International Journal of Plant & Soil Science

18(4): 1-11, 2017; Article no.IJPSS.35813
ISSN: 2320-7035

Morphological Characterization of Cameroon Cowpea Genotypes for Nitrogen Fixation Related Traits in Low Phosphorus Soils

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Authors' contributions

This work was carried out in collaboration between both authors. Author MFA designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author TCY collected data for the study. Both authors read and approved the final manuscript.

Article Information

DOI: 10.9734/IJPSS/2017/35813
Editor(s):
(1) Adamczyk Bartosz, Department of Food and Environmental Sciences, University of Helsinki, Finland.
Reviewers:
(1) ABA-Toumnou Lucie, University of Bangui, Central African Republic.
(2) Essam Fathy Mohamed El-Hashash, Al-Azhar University, Egypt.
Complete Peer review History: http://www.sciencedomain.org/review-history/20923

Original Research Article

Received 30th July 2017
Accepted 4th September 2017
Published 11th September 2017

ABSTRACT

Aims: The objective of this study was to characterize cowpea genotypes based on grain yields and nitrogen fixing potentials.
Study Design: The experimental design was a two-factor factorial experiment in a randomized complete block with two replications.
Place and Duration of Study: This study was carried out in the International Institutue of Tropical Agriculture (IITA), Cameroon between June and December 2013.
Methodology: Fifty genotypes of cowpea were collected in 2012 from various sources. The exotic genotypes came from the International Institute of Tropical Agriculture (IITA), Senegalese Agricultural Research Institute (ISRA), University of California, Riverside (UCR); and one genotype evaluated previously at the study site (Danilla). The experiment was carried out in pots in the screen house at Nkolbisson IITA, Cameroon. Soil samples were analyzed for pH, organic C, N, exchangeable Ca, Mg, K, and extractable P .Data was collected for 20 morphological traits related to grain yields, nitrogen fixation and plant growth. Analysis of variance (ANOVA) was performed on

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all the traits using the GLM of SAS version 9.2. Cluster analysis was done and dendrogram constructed based on the hierarchical method using Euclidean test in SAS version 9.2.
Results: The combined analysis of variance across environments (P-fertilization levels) showed significant effects for most of the traits. In this study, the interaction between genotype and P-fertilization was significant ($p< 0.05$) for Nsize and very significant ($P< 0.01$) for LCS, SFW, SDW, RDW, shootp, NN, NFN, FN, NDW, NI, delta N of the legume (δ15N leg), %Ndfa, Shoot N, N-fixed and YLDplt. The genotypes were significantly different for all the traits studied at $P< 0.01$.Under low P at a dissimilarity coefficient of 1.8, the phenogram distributed the 50 accessions into two major clusters (I, II). Cluster II has only one accession (Kodek. When P fertilizer was applied, the number of clusters and structure of phenogram II did not change but the accessions were regrouped compared to grouping under low P. Here at a dissimilarity level of 1.8, the phenogram also divided the 50 accessions into two major clusters (I, II). Under high P and high N environments, the maximum dissimilarity level between the accessions was 1.6 so grouping was done only at a dissimilarity level of 0.6. The addition of N fertilizers to the high P pots completely changed the phenogram distributing the accessions into 5 clusters.
Conclusion: Cluster analysis substantiated the existence of diversity among the 50 accessions for the morphological traits studied. The clustering pattern showed that the Cameroon landraces were not distant from each other like the exotic accessions. Furthermore, dissimilarities were observed among accessions from the three geographical regions, presenting a great possibility for the development of suitable varieties for the various agro-ecological zones of Cameroon.

Keywords: Morphological traits; cowpea; Phosporus soils; nitrogen fixation; clusters.

1. INTRODUCTION

Biological nitrogen fixation (BNF) potentials of legumes including cowpea is limited in heavily weathered acid soils that prevail in the humid forest zone (HFZ) of Cameroon. In these soils, phosphorus (P) an important element in nodulation, is generally deficient [1]. Challenges of recurrent climate change and soil fertility decline demands the search for crop varieties that will have the intrinsic capacity to withstand these stresses and still be highly productive. It is reported that cowpea [*Vigna unguiculata* (L.) Walp] has high protein value, high variability and high adaptability [2,3,4]. However, its landraces have not been fully exploited in research, which has caused serious losses of superior genes that could have been used during crop genetic manipulation. The availability of knowledge on genetic diversity of indigenous and cultivated germplasm can greatly enhance the genetic improvements of cowpea. Cowpea as a single crop species has varietal requirements in terms of plant type, maturity date, seed type (colour preference), and its uses are extremely diverse from region to region, making breeding programmes for cowpea more complex than for other crops [5,6,7,8,9,10]. Since no single variety can suit all conditions, there is a need to develop varieties with different attributes and resistance to major biotic and abiotic constraints, to suit the specific needs of different regions and cropping systems. Therefore, characterization of diversity in germplasm collections is important for plant breeders, germplasm curators and farmers.

Recent advances in biotechnology and other related fields have enhanced the improvement of crops for adverse conditions. This notwithstanding, conventional techniques involving the use of biometrical approaches with morphological markers has also proved indispensable as a complementary tool to the modern techniques [11,12]. Distance measures between entries can be based on biochemical marker traits, pedigree information, and quantitative or qualitative morphological traits. Although variation in morphological traits is influenced by environmental factors, their efficiency for selection is still extremely useful. Morphological attributes have traditionally been employed in establishing phylogenetic relationships among genotypes between and within species and for various other purposes including identification of duplicates, studies of genetic variation patterns, and correlation of characteristics of agronomic importance. Marechal et al. [13] used morphological diversity to study taxonomic relationships between genotypes belonging to the genera Phaseolus and Vigna. Lush [14] carried out a study on the flower morphology of wild and cultivated cowpea. Pasquet [15] carried out an intraspecific classification on Vigna unguiculata using their morphological traits. Obute [16] used morphological traits (plant height, number of

leaves, leaf length, the number of pods per plant, pod length and number of seeds per pod) to characterize an aneuploid Vigna unguiculata from the other cytotypes. So far, several types of Cameroonian cowpea have been promoted by IITA, IRAD and other Non governmental organizations (NGOs). However, information on the genetic diversity of these cowpea germplasm is limited. Recently, Gonné et al. [17] evaluated the diversity of 18 traditional cowpea varieties grown by farmers in the Soudano-Sahelian Zone of Cameroon. Their results indicated that the genotypes were predominantly of the spreading type with rough and white seeds and formed five distinctive groups on the basis of morphological discriminant descriptors. The findings are interesting but further studies are necessary as only landraces from northern Cameroon were evaluated. Traditionally, farmers conserve and develop local phytogenetic resources by preserving landraces and associated local knowledge. However, ex situ conservation of potentially useful populations requires a clear understanding of the genetic variation and distinctiveness of these populations. Careful characterization of Cameroon cowpea genotypes is a necessary first step to guide efforts to conserve biodiversity and facilitate breeding.

2. METHODOLOGY

2.1 Plant Material

A total of 50 genotypes of cowpea were collected during 2012 from various sources (Table 1). Among the collection were seeds of ten Cameroon landraces obtained from farmers in the different agro-ecological zones, research stations, and village markets in Cameroon. The exotic genotypes came from the International Institute of Tropical Agriculture (IITA), Senegalese Agricultural Research Institute (ISRA), University of California, Riverside (UCR); and one genotype evaluated previously at the study site (Danilla). The seeds were conserved ex situ, dried and stored at -20°C and then multiplied at IRAD Nkoemvone, during which each landrace was self-pollinated for purification.

Table 1. Source of 50 *Vigna unguiculata* germplasm characterized

No.	Accession	Source	No.	Genotype	Source
1	58-77	Senegal	28	IT08K-161-2	IITA
2	Ahel	North Cameroon	29	IT08K-162-3	IITA
3	Bambey 21	UCR	30	IT08K-189-8	IITA
4	CB46	UCR	31	IT08K-193-14	IITA
5	DANILLA	UCR	32	IT08K-193-6	IITA
6	Dsch MMBL	West Cameroon	33	IT08K-215-2	IITA
7	FRIJOL BAYO	UCR	34	IT84S-2246-4	IITA
8	Grif 14286	UCR	35	IT89KD-288	IITA
9	IT00K-1263	IITA	36	IT90K-277-2	IITA
10	IT00K-835-45	IITA	37	IT95K-105-2	IITA
11	IT04K-227-4	IITA	38	IT95K-1090-12	IITA
12	IT06K-111	IITA	39	KODEK	North Cameroon
13	IT06K-275	IITA	40	LORI-Niebe	North Cameroon
14	IT07K-202-2-10	IITA	41	Mouride	UCR
15	IT07K-210-1-1	IITA	42	Mozongo	North Cameroon
16	IT07K-241-1-2	IITA	43	NKOL CR white	Centre Cameroon
17	IT07K-243-1-10	IITA	44	Tiko	Southwest Cameroon
18	IT07K-298-9	IITA	45	UCR 287	UCR
19	IT07K-302-10	IITA	46	UCR 3373	UCR
20	IT07K-309-44	IITA	47	Vya-NIEBE	North Cameroon
21	IT08K-125-18	IITA	48	YACINE	Senegal
22	IT08K-126-2	IITA	49	yde 2	Centre Cameroon
23	IT08K-134-11	IITA	50	Yde 6	Centre Cameroon
24	IT08K-134-12	IITA			
25	IT08K-149-1	IITA			
26	IT08K-149-3	IITA			
27	IT08K-150-24	IITA			

IITA = International institute of tropical agriculture, UCR = University of California Riverside

2.2 Experimental Design and Layout

The experiment was carried out in pots in the screen house at Nkolbisson (11° 36' E; 344' N) IITA, Cameroon. The experimental design was a two-factor factorial experiment in a randomized complete block with two replications. Each entry was represented by two pots in each replication. The factors were fertilization level and the genotypes. Plant nutrient application was as follows: N0P0 (no Nitrogen and no Phosphorus applied; N0P30 (0 mg N and 30 mg P per kg of soil); and N90P30 (90 mg N and 30 mg P per kg of soil). All pots received potassium fertilizer at equal dose (80 mg K per kg of soil). Nitrogen, phosphorus and potassium were supplied as ammonium sulphate $(NH_4)_2SO_4$, KH_2PO_4 and muriate of potash, respectively. For each treatment, ten pots were planted with early maize variety (TZEEI 42) from IRAD Maroua, to serve as reference crop for the quantification of nitrogen fixation.

2.3 Soil Sampling

A composite soil sample was collected from the field in Nkoemvone on low N plots to a depth of 20 cm after removing surface litter. The low N plot was created by planting maize for two seasons at very high densities (100,000 plants /ha).

2.4 Plant and Soil Analyses

Soil samples were analyzed for pH, organic C, N, exchangeable Ca, Mg, K, and extractable P. For plant samples, all fresh biomass samples were oven-dried at 65o C, then ground to pass through a mesh of 0.5 mm, and digested according to Novozamsky et al. [18] Total N was determined with an ammonium sensitive electrode [19] Total P was determined by the malachite green colorimetric procedure [20]. Soil particle size was also determined as presented in section 4.2.3. For P, 0 – 20 ppm is classified as low, 20 – 40 as moderate and more than 40 as high. Soil N less than 0.2% is classified as low, 0.2 – 0.4 % as moderate and more than 0.4 % as high.

2.5 Data Collection

Data on the following traits were collected:

1) Days to 50% flowering (Dflw) – Data was collected on number of days from sowing to when 50% of the plants of same genotype in a treatment showed the first fully open flower.
2) Leaf Colour Score (LCS) - Three leaves per genotype in a replication were sampled at six weeks after planting and chlorophyll content measured. The LCS was measured with a chlorophyll meter [21].
3) Nodule score (NSC) – One plant per genotype in a replication was harvested at early podding stage (5-8weeks after planting). To examine the roots, the plants were carefully removed from the pots manually. The soil was separated from the roots by carefully shaking to loosen the soil followed by washing with tap water. The nodule scoring followed a 0-5 scale [22,23].
4) Nodule Number (NN): After scoring, the total number of nodules per plant was counted.
5) Number of non-functional nodules (NFN): The functional nodules were identified by their color after cutting the nodules. All nodules with green or white colour were considered non-functional.
6) Number of functional nodules (FN): After selecting the non-functional nodules the rest of the nodules were counted as functional.
7) Nodule dry weight (NDW) - Dry weight was obtained by drying nodules in an oven at 70°C for 72 h. The value was expressed in milligram per plant.
8) Shoot fresh weight (SFW): At harvest, the fresh weight of the shoot was taken on the field and expressed in grams per plant.
9) Shoot dry weight (SDW) – At the early podding stage, after collecting data on nodulation, above ground parts were harvested and dried in the oven at 70°C for 72 h. These dried plants were weighed and value recorded in grams per plant.
10) Root dry weight (RDW): Roots were dried in the oven at 70°C for 72 h. These dried plants were weighed and value recorded in gram per plant.
11) Nodule size (Nsize): The nodule size [24,25] was estimated as nodule dry weight divided by nodule number (NDW/NN).
12) Nodulation index (NI): The nodulation index was calculated as number of nodules per gram of the total fresh biomass [26].
13) Number of pods per plant (Npod) – At harvest, when pods turned completely brown on a plant, the pods per pot were counted. The dry pods were threshed by hand and seeds dried for 24 h at 40°C.

14) Number of seeds per pod (Sdpod): The number of seeds from 10 randomly selected pods per plant were counted and the average recorded. The moisture content of seeds was 12% after oven drying.
15) Grain yield per plant (YLDplt) – Grain yield was measured per pot and expressed in gram plant-1.
16) Shoot phosphorus content (Shoot P) – Dry shoots were ground and analysed for P. Total P was analyzed using Murphy Riley reagent and read on a UV-VIS spectrophotometer.
17) Delta N of the legume (δ15N leg): is the 15N natural abundance of legume. It was calculated according to Unkovich et al. [27] δ15N = [(atom%15Nsample - atom%15Nair)/atom%15Nair] *1000.
18) Percentage N derived from atmosphere (%Ndfa) – Maize was used as the reference crop. After drying the plants, 5 g of finely ground and sieved (2mm) plant material (Leaves of cowpea and maize reference plant) were weighed into labeled plastic bags and mailed to the stable light isotope laboratory, University of Cape Town for quantification of δ15N and Shoot N (mg N per g plant) and eventual estimation of nitrogen fixed by the δ15N natural abundance method [28,29,30,31]. Samples were collected from the legume and reference plants at the same period, as described by Unkovich et al. [27]. The 15N natural abundance (δ15N) of the plant sample was calculated as [27] δ15N = [(atom%15Nsample - atom%15Nair)/atom%15Nair] *1000.

    The proportion of N derived from fixation (%Ndfa) was estimated as [30;27]: %Ndfa = [(δ15N ref - δ15N leg)/ (δ15Nref - B)] *100.

    Where δ15Nref is the 15N natural abundance of reference plants, δ15Nleg is the 15N natural abundance of legume, and B represents the δ15N of legume plants relying entirely on symbiotic N_2 fixation for their N nutrition. The B value incorporates the isotopic fractionation associated with nitrogenase activity during N2 fixation and replaces the value of atmospheric N_2 [30,27]:

    The B value was calculated by taking the average value from five accessions collected from different geographic areas. This measurement was done on sterile-grown plants of each accession raised in pots [32] and inoculated with rhizobia isolated from the nodules of that accession. Shoot 15N was analyzed, and the δ15N value used as the B value in the calculation of %Ndfa of the accessions.

19) Shoot N (mg N per g plant): Dry shoots were ground and analysed for N along side the 15N isotope.
20) Nitrogen fixed (N-fixed): The amount of N-fixed was calculated as the product of %Ndfa and legume shoot N.

2.6 Data Analysis

Analysis of variance (ANOVA) was performed on all the traits using the GLM of SAS version 9.2. For cluster analysis, scatter plots of each variable in a pair-wise manner was performed to check if the data needed transformation prior to cluster analysis. The data was linearly transformed as the scatter plots showed poorly separated and in some cases elongated elliptical patterns. Some of the variables also had different units warranting transformation. The Proc Aceclus program of SAS was used for transformation. The dendrogram was constructed based on the hierarchical method using Euclidean test in SAS version 9.2.

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Soil characteristics

The soil was low in available P (7.51 ± 0.91 ppm) and N (0.12 % ± 0.03) according to Nelson et al. [33] and had a pH water of 4.52 (Table 2). It was also characterized as clay with bulk density of 1.29Mg/m3 and field capacity of 0.360 cm^3 water/cm^3 soil (Table 3).

3.1.2 Variation in nitrogen fixation and yield related traits among 50 genotypes of cowpea

The combined analysis of variance across environments (P-fertilization levels) showed significant effects for most of the traits (Table 4, 5 and 6). In this study, the interaction between genotype and P-fertilization was significant ($p< 0.05$) for Nsize (Table 6). This interaction was

also highly significant (P< 0.01) for LCS, SFW, SDW, RDW, shootp (Table 4), NN, NFN, FN, NDW, NI, delta N of the legume (δ15N leg), %Ndfa, Shoot N, N-fixed (Table 6) and YLDplt (Table 5). The genotypes were significantly different for all the traits studied at P< 0.01.

3.1.3 Phylogenetic relationships between cowpea in different phosphorus environments

The phenogram shows the dissimilarity among the accessions grown under low P condition (Fig. 1). The phenograms for high P and high P and N are not presented but the information is summarized on Table 7.Under low P (Figure 1), at a dissimilarity coefficient of 1.8, the phenogram divides the 50 accessions into two major clusters (I, II) as sumarrised in Table 7. Cluster II has only one accession (Kodek). The first major cluster (I) had four sub clusters (A, B, C, and D) at a dissimilarity coefficient of 0.6. Sub cluster A of cluster I comprised of 10 accessions and were the high nitrogen fixers, sub cluster B, 31 accessions, sub cluster C only one accession (Vya niebe with high grain yield), and sub cluster D, 7 accessions (with low nitrogen-fixing potentials). The genotypes 58-77 and Danilla that were among the top 20% based on N-fixed fell in sub cluster I -A while yacine and Lori-niebe that belonged to the bottom 20% group were in sub cluster I -D.

When P fertilizer was applied, the grouping changed. The number of clusters and structure of phenogram II did not change but the accessions were regrouped compared to grouping under low P. Here at a dissimilarity level of 1.8, the phenogram also divides the 50 accessions into two major clusters (I, II) as indicated in Table 7. Cluster II had only one accession (Frijol Bayo). Considering sub-clustering at a dissimilarity level of 0.6, the first major cluster (I) had five sub-clusters (I-A, 1-B, 1-C, 1-D and 1-E). In sub-cluster A of cluster I, there are 3 accessions, in sub cluster B, 25 accessions, sub clusters C only two accessions, sub cluster D, 15 accessions, and sub cluster E, 4 accessions.

Table 2. Soil chemical properties of Nkoemvone at 0-20cm

Characteristic	Nkoemvone
pH (water)	4.52
Ca cmol(+) /kg	0.58
Mg cmol(+) /kg	0.32
K cmol(+) /kg	0.18
Na cmol(+) /kg	0.28
Al cmol(+) /kg	2.33
CEC cmol(+) /kg	8.07
Available P ppm or ug/g	7.51
Mn ppm or ug/g	1.26
Fe ppm or ug/g	106.00
Org C %	1.73
Total N %	0.12
C/N	13.92

Table 3. Soil physical properties of Nkoemvone 0-20cm

Site	Sand (%)	Clay (%)	Silt (%)	Textural class	Bulk density (Mg/m^3)	Field capacity (cm^3 water/cm^3 soil)
Nkoemvone	42.3	46.4	11.4	clay	1.29	0.36

Table 4. Analysis of variance for cowpea agronomic traits from 50 genotypes

Trait	Source of variation			Coeff Var	R-Square
	Genotypes (G)	P-fert	Genotype * P-fert		
Dflw	83.279**	978.509**	19.193ns	7.1	0.76
LCS	92.015**	4657.409**	32.834**	9.9	0.85
SFW	88.219**	7957.470**	75.224**	27.3	0.90
SDW	6.244**	223.149**	4.934**	46.0	0.79
RDW	0.084**	0.953**	0.056**	36.6	0.80
Shootp	0.007**	0.009*	0.004**	20.3	0.69

*** Significant at the probability 0.01 level; * Significant at the 0.05 probability level, ns non significant. Dflw = Days to 50% flowering, LCS = Leaf Colour Score, SFW = Shoot fresh weight, SDW = Shoot dry weight, RDW = Root dry weight and Shoot P= Shoot phosphorus content*

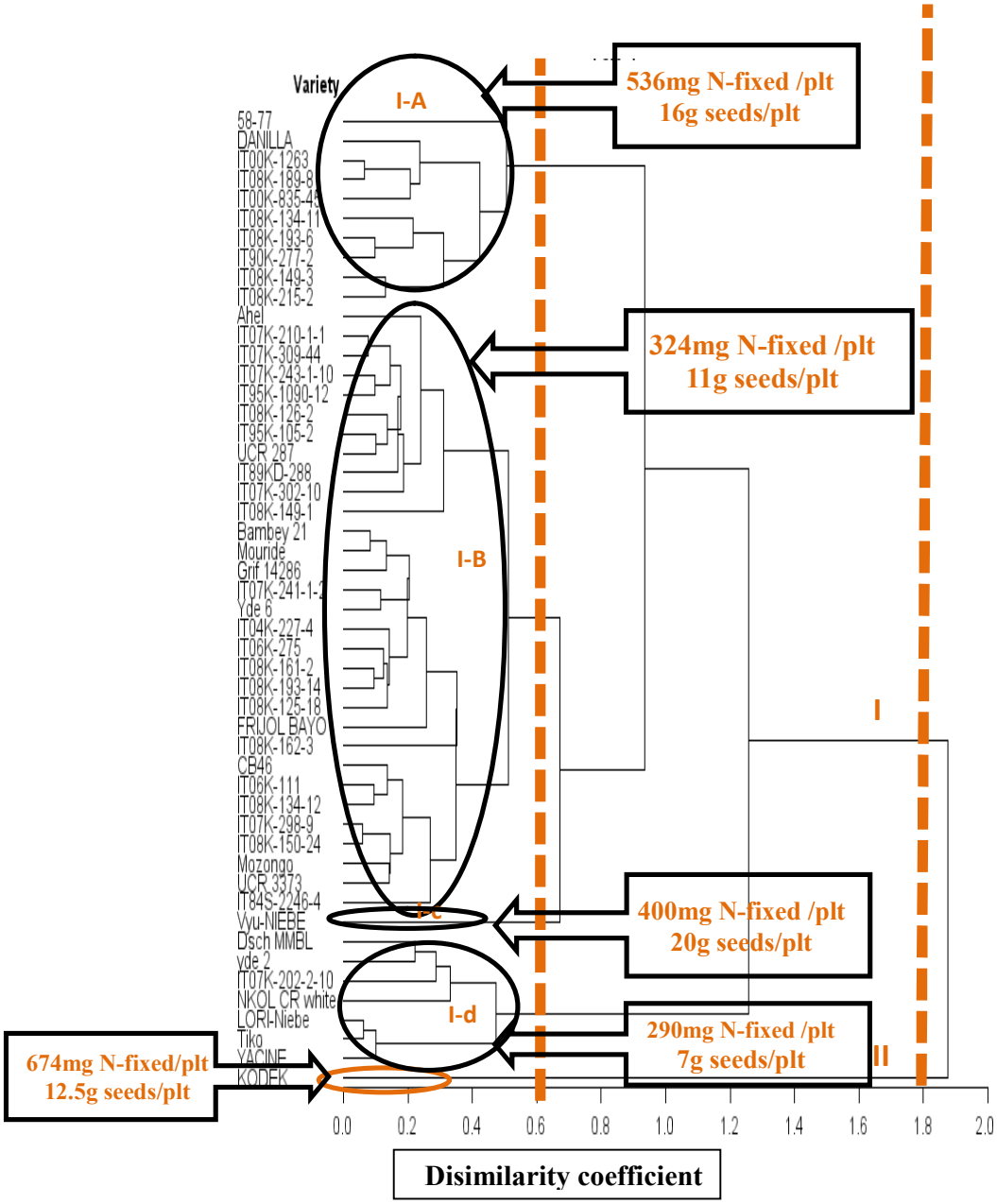

Fig. 1. Phenogram of 50 cowpea accessions grown in low P

Table 5. Analysis of variance for cowpea Yield and yield components using 50 genotypes

Trait	Source of variaton			Coeff Var	R-Square
	Genotypes (G)	P-fert	genotype * p-fert		
Npod	7.469**	153.840**	0.679ns	34.4	0.87
Sdpod	17.354**	258.757**	3.547ns	31.7	0.74
YLDplt	123.167**	800.337**	12.382**	13.5	0.91

*** Significant at the probability 0.01 level; * Significant at the 0.05 probability level, ns = non significant. Npod = Number of pods per plant, Sdpod = Number of seeds per pod, and YLDplt = Grain yield per plant*

Under high P and high N environments, the maximum dissimilarity level between the accessions was 1.6 so grouping was done only at a dissimilarity level of 0.6. The addition of N fertilizers to the high P pots completely changed the phenogram. The accessions were grouped into 5 clusters (Table 7). Cluster I had 18 accessions, cluster II, 24 accessions, cluster III, 3 accessions, cluster 4, 1 accession, and cluster 5, 5 accessions.

3.2 Discussion

According to Nelson et al. [33] P soil content of 0 – 20, 20 – 40 and more than 40 ppm is classified as low, moderate and high, respectively. Whilst soil N less than 0.2% is classified as low, 0.2 – 0.4 % as moderate and more than 0.4 % as high. The physico-chemical properties of the soil used indicated that it was a low P and N soil making it appropriate for screening for the efficient use of those traits. This is following recommendation by Bliss [34] that when selecting plants for increased N_2 fixation in grain legumes, selection must be practiced under low soil Nitrogen conditions that allow discrimination between high- and low-fixing lines. Moreover, according to [35], one of the prerequisites of varietal screening for mineral stress is that the growth medium should be deficient in the nutrient under study. The low available P concentration in the soil can be attributed to low pH (5.0) in H_2O. At this acidic pH, orthophosphate anions H_2PO^{4-} and $HPO4^{2-}$ will be in soil solution just as Al and Fe. The Fe and the Al may have, therefore, precipitated the orthophosphate anions from solution. The two cycle of soil depletion with high density maize population also greatly reduced the soil N content. Genotypic, environmental factors and genotype by environmental (GxE) interaction differences were observed in this study. These differences affected crop yields and yield components [36]. The existence of significant effects of G x E interaction on quantitative and qualitative traits response in cowpea has previously been reported [37; 38; 39 and 40]. Crop genotypes also have different efficiencies in their use of available growth resources [41]. This variation in genetic constitution of crop genotypes influences the expression and level of heritable traits between and within environment [42]. The present findings are in agreement with these reports wherein genotype and P-fertilization effects had significant effect on yield and yield components and nitrogen fixation related traits. Because of the variation of trial environment as seen in the contrast analysis, the accuracy of the results will depend on repetitions across representative environments.

Under low P, the phenogram grouped accessions according to their geographic origin or specific collection sites. For example, the sub-cluster I-D had seven accessions, with five (5/7) of them constituting Cameroon landraces. Sub-cluster B of cluster I, grouped 31 accessions including all the accessions from the University of California Riverside (UCR) and the majority (22/30) of the accessions from IITA. The two accessions (Vya niebe and Kodek) that were singled out in sub-cluster I-C and cluster II are from the northern part of Cameroon. Vya niebe is a high grain yielding genotype while Kodek

Table 6. Analysis of variance for cowpea nitrogen fixation related traits

Trait	Source of variation			Coeff Var	R-Square
	Genotypes (G)	P-fert	genotype * p-fert		
NSC	38.449ns	77.810ns	35.715ns	259.6	0.51
NN	453.821**	10830.721**	106.820**	32.6	0.91
NFN	30.437**	670.925**	20.957**	86.3	0.73
FN	316.255**	16333.238**	137.196**	41.1	0.93
NDW	5951.260**	135655.518**	1725.713**	48.5	0.90
Nsize	12.672**	46.622**	7.222*	94.2	0.64
NI	1.780**	109.761**	1.752**	30.4	0.94
δ^{15}N leg	0.046**	11.263**	0.033**	4.7	0.99
%Ndfa	38.063**	24586.748**	26.727**	0.8	0.99
Shoot N	24939.189**	2697688.528**	23900.977**	0.8	0.99
N-fixed	15763.463**	142502.777**	10953.339**	0.7	0.99

*** Significant at the probability 0.01 level; * Significant at the 0.05 probability level, ns non significant. NSC= Nodule score, NN = Nodule Number, NFN= Number of non-functional nodules, FN = Number of functional nodules, NDW= Nodule dry weight, Nsize = Nodule size , NI = Nodulation index, δ^{15}N leg = Delta N of the legume, %Ndfa = Percentage N derived from atmosphere, ShtN = Shoot N, and N-fixed = Nitrogen fixed*

Table 7. Relatedness of 50 cowpea accessions based on 20 traits in three environments

Environment					
Low P		High P		High P and N	
Cluster	**No. of accessions**	**Cluster**	**No. of accessions**	**Cluster**	**No. of accessions**
sub cluster **I** -A	10	sub cluster **I** -A	3	**Cluster I**	18
sub cluster **I** -B	31	sub cluster **I** -B	25	**Cluster 2**	24
sub cluster **I** -C	1	sub cluster **I** -C	2	**Cluster 3**	3
sub cluster **I** -D	7	sub cluster **I** -D	15	**Cluster 4**	1
Cluster II	1	sub cluster **I** -E	4	**Cluster 5**	5
		Cluster II	1		

is a good N_2 fixer under low P conditions. As was the case of grouping under low P, the phenogram under high P grouped accessions according to their geographic origin or specific collection sites. This can be observed in the sub-cluster I-B with 25 accessions, including the majority (7/10) of Cameroon landraces. The peculiarity of phenogram III is that all the landraces fell in two of the clusters. Cluster I with 4/10, and cluster 2 with the remaining 6/10 landraces. Previous studies on cowpeas using morphological traits such as plant pigmentation, plant habit, root traits, leaf traits, pod traits, seed traits, grain quality, and yield have been done. These traits were all found to be of great importance to distinguish genetic variability, and have led to a better classification of cowpea genotypes [17; 43; 44; 45]. As in earlier studies, this study also found that agro-morphological traits especially those related to nitrogen fixation are still valuable tools for cowpea genetic diversity studies.

4. CONCLUSION

Highly significant differences among genotypes for all the traits were recorded indicating the presence of genetic variability which is necessary for an effective breeding programme. Cluster analysis substantiated the existence of diversity among the 50 accessions for the morphological traits studied. The clustering pattern showed that the Cameroon landraces were not genetically more distant from each other like the exotic accessions. Furthermore, dissimilarities were observed among accessions from the three different geographical regions, presenting a great possibility for the development of suitable varieties for the various agro-ecological zones of Cameroon. That is to say, different cultivars can be developed for specific agro-ecological regions by making use of the available potential of the germplasm. It can therefore be concluded that in this study, the clustering process did reflect the source of the accessions when grown in the natural environment (low P conditions). In addition, the use of materials from different geographical origins in any cross breeding programme aiming to develop suitable varieties with specific characters is therefore strongly recommended.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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