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Genetic Screening of Cowpea Varieties [*Vigna unguiculata* (L.) Walp.] for *Aphis craccivora* Resistance in the Sudano-Sahelian Zone of Cameroon

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Authors' contributions

This work was carried out in collaboration between all authors. Author LI designed, analyzed and interpreted and prepared the manuscript, authors JBN and PMM managed the literature, author GS wrote the protocol, author BD managed a correction for the manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Cowpea (*Vigna unguiculata* L. Walp) is a highly sought after legume of populations in the Sudano-Sahelian zone of Cameroon. Its multiple uses meet the needs of farmers because it contributes to food security and contributes to people's incomes. The use of resistant varieties is the most advantageous method because of its compatibility with integrated pest management. The objective of this study was to identify with the marker CP171 / 172 the cowpea varieties resistant to *A. craccivora* .Ten (10) varieties are conducted in Greenhouse for tested of resistance to aphids. The seedlings were artificially infested with five aphid nymphs and the assessment of their responses to symptoms of aphid damage was made on the basis of qualitative and quantitative criteria, namely: stem height, number of leaves, leaf surface primary, number and damage of *A. craccivora.* After

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DNA Extraction and Polymerization Chain Reaction in the Laboratory, two varieties (ENMD2 and NML50) revealed aphid resistance: these results show the effectiveness of Marker Assisted Selection in Varietal Selection programs to conserve and sustainably manage these resources.

Keywords: Vigna unguiculata; microsatellite markers; Aphis craccivora Koch; varietal resistance; Sudano-Sahelian zone.

1. INTRODUCTION

The main food crops grown in the Northern Cameroon Region are ideal for this purpose: maize, millet, sorghum, cowpea, groundnuts, yams and vegetables MINRESI. [1]. Among these, cowpea (*Vigna unguiculata* (L.) Walp), shows more interest. In fact, cowpea plays an important role in the population's food balance Amevoine et al. [2]. Genetic improvement of cowpea began in the early 60st with the identification of highly productive local varieties. Cowpea (*V. unguiculata*) is the most important seed legume in the tropical savanna areas of Africa. To come from South-East Africa, he has spread all over the world. Cowpea is a staple diet in Africa because its leaves, green pods and dry seeds can be eaten and market. The main role of cowpea is to provide cheaper proteins than meat or fish in dishes such as daxin and not as a substitute for rice or millet as a source of energy. Some short-cycle varieties ripen early, making it possible to have a good quality food during the "lean" periods. In the Sahelian regions of Africa, this period during which food is rare, corresponds to the months of August and September IITA [3]. World production of cowpeas amounts to more than 5.7 million tons of dry seed per year over 7.5 million ha; In Africa, it accounts for more than 70% of this production and occupies 80% of the world's surface area for cowpea production Tengomo [4]. In Cameroon, cowpea production is estimated at 1% of world production, about 112 501 tons of cowpea Moussa [5]. Nevertheless, one of the major problems in cowpea production around the world is the attack of insects and mainly of aphids Rachie [6]. *Aphis craccivora* is of great importance for cowpea cultivation. Singh and Allen [7] estimated cowpea yield losses due to this insect from 20% to 40% in Asia and over 35% in Africa. *A. craccivora* primarily attacks seedlings where it feeds by taking sap from succulent stems and young leaves. Afterwards, he attacks the flowers and pods. A severe infestation of *A. craccivora* causes stunting of the plant, deformation and early defoliation of leaves, reduction of pods ... In case of extreme attack, the infested plant dies and dies. In addition to this direct damage, *A. craccivora* transmits the cowpea mosaic virus Atiri [8].

However, studies on genetic analysis of cowpea resistance to *A. craccivora* are rare except for the few studies by Bata et al. [9]; Githiri et al. [10]; Kusi et al. [11] in Ghana. In Cameroon, and more particularly in the Far North Region, no work has been done on this subject. Efforts must be made to improve the production of this crop in this country. This marker-assisted selection is therefore of definite interest to the breeder because it offers the advantage of efficient, rapid and early selection, and becomes a necessary complement to traditional methods of genetic improvement of legumes. The present study is a response to the urgent need for the collection and characterization of genetic resources, and can be an important contribution to socio-economic development and the fight against poverty.

The main objective of this study is to genetically analyze the resistance of cowpea to aphids in order to select aphid-resistant varieties.

2. MATERIALS AND METHODS

2.1 Study Zone

The experiment was conducted at the greenhouse of the cowpea section of the IRAD Regional Center of Maroua located in Djarengol, a district of the Maroua first district, Diamaré department, Far North Cameroon region. The study site is located between Latitude 10 degree and 35 minutes North and Longitude 14 degree and 17 minutes East at an elevation of 412 meters. The Far North region is Cameroon's second most populous region with 3,480,414 inhabitants, or about 17.9 percent of Cameroon's population. It covers an area of 34,263 kilometers and is between longitudes 10 degree and 13 degree North and between latitude 13 degree and 15 minutes and 15 degree and 45 minutes East OMD. [12]. The climate prevailing in the study area is the Sudano-Sahelian climate of dry tropical type that extends from the south to the center of the region and Sudano-Sahelian type to the north. This climate is characterized by

recurrent droughts and annual rainfall averages decreasing over time Nchoutnji et al. [13]. The Far North region is the part of the country that is lightly watered by the rains. The annual rainfall in this region ranges from 500 to 900 milimeters per year, averaging between 750 and 800 milimeters Bring [14]. The annual temperature is about 27 degree celsuce with a maximum of 15 degree to 38 degree from March to April and a minimum of 18 degree C from December to January. The relative humidity is 80 percent in the rainy season and it drops to 30 to 40 percent, sometimes 10 percent in the dry season Bring [15]. The soils of the Far North region are varied but unstable and predominantly sandy-clay. In Far-North Cameroon, vegetation is characterized by the presence of thorny steppes made up of trees and shrubs. The most common woody species are *Acacia spp., Balanites aegyptiaca, Piliostigma reticulatum, Ximenia americania, Tamarindus indica and Ziziphus spp* Letouzey [16].

2.2 Methodologies

The Cowpea genotypes used for this study consisted of 10 cowpea genotypes obtained from my collection. The experiment is laid out in completely randomized design with three replications and are placed in the greenhouse for evaluation of aphid damage. *A. craccivora* it collected in fields used to infest the APAGBALA genotypes which is susceptible to aphids and is considered aphid bank. These adult aphids are carefully removed from cowpea seedlings with small bushes to prevent mechanical injury and are immediately transferred to infest two-week-old APAGBALA seedlings. Four days after infestations aphids flourish. All adult aphids are removed and destroyed after the production of the first generation of nymphs. It is in the second generation that the nymphs thus obtained, four days old are used to infest our different growing varieties in vegetation pots at the two-leaf stage, that is, four days after emergence or eight days after sowing. Regular checks are made to avoid parasitoids and predators (ants, spiders ...) Kusi et al. [17]. Four-day-old aphid nymphs are collected very carefully with small brushes and are kept in the Petri dishes. Using these same brushes, five nymphs are taken from the boxes and carefully deposited on the upper surface of the leaves. They migrate progressively towards the lower face.

A check is made two days after infestation to verify that each plant contains its five aphids. The number of aphids in individual plants is count at 5, 9, 13, 17 and 21 days after infesting seedlings with aphids. Aphids population pressure on each plant are weighe using in 1-5 rating scale (1= a few individual aphids, 2= a few small individual colonies, 3= several small colonies, 4= large individual colonies, 5= large continuous colonies) given by Souleymane et al. [18]. . The scale (from 1 to 5) defined by Singh et al. [19] evaluated the damage of aphids and categorized different varieties. This scale states that at the end of the assessment, varieties with average damage between 0 and 2 are healthy and considered resistant; between 2.1 and 3 are moderately healthy and considered tolerant; between 3.1 and 5 are heavily infested and considered as susceptible to aphids. The main symptoms of damage observed were: stunting of the plant, deformation of the leaves, early defoliation and dieback of seedlings.

Tabel 1. Notation of the dett of the sytegors and categiation on of varieties

Rating scale	Description of symptoms
0	Visual damage < 10%
1	Visual damage of 10 - 20%
2	Visual damage 21 - 40%
3	Visual damage of 41 - 60%
4	Visual damage 61 - 80%
5	Visual damage of 81 - 100%

On the basis of these observations, we thus had the categorization of the following varieties.

Classification scale	Categories
0 ≤ Average damage ≤ 2	Resistant
2.1 ≤ Average damage ≤3	Tolerant
3.1 ≤ Average damage ≤ 5	Sensitive

Then we did the Genome Screening at the Laboratory of Molecular Biology according to the FTA Plant Card method defined by Karle et al. [20]. This step takes place in two main phases: DNA Extraction and Polymerization Chain Reaction (PCR).

DNA extraction: it requires high aseptic conditions and proceeds according to the following steps: Take in each pot two thirds of the upper part of the leaflet, cut with the chisel; Squeeze the leaves of the plants thus cut on FTA Plant Card (Watman paper) using a plastic pestle and a Parafilm, in order to extract the juice containing the DNA; Collect the DNA discs using Harris Uni-Core and put them in 2 ml Eppendorf

Photo 1. Photography of assessment of the responses of 10 varieties of cowpea seedlings with symptoms of aphid damage

tubes at the rate of four (4) discs per tube; Wash the disks twice with the FTA Purification solution (pipette 100 µl into each tube) by vortexing it for 4 to 5 minutes (at the rate of four DNA disks per tube per 100 µl of the FTA purification solution). ; Rinse twice with the TE Buffer solution (1X) which is a solution of Tris-EDTA: 10 Mm Tris-HCl Ph = 8.0 [tris (hydroxymethyl) aminomethane)] and 1 Mm EDTA (ethylene diamine tetraacetic acid). Pipette 100 µl into each tube by subjecting it to the Vortex Genie for 4 to 5 minutes; Dry the discs obtained for at least 1 hour on the healthy paper.

PCR (Polymerisation Chain Reaction): So, to do the PCR, we use the 0.2 ml tubes called PCR tubes (Accupower PCR PRE-MIX) or GE Healthcare still called illustra TM, puretaq ready-to-go TM or PCR Beads; they are 0.2 ml tubes, in each carton we have five sachets and each sachet contains ninety-six tubes. Each tube contains 1.5 Mm of Magnesium Dichloride of formula MgCl 2. After opening the Kit, you must first number the tubes and make the correspondence. To carry out the PCR, we first have: Reconstituted the pair of primer CP171 / 172. This marker is a co dominant marker developed for aphid resistance biotype in Tamale and Ghana Omoigui et al. [21]; pipetted 50µl of buffer solution (1 x TE) and put it in the tube containing the freeze-dried primer pair for dissolution. This step takes place on the ice to prevent denaturation of the primer by heat. Everything is kept cool for two hours; on the other hand, we have the primer pairs containing on the one hand the sense primer and on the other hand the antisense primer, these tubes contain 226 number of moles, to reconstruct them: pipette 226 µl of the solution TE in two different 1.5 ml Eppendorf tubes and add to each of the primers and pass them to the Centrifuge, this solution thus prepared constitutes the stock solution; take 50 µl of the stock solution each time add 450 µl of the TE solution and mix it with the Vortex Genie, this is the solution to use. Then, the PCR mixture is primed. The PCR was carried out using the Ready-To-Go ™ PCR Beads kit in 96-well microplates, the length of which has a dimension of 12 tubes and the width has a capacity of 8 tubes. We used 11 tubes numbered from 1 to 11.

In our case study, we prepare a solution with a total volume of 25 µl, the procedure is as follows: In each tube Pipetted 2.5 µl Buffer B solution or BD, which serves to neutralize the medium; Put 0.5 µl of the DNTP solution in each PCR tube, to have as many nucleotides in the solution; Pipette 1.5 µl of the magnesium dichloride solution, which will increase the ions in the reaction; Next, introduce a single DNA disk into each PCR tube using a needle; Pipette 0.5 µl of DNA Polymerase, which is the enzyme that will catalyze the reaction; Put 2 µl Primer CP171 / 172 containing both Forward and Reverse - Finally, pipette 20 µl of biological water (Molecular Biology Water); Place the resulting solution in the Applied Biosystems 2720 Thermal cycler with a capacity of 96 wells for a time indicating the start and end of the reaction and the program.

In this phase, genomic DNA is digested withTaq polymerase T, then there is release of fragments with*Thermus aquaticus* , then Ligation with known sequence adapters (*Thermus aquaticus*) adapters, after this phase we have the first amplification and finally the second amplification and we pass to electrophoresis on 2% agarose gel agarose gel electrophoresis 2% Preparation of the agarose gel : Weigh 2.7 g of the agarose salt using a sensitive electrical balance and pour into a 250 ml graduated burette; Add 150 ml of the 10 X TAE solution into the burette; Heat the mixture obtained in the Microwave (Russell hobbs) by adjusting the time between 4 and 5 minutes and thus obtain a homogeneous and clear solution; Next, pipette 6 µl of Ethidium bromide concentration of 10 mg / ml into the resulting solution and homogenize the resulting solution; this to make the bands visible during the observation Ultra Violet rays Then pour the solution in the electrophoresis tank containing the combs (Comb) for 30 to 45 minutes of solidification and thus obtained the Agarose Gel; After the PCR, pipette into each amplicon 2 µl of Bromotimol Blue to stain the solution and to observe the migration of the bands in the electrophoresis tank; Then, introduce the amplicon from the PCR in the holes left by the combs contained in the tank, the first hole is left to put 10 ul of standard marker (50 or 100 Pb DNA ladder RTU); Fill the tank with the 10 X TAE solution and then connect the tank to the 64 Volt Power Generator, with a current of 120 milliamps and releasing 8 Energy; the migration of the DNA bands is from the positive (-) pole to the (+) pole for 45 minutes to 1 hour of the time. Remove the Gel and go to the observation with the Ultra Violet ray camera and film using the camera.

2.3 Data Processing and Analysis

Data collected included the length and width of the two primary leaves, the size of the plants, the number of leaves and the number of aphids. The analysis of variance of these different parameters was carried out using the software Statgraphics plus 5.0 and XL STAT. As for the molecular analysis of the results, it was based on the visual interpretation of the different profiles obtained.

3. RESULTS AND DISCUSSION

3.1 Descriptive Statistics of Measurement Parameters

A total of four parameters were measured after infestation of seedlings with aphids.

3.2 The Leaf Area

The figure opposite shows the leaf area of each of the ten varieties of cowpeas thus tested.

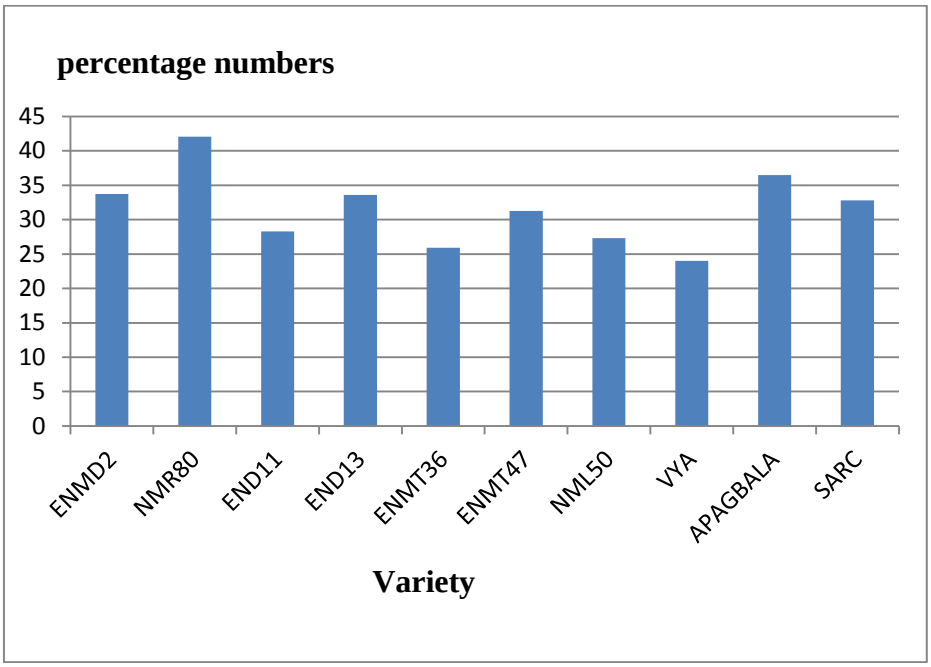

Fig. 1. Curve of variation of leaf area according to varieties

Table 2. Statistical analysis of leaf area of different varieties

Rank	Varieties	Average	Coef. Of variation
Number of varietes	ENMD2	33,73 ±6,16 ^{abc}	18,25%
	NMR80	42,06 ±11,95 ^{c}	28,42%
	END11	28,30 ±6,30 ^{ab}	22,25%
	END13	33,6 ±4,75 ^{abc}	14,15%
	ENMT36	25,9 ±8,18 ^{ab}	31,56%
	ENMT47	31,25 ±7,44 ^{abc}	23,80%
	NML50	27,33 ±5,41 ^{ab}	19,78%
	VYA	24,0 ±3,55 ^{a}	14,80%
	APAGBALA	36,46 ±2,92 ^{bc}	8,02%
	SARC	32,82 ±1,46 ^{abc}	4,43%
Total		31,55 ±7,48	23,72%

The highest average primary leaf area is obtained with the variety NMR80 (42.06 ± 11.95 cm2). The average damage of aphids on this variety is (3,33 ± 1,01). This result shows that plants with large leaves attract more aphids than those with narrow leaves. This result is similar to that obtained by Laamari et al. [22]. The following table presents the statistical analysis of the surface of the primary leaves in cm2 of the ten varieties tested.

The average numbers of the leaves followed by the same letter are statically identical.

The maximum number of leaves is observed in the variety 2 (NMR80: 42, 06 ±11, 95). The minimum number of leaves is observed in varieties 8 (VYA: 24,0 ± 3,55) so the number of leaves varies by one variety to the other.

The following table presents the statistical analysis of the surface of the primary leaves in cm2 of the ten varieties tested.

Based on the analysis of variance, there are no significant differences among the ten varieties in the area of primary leaves (P = 0.0809> 0.05).

3.3 The Number of Leaves

The following figure shows the number of leaves of each of the ten varieties of cowpeas thus tested.

The maximum number of leaves is observed in the variety NMR80 (8 ± 2). The minimum number of leaves is observed in varieties: ENMD2 (5 ± 0), END11 (5 ± 2), ENMT47 (5 ± 1) and SARC (5 ± 1), so the number of leaves is the same.

The following table presents the statistical analysis of the number of the leaves of the ten varieties tested.

The average numbers of the leaves followed by the same letter are statically identical.

The maximum number of leaves is observed in the variety NMR80 (8 ± 2). The minimum number of leaves is observed in varieties: ENMD2 (5 ± 0), END11 (5 ± 2), ENMT47 (5 ± 1) and SARC (5 ± 1), so the number of leaves varies by one variety. to the other. Adeoti et al. [23] on *Sesamum radiatum* shows that a distinction can be made according to the number of leaves of annual accessions.

Table 3. Statistical analysis of the surface of the primary leaves

Source	Sum of squares	DDL	Middle square	F	Probability
Inter-groupes	787,561	9	87,5068	2,09	0,0809
Intra-groupes	835,526	20	41,7763		
Total (Corr.)	1623,09	29			

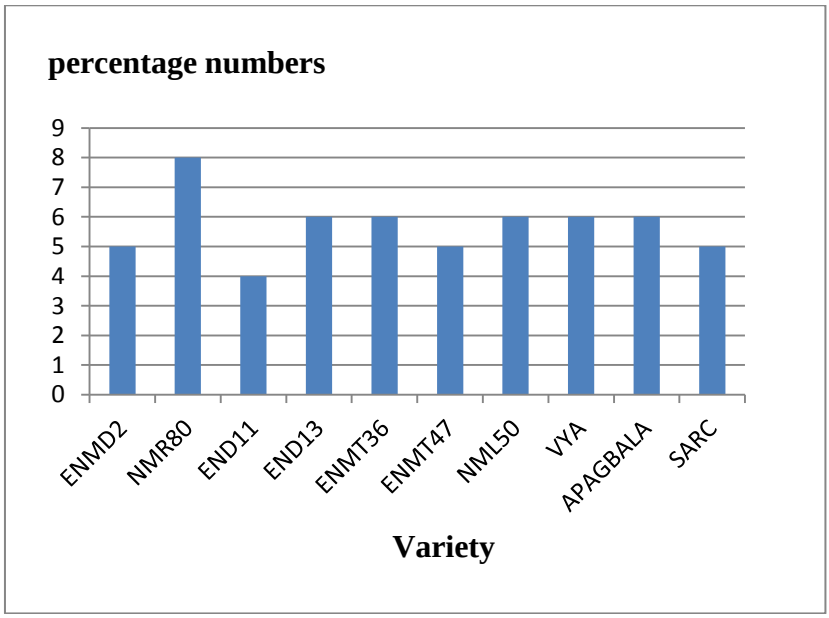

Fig. 2. Average number of leaves of cowpea varieties tested

Table 4. Statistical analysis of the number of the leaves

Scale	Variéties	Medium	Coef. Of variation
Number of varieties	ENMD2	5 ± 0 [a]	0%
	NMR80	8 ± 2 [b]	27,15%
	END11	5 ± 2 [a]	32,73%
	END13	7 ± 1 [ab]	9,12%
	ENMT36	6 ± 2 [ab]	28,87%
	ENMT47	5 ± 1 [a]	10,82%
	NML50	6 ± 1 [ab]	16,67%
	VYA	6 ± 1 [ab]	16,67%
	APAGBALA	6 ± 1 [ab]	18,23%
	SARC	5 ± 1 [a]	20,0%
Total		6 ± 1	22,55%

Table 5. Analysis of variance for number of sheets

Source	Sum of squares	DDL	Middle square	F	Probability
Inter-groupes	20,8333	9	2,31481	1,58	0,1889
Intra-groupes	29,3333	20	1,46667		
Total (Corr.)	50,1667	29			

Based on the analysis of variance, there were no significant differences between varieties in average number of leaves (P = 0.1889> 0.05).

3.4 The Height of the Plant

The following Fig. 3 presents the evolution of the diagram presenting the height of the stem.

Based on the foregoing, the highest average height is observed in the NMR80 (13.88 cm) and APAGBALA (13.74 cm) varieties. This could be explained by the fact that all the seedlings of these varieties were well emerged (four days after sowing) and especially because these varieties had tolerated the presence of aphids. This result is consistent with the work of IITA [23] in Nigeria.

The Table 6 presents the statistical analyzes of the stem heights of the varieties thus tested.

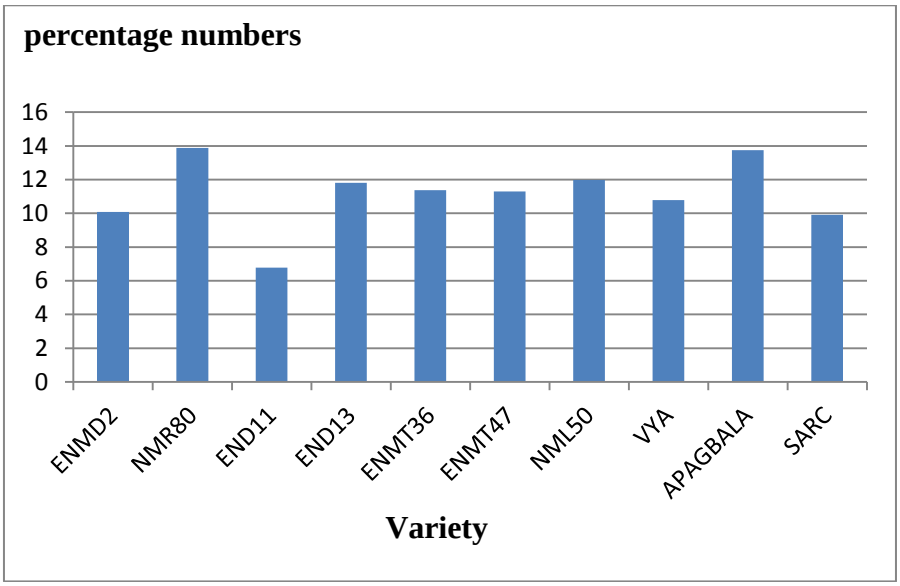

Fig. 3. Diagram of the height of the plant according to the varieties

Table 6. Statistical analysis of stem height of different varieties.

Scale	Varieties	Medium	Coef. of variation
Number of varieties	ENMD2	10,08 ±3,64 [ab]	36,13%
	NMR80	13,88 ±2,99 [b]	21,53%
	END11	6,77 ±2,73 [a]	40,34%
	END13	11,80 ±2,40 [b]	20,31%
	ENMT36	11,38 ±0,90 [b]	7,911%
	ENMT47	11,30 ±3,78 [b]	33,50%
	NML50	11,98 ±0,77 [b]	6,40%
	VYA	10,79 ±2,17 [ab]	20,09%
	APAGBALA	13,74±2,78 [b]	20,20%
	SARC	9,91 ±1,01 [ab]	10,17%
Total		11,16 ±2,88	25,83%

Table 7. Analysis of variance for stem height

Source	Sum of squares	DDL	Middle square	F	Probability
Inter-groupes	112,019	9	12,4466	1,93	0,1061
Intra-groupes	129,064	20	6,45322		
Total (Corr.)	241,083	29			

The averages of stem lengths followed by the same letter in a column are statically identical.

The mean maximum height is 13.88 ± 2.99 cm and is observed in the variety NMR80. The average minimum height of 6.77 ± 2.73 cm is observed in the END11 variety. This result corroborates that of IITA [24] in Nigeria.

Based on the analysis of variance, there were no significant differences in seedling height between the varieties (P = 0.1061> 0.05).

3.5 Aphid Damage for the Ten Varieties Tested

The Fig. 4 shows the evolution of aphid damage on the ten varieties tested.

The damage caused by aphids is highest in the ENMD2 variety (4). And the minimum number of damage is observed in ENMT36 varieties (2, 13).

The Table 8 presents the statistical analysis of aphid damage on the ten varieties.

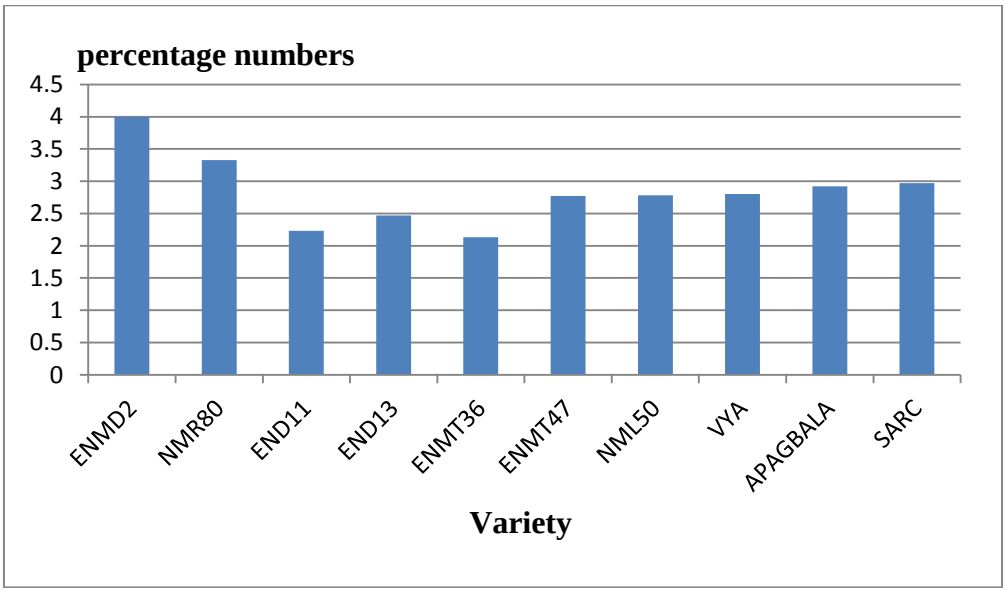

Fig. 4. Diagram of damage caused by aphids

Table 8. Summary statistics for aphid damage

Scale	Varieties	Medium	Coef of variation
Nomber of varieties	ENMD2	4,0 ± 0,87[b]	21,79%
	NMR80	3,33 ± 1,01[ab]	30,20%
	END11	2,23 ± 0,97 [a]	43,49%
	END13	2,47 ± 0,95 [a]	38,53%
	ENMT36	2,13 ± 0,81 [a]	37,89%
	ENMT47	2,77 ± 0,25[ab]	9,10%
	NML50	2,78 ± 0,80[ab]	28,76%
	VYA	2,8 ± 1,39[ab]	49,49%
	APAGBALA	2,92 ± 0,25[ab]	8,46%
	SARC	2,97 ± 0,45[ab]	15,20%
Total		2,84 ± 0,88	30,82%

Table 9. Analysis of variance for aphid damage

Source	Sum of squares	DDL	Middle square	F	Probability
Inter-groupes	7,88367	9	0,875963	1,22	0,3354
Intra-groupes	14,3233	20	0,716167		
Total (Corr.)	22,207	29			

The maximum damage is 4.0 ± 0.87 and is observed in the ENMD2 variety. The minimum damage is 2.13 ± 0.81 and is observed in the variety ENMT36.

From the analysis of variance, it can be seen from this table that there are no significant differences among the ten varieties in aphid damage.

3.6 Summary Statistics for the Measurement Parameters in Greenhouse

A total of 4 morphological descriptors are used to characterize the 10 varieties. None of these morphological descriptors proved significant.

3.7 Correlations between the Parameters

The following table shows us the correlations that exist between the different parameters measured in Greenhouse.

Table 10. Agro-morphological descriptors of varieties and their degree of significance

Measured parameters	Degree of significance
leaf area (cm2)	0,0809 ns
The number of leave	0,1956 ns
The size of the	0,5600 ns
plant(cm)	0,3354 ns
Aphid damage	
Descriptors	Not significant

** = 0.05 (significant); ** = 0.01 (very significant); *** = 0.001 (highly significant); ns = not significant; g: gram; cm: centimeter*

This table shows that the correlations are negative between (stem height and aphid damage, number of leaves and leaf area, stem height and leaf area) and other it is positive between (aphid damage and number of leaves, leaf area and aphid damage, number of leaves and height of stem). We find that aphid damage is higher in the juvenile stage and decreases with seedling development. Resistance to insects in general and to aphids in particular increases with age Nair et al. [24]. This result corroborates with those obtained by Nair et al. [24]. All significant correlations reflect the fact that the aphid *Aphis craccivora* does not alter the physiology of the host plant, especially the quality of the phloem, as do other aphids Nair et al.[24].

Table 11. Matrix of correlation between the different parameters measures

Variables	Degat puce	Nbre feui	Haut tige	Surf foli
DEGAT PUCE	1	0,028	-0,156	0,289
NBRE FEUI	0,028	1	0,305	-0,138
HAUT TIGE	-0,156	0,305	1	-0,422
SURF FOLI	0,289	-0,138	-0,422	1

3.8 The Spatial Arrangement of the Measured Parameters in Greenhouse

The following figures present the spatial representation of the different varieties of cowpeas thus studied in Serre.

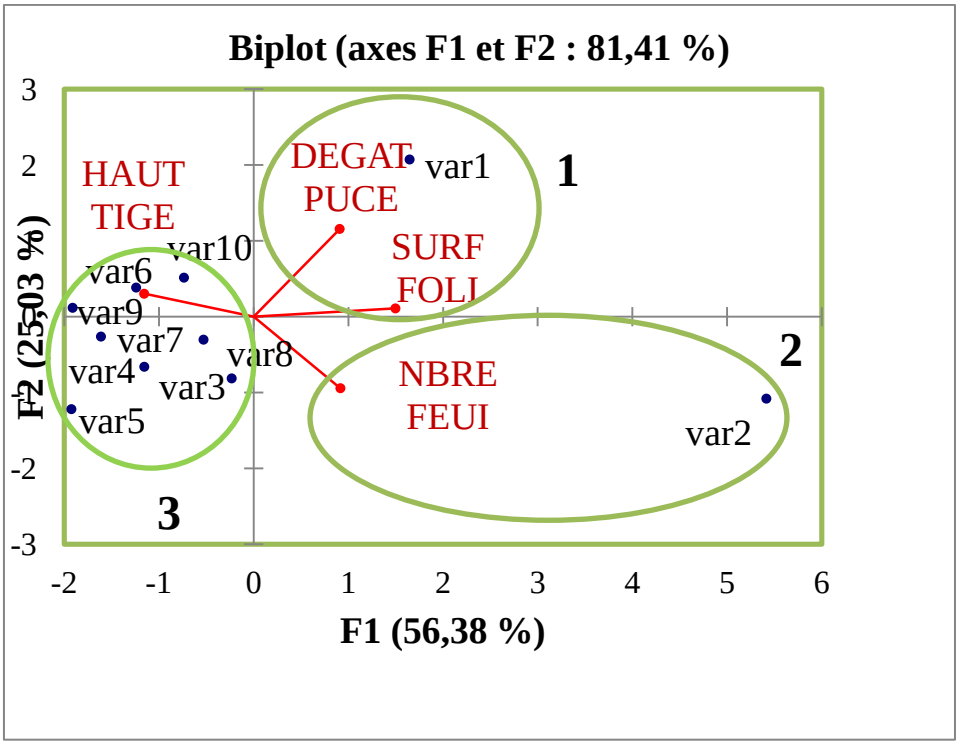

Fig. 5. Spatial layout of the ten varieties tested in Greenhouse

The representation of the cowpea varieties studied according to the measured parameters shows that their distribution is not uniform in space. The varieties analyzed in the F1 x F2 plane are visible at 56.38%, unlike the other axes.

In the F1 x F2 plane, three (3) scatterplots are observed. The first cloud shows that variety # 1 is related to aphid damage and leaf area. The second cloud shows that variety No. 2 is close to the number of leaves. And in the third cloud, we find that the varieties No. 3, 4, 5, 6, 7, 8, 9 and 10 gather around the height of the stem.

The previous arrangement is confirmed by the hierarchical ascending classification which shows 3 morphotypes. The figures below show the different classes of cowpea varieties tested and their similarities.

Morotype 1 is the most represented 70% of cowpea varieties with the highest aphid damage (4);

Morphotype 2 contains 20% of cowpea varieties with the highest number of leaves (7) and leaf area (36.11 cm2);

Morphotype 3 is represented by 10% of cowpea varieties with the highest stem height (11.56 cm). Kamau et al. [25] obtained similar results by testing the resistance of a few accessions of *Lablab purpureus* to cowpea aphid *Aphis craccivora* Koch in Kenya at different stages of growth.

3.9 Assessment of Aphid Damage

The assessment of aphid damage and categorization on the different varieties of cowpeas tested was done according to the scale (from 1 to 5) defined by Singh et al. [26].

The minimum number of aphids (2) is observed in END11, END13, ENMT36, ENMT47, NML50, SARC, VYA and APAGBALA varieties. The latter would probably have acquired certain characteristics that would remove the aphids allowing them to tolerate aphid damage including an early accumulation of toxic substances in their sap. These tolerant varieties would have lost certain resistance characteristics (SARC), hence the absence of true resistance to aphid attacks, a result that corroborates with that of Kumar [27]. The susceptible varieties (NMR80 and ENMD2) which had the highest number of aphids (4) as the other susceptible varieties would have in addition to the morphological characteristics, other advantages such as the succulent nature of the stems and easy penetration in case removal of non-toxic substances from their sap that would attract more aphids Kumar [27].

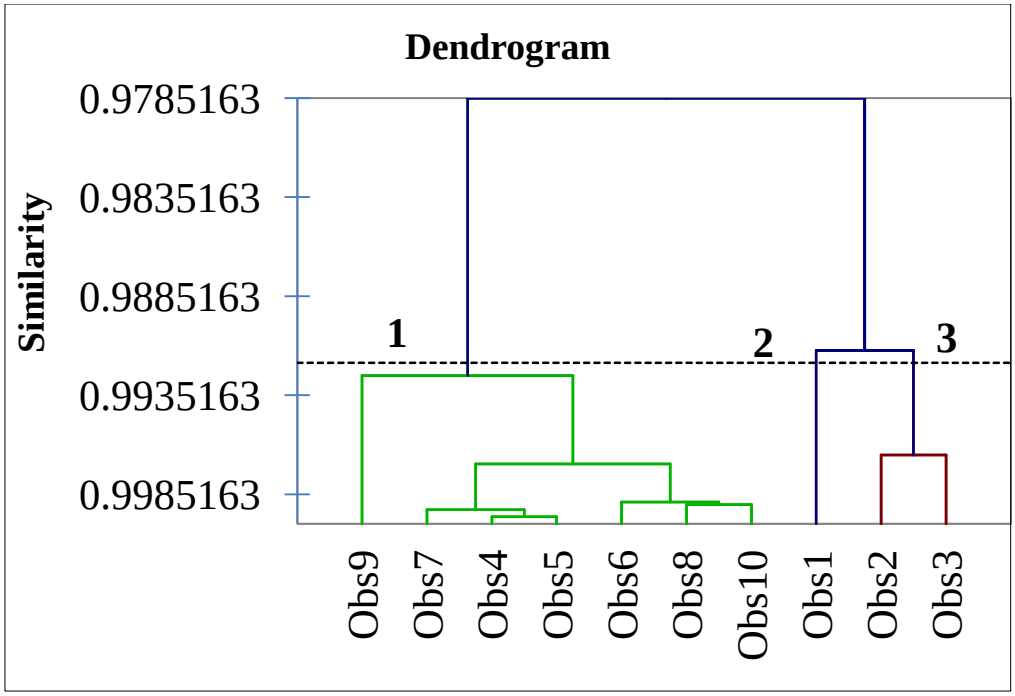

Fig. 6. Dendrogram; morphotypes (1, 2, and 3) for the ten varieties

Table 12. Categorization of cowpea varieties according to the method defined by Singh et al. [28]

Classification scale	Categories	Varieties	Number
0 ≤ Average damage ≤2	Resistant	-	0
2,1≤ Degate average ≤3	Tolerant	END11, END13, ENMT36, ENMT47, NML50, SARC, VYA, APAGBALA.	8
3,1≤ Middle degraded ≤5	Sensitive	ENMD2, NMR80	2
Total			10

Table 13. Rating scale of aphid damage symptoms

Varieties	Damage observed	Class or Category	Number
-	Visual damage 10 – 20%	Resistant	0
-	Visual damage 21 – 40%	Resistant	0
END11, END13, ENMT36, ENMT47, NML50, SARC, VYA, APAGBALA	Visual damage 41 – 60%	Tolerant	8
NMR80	Visual damage 61– 80%	sensitive	1
ENMD2	Dégâts visuels 81– 100%	sensitive	1
Total			10

The minimal damage of aphids observed in varieties: END11 (2.23 ± 0.97), END13 (2.47 ± 0.95), ENMT36 (2.13 ± 0.81), ENMT47 (2.77 ± 0) , 25), NML50 (2.78 ± 0.80), VYA (2.8 ± 1.39), APAGBALA (2.92 ± 0.25) and SARC1-57-2 (2.97 ± 0.45).); could be explained by the morphological characteristics (small leaves and an early accumulation of toxic substances in the sap of these varieties that would remove aphids. These values are not very different.) This allows us to say that the variety SARC1- 57-2 is resistant to aphids but has lost these traits. This result does not support that of the varietal screening for aphid resistance conducted at SARI in Ghana by Kusi. F. [29] who used the variety SARC1-57- 2 as a source of genes for aphid resistance in its work, whereas the varieties ENMD2 and NMR80 which presented the maximum damage (4.0 ± 0.87 and 3.33 ± 1.01) would have the characteristics morphological, other advantages (succulent stem, easy penetration when non-toxic sap is taken ...) that would attract more aphids. This further confirms the susceptibility of the latter to the aphids.

According to the scale defined by Singh et al. [30] Table 11 shows that, at the end of this test, no variety was found to be resistant to seedling aphids (0 ≤ average damage ≤ 2). 8 varieties were tolerant to aphids. This is based on the observed decreasing tolerance level of SARC1-57-2, APAGBALA, VYA, NML50, ENMT47, END13, END11 and ENMT36. The two remaining varieties showed a very high degree of susceptibility to aphids. This is based on the decreasing sensitivity of ENMD2 and NMR80. This finding allows us to note that true resistance occurs at low percentages and, if so, does not exist in the evaluated plant material Smith [31], Hill et al. [32], Mensah et al. [33] Diaz-Montano [34]. This lack of resistance observed in the varieties could be explained by the development over time of new biotypes of insects able to bypass the resistance thus rendering ineffective the genes of resistance contained in the genome of certain varieties Smith [35]. Kamau et al. [36] obtained similar results by testing the resistance of a few accessions of *Lablab purpureus* to cowpea aphid *Aphis craccivora* Koch in Kenya at different stages of growth. III-5-) Molecular analysis of the bands using the marker CP171 / CP172 At the molecular level, the marker CP171 / CP172 is a polymorphic marker that has shown efficacy in gene detection. The following figure shows the migration of the bands obtained with the marker CP171 / CP172.

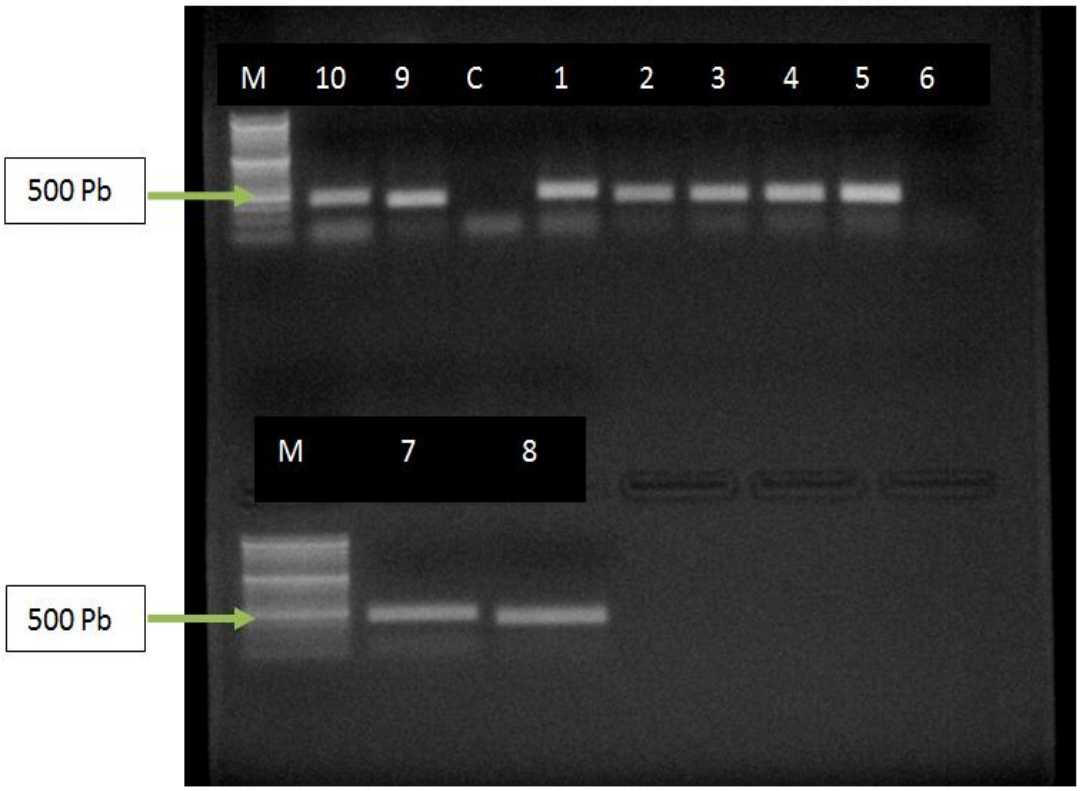

Fig. 7. Band profiles obtained with CP171 / CP172 primer pair
Forward and reverse sequences of CP171/172 primer for PCR amplification;
CP171/CP172 5'- CAACCGATGTAAAAAGT GGACA-3'
5'- TGAAGCTGATTGTGGAA CCAT-3'

M: marker; 10: SARC-1-57-2 resistant parent; 9: APAGBALA sensitive parent; C: the control, 1: ENMD2; 2: NMR80; 3: END11; 4: END13; 5: ENMT36; 6: ENMT47; 7: NML50; 8: VYA. Primer pair CP1711 / CP172 to reveal amplification. The amplified band corresponds to the value 500 Pb. Here we find that there are three cases of situations that arise: First case: the varieties 1 (ENMD2) and 7 (NML50) have migrated at the same distance as the resistant parent SARC -1-57-2 (500 Pb), therefore they have the aphid resistance gene; for the second case: varieties 2 (NMR80), 3 (END11), 4 (END13), 5 (ENMT36), and 8 (VYA) migrated at the same distance as the sensitive parent 9 (APAGBALA), they are therefore sensitive; finally, for the third case, the absence of bands in variety 6 (ENMT47) is therefore identical to that of control. On the molecular level, the varieties ENMD2 and NML50 are resistant or phenotypically no variety has presented the resistance gene. In contrast to us, Benchasri et al. [36] had better phenotypic resistance to aphids (in-station) with the resistant parent (IT82E-16), which were confirmed by molecular analyzes using SSR primer (VM37).

4. CONCLUSION

In summary, we can say that the cowpea is a legume very popular with the populations of the Sudano-Sahelian zone of Cameroon, because its fruits, its stem and its leaves are consumed and marketed in the markets. All varieties tested did not have the same reactions to symptoms of aphid damage. Phenotypically, two (2) varieties: ENMD2 and NML50 are susceptible to aphids; Eight (8) other varieties (END13, ENMT36, NMR80, ENMT47, END11, VYA, SARC-1-57-2 and APAGBALA) have a form of tolerance to aphids. In addition, it has been found that aphid infestation is more pronounced at the seedling stage and that varieties with large leaves are most attractive to aphids. Molecular analysis using the marker 171/172 for the resistance of cowpea (*Vigna unguiculata*) to aphids (Aphis craccivora) allowed us to identify two (2) varieties (ENMD2 and NML50) presenting the resistance genes among the eighty-five (85) varieties tested. Thus, these varieties will serve as a donor parent in future varietal breeding programs for cowpea resistant to aphids.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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