	0.174*	0.416**
SDN22	0.073	0.079	0.124	0.053	0.064	0.073	0.041		0.427*	0.543*	0.380**	0.405**	0.527***
SDN25	0.010	0.019	0.054	0.038	0.002	0.075	0.032	0.057		0.389^{NS}	0.156^{NS}	-0.069^{NS}	0.318**
SDN26	0.024	0.032	0.068	0.059	0.018	0.095	0.073	0.090	0.022		0.463**	0.369**	0.427**
SDN27	0.031	0.032	0.084	0.037	0.026	0.066	0.013	0.058	0.016	0.051		0.014^{NS}	0.319**
SDN30	0.016	0.025	0.064	0.039	0.009	0.077	0.022	0.055	0.003	0.032	0.007		0.288**
SDN33	0.033	0.013	0.071	0.035	0.020	0.057	0.057	0.089	0.025	0.038	0.036	0.026	

Pairwise Fst significances: *, significance at the 0.1% nominal level; **, significance at the 1% nominal level; *, significance at the 5% nominal level; NS marking depicts non-significant values.**

level close levels of overall diversity (42.9%, 1.62 and 0.084) compared to other selfing species (41.8%, 1.69 and 0.124) (Hamrick and Godt, 1990). At the species level, the total gene diversity obtained (0.084) is low when compared to other reported data on wild *V. unguiculata* using allozymes. Panella and Gepts (1992) reported 0.110; Vaillancourt et al. (1993) 0.168 and Pasquet (1999) 0.290. This difference is explained by the fact that their studies included several subspecies, representing much larger part of the crop gene pool in contrast of the present study that involves only one subspecies (*ssp. unguiculata var. spontanea*). Limited allozyme variation may reflect restricted habitat range or homogeneity of the environment (Hedrick et al. 1976). The breeding system of a species is an important determinant of variability at both the species and population levels. The relatively low level of genetic variation found in *V. unguiculata* is consistent with its breeding system. *V. unguiculata* is a highly inbreed and insect-pollinated species, a combination well-known to be associated with low levels of allozyme variation (Brown 1979, Gottlieb 1981, Hamrick and Godt, 1990).

Genetic differentiation among populations is principally a function of gene flow among populations via pollen and seed dispersal (Loveless and Hamrick 1984). Estimates of genetic differentiation indices (F_{ST} = 0.477; G_{ST} = 0.409) indicated highly significant degree of differentiation ($P < 0.001$) among populations of *Vigna unguiculata* in Sudan. Since the species of concern is a tropical inbreed and bees pollinated (Lush 1979, Pasquet et al. 2008), this result may fit with the general observation that inbreeding species maintain high and significant level of population differentiation (Hamrick and Godt 1990). Other studies that have assessed genetic variation in natural populations of tropical inbred plant species using allozyme exhibited similar high levels of genetic differentiation: G_{ST} = 0.519 for wild lima bean (Bi et al. 2003) and G_{ST} = 0.712 in bambara groundnut (Pasquet et al. 1999). This high level of genetic differentiation suggests that gene flow among populations is low (Nm^W = 0.274). The distribution of genetic diversity within and among populations of *V. unguiculata,* however, follows an unexpected pattern. Most of the species genetic variation (about 59%) occurs within populations. We found highs, significant and positives F_{IS} and F_{IT} values (0.684 and 0.835 respectively) indicating that homozygotes were significantly in excess. This

high level of inbreeding can result from several causes: family structuring within a restricted neighbourhood causing mating between relatives (Levin and Kerster 1971, 1974), selection for homozygotes, Wahlund effect and positive assotative mating (preferential mating between individual of similar genotypes) (Crow and Felsenstein 1968). Other authors have also observed positive and significant inbreeding coefficient values and attributed it to populations' substructure and inbreeding (Bi et al. 2003, Ueno et al. 2002). An important feature of the floral biology of *V. unguiculata* is the position of stigma and anther that are in contact with the pollen that usually shed before the full opening of the flower (Lush 1979). This may reduce cross-pollination and provide high reproductive assurance even in the absence of pollinators. Hence, the high levels of inbreeding in *V. unguiculata* appear to be due to autonomous selfing due to the close proximity of the stigma and anthers in this species (Lush 1979). Geography did not correlate significantly with the genetic relationship among populations of *V. unguiculata*. The lack of significant association between geographic and genetic distances found in *V. unguiculata* may be explained by the expectation of a model of differentiation by founder effects (Mayr 1963) where small number of individual from an existing population colonize a new habitat forming a new population genetically close to the former. It might also be the result of genetic drift (Chakraborty et al. 1978) coupled with a highly selfed-mating system and restricted pollen flow among local populations. These factors could all result in the low levels of allozyme diversity within populations and a high degree of genetic divergence between populations of *V. unguiculata*. However, it is difficult to determine which factor played a major role in shaping population genetic structure in *V. unguiculata* in the region.

Gene flow is the movement of gene within and among populations. It has a significant influence on the distribution of the genetic variation (Hamrick 1989). Indirect population genetics statistics estimates the number of individual migrating from one population to the other (Nm). The estimates of *Nm* based either on the level of population differentiation or the private allele approach were very low: $Nm^W = 0.274$ and $Nm^S = 0.043$. In general, if *Nm* is less than one, then local differentiation of populations will result and if *Nm* is grater than one, there will be little differentiation among populations (Wright 1951). Between *V. unguiculata* populations, gene flow (Nm) is very low, less than unity like in other endemic and endangered plant species (Hamrick and Godt 1996) and appears insufficient to counter divergence due to the effects of random genetic drift according to population genetics theory (Wright 1951, Real 1994). Although both low, we noted that Nm^W is about 6 times higher than Nm^S. Such disparity could be attributed to the general high frequencies of private alleles (Idh_1^{090} in SDN03 with 0.4 frequency; Pgm_2^{096} in SDN26 with 0.6 frequency and Pgi_3^{096} in SDN09 with 1 frequency). Similar low level of gene flow were reported by Bi et al. (2003) and Hardy et al. (1997) after studying the population genetic structure of *Phaseolus lunatus*, an autogamous plant species. Two main mechanisms of gene flow exist for cowpea: seed and pollen dispersal. Seed migration, is very unlikely given the extensive spaces that generally separate neighbouring wild populations. Pasquet et al. (2008) reported that bees are able to disperse cowpea's pollen to few km with a low probability of long pollen transport events. Pollen dispersal by bees clearly is the most likely mechanism of gene flow between populations of cowpea.

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