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EVALUATION OF THE VARIETAL RESISTANCE OF COWPEA [*VIGNA UNGUICULATA* (L.) WALP.] TO THE APHIDS (*APHIS CRACCIVORA* KOCH) IN THE FAR NORTH OF CAMEROON.

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Abstract: Cowpea is one of the food products that ensure food security in the Far North Region of Cameroon. However, production yields remain low due to many parasitic constraints, due particularly to *Aphis craccivora*. The commonly used control strategy is based mainly on chemicals whose usage is polluting, onerous and restrictive. This work was undertaken to assess the resistance of 10 varieties of cowpea *A. craccivora*, pest of cowpea in the Far North Region of Cameroon. For this purpose, after infestation of the seedlings by 5 aphids nymphs each, stem height, the number, length and width of primary leaves on the one hand and the number and the damage of the other aphids were evaluated. Thereafter, 40 pairs of primers were tested for identification of closely linked microsatellites resistance gene to aphids. The results obtained show that the varieties NGT115, SARC-1-57-2, KVX-165-14-1, LORI, IT97K-556-6 IITA, B-301 and APAGBALA are tolerant. The varieties KVX-295-2-124-99 and BR1 are sensitive and variety VYA is very sensitive.Two pairs of primers CP51 / CP52 and CP53 / CP54 identified morphic single markers which can therefore not be used for the detection of the gene for resistance to aphids. Other primers have not identified anything. These results demonstrate the need to find sources of resistance in the native material. They show the need to further look for polymorphic markers closely associated with resistance genes to aphids in order to integrate the marker-assisted breeding programs.

Key words: *Vigna unguiculata*, microsatellite markers, *Aphis craccivora*, varietal resistance.

Introduction: Cowpea is one of the food crops that ensure food security in the Northern Region of Cameroon (Kosma *et al.*, 2014). It is a legume with high protein levels which the population uses to balance its nutrition (Kouebouet *et al.*, 2013; Djile *et al.*, 2016). Indeed, its seeds constitute a precious source of protein and vitamins (Dugje *et al.*, 2009; Charassri *et al.*, 2013). Its young immature leaves and pods are eaten as vegetables (Ouédraogo, 2003; Dudje *et al.*, 2009). In addition to its nutritional qualities, it plays an important agronomic role in improving soil

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fertility through its ability to fix atmospheric nitrogen (Adeotiet *et al.*, 2002; Ouedraogo 2003; Moussa, 2011). In addition, cowpea tops constitute an excellent fodder which is very much appreciated. It also represents a substantial source of income for many people and the actors in the rural and urban marketing chain (Kosma *et al.*, 2014). Despite its importance in food, improving the fertility of soils and producers' incomes, production remains low. Indeed, the average yield obtained by farmers is 600 kg / ha, while the potential yield can reach 2 tonnes / ha (C2D, 2013). Moreover, it has been found that a significant proportion of this production is reduced each year by diseases, parasitic plants but especially insect pests (Djile, 2005). Among the insect pests of cowpea, the shiny black aphid, *Aphis craccivora*, is the most devastating. The latter lives in dense colonies on cowpea plants and other plants, infecting all organs and causing considerable damage. Severe attacks of *A. craccivora* cause plant depletion, leaf deformation, early defoliation and seedling decay (Appert and Deuse, 1982). In addition to these direct attacks, *A. craccivorat* transmits viruses such as the mosaic of cowpea and produces honeydew which attracts saprophytic fungi to the plant, thereby disrupting photosynthesis (Scheppers, 1989). The total annual loss of agricultural production from aphids is approximately 1.2 x 107 tons; this represents about 2% of the losses due to insect pests (Alavo, 2000). In the face of this substantial damage, the most widely used control strategy against aphids is mostly the intensive use of chemicals (Scheppers, 1989). Despite its effectiveness, the regular use of chemicals has disadvantages. Apart from their relatively high cost of application, they disturb the ecological balance of the treated milieu, pollute the environment (water, soil and air) and foodstuffs, have adverse effects on human health and animals, and cause the development of resistant strains (Devonshire, 1989; Glitho *et al.*, 2008; Nerio *et al.*, 2010). Genetic control is beneficial to small farmers because it does not require expensive inputs and is a sound, economical and environmentally sound method of control. The rapid accumulation of several beneficial genes in a single elite variety for more effective resistance is now possible thanks to molecular markers (Hospital, 2001). Indeed, new techniques, particularly molecular markers, appear to be indispensable tools in support of conventional programs which meet these challenges. The search for molecular markers closely related to genes of agronomic interest, including resistance to aphids (marker screening) is therefore an important step before their practical exploitation (Moreau *et al.*, 2001, Eagleset *et al.*, 2009, Dekkers and Hospital, 2002). The development of molecular markers has led to the extensive study and genetic characterization of several plants such as tomato and maize (Coe and Gardiner, 1994). Microsatellites have already been adopted for mapping studies in maize (Senior and Heun, 1993), rice (Wu and Tanksley, 1993) and soybean (Morganteetal., 1994), as well as for diversity analyzes in rice (Yang *et al.*, 1994) and soybean (Powell *et al.*, 1996). Cowpea is one of the crops that have been left behind in this area. It is by considering this that we proposed to evaluate the resistance of 10 varieties of cowpea to aphids by identifying microsatellite markers that are closely related to resistance genes in the Far North Region of Cameroon.

Materials and methods

Presentation of the study area: This study was carried out in the greenhouse of the Cowpea Section of the Regional Center of the Institute of Agricultural Research for Development (IRAD) of Maroua, located in Djarengol, in Maroua I Sub-Division of the Diamaré Division in the Far North Region of Cameroon. The study site is located between 10° 35 North latitude and 14° 17 longitude East and at an altitude of 412 m.

Materials: The plant material used in this study consists of 10 varieties of cowpea of different origins and provided by the Cowpea Section of the Maroua Regional Center of IRAD. Table 1

shows the morphological characteristics of the varieties of the seeds used.

The animal material consists of adult aphids. The latter, of medium shiny black color, were used to develop nymphs (four days old) from which the infestation was made.

The primer library used to amplify SSR closely related to aphid resistance genes in this study consists of 96 pairs of preconceived, lyophilized and silicone-protected primers (Cowpea Primers prepared in Mike Timko's lab, USA). From this range, only 40 pairs have been tested.

Method

Implementation of the test: The study began with the preparation of the soil, which consisted of making a homogeneous mixture of 80% clay and 20% sand. This moistened soil of clay-sandy texture was used to fill the 2/3, 56 pots of vegetation of 28 cm of depth and 28 cm of diameter of which 50 were used for the sowing of the different varieties tested; 4 for the cultivation of aphid nymphs and the latter for sowing a susceptible variety (VYA) and resistant variety (SARC-1-57-2) to aphids for genetic analysis in the molecular biology laboratory. The varieties of cowpea used were stored in the bags after they had been sown, classified by variety in labeled petri dishes.

The study was carried out using a completely randomized block device comprising 10 treatments (the number of varieties used) and 5 repetitions. Each block consisted of ten plastic cylindrical pots 28 cm deep and 28 cm of diameter for sowing the different varieties tested.

For the aphid bank, using a string and a hoe, 4 semi lines were prepared that constitute the aphid bank. A semi-progressive was carried out at a density of 20 cm between APAGBALA plants, a variety susceptible to aphids. Aphids collected from peasant fields and kept in petri dishes served to infect APAGBALA seedlings for 4 days after emergence. This progressive sowing made it possible to keep the aphids available throughout the period of the study. Watering of the seedlings was done every morning when need be.

A superficial seedling of 4 seeds per pot of the 10 varieties tested was carried out. Prior to sowing, the pots of vegetation were watered and water gradually infiltrated. The pots where the seeds did not sprout were replanted 4 days after sowing. The maintenance operations carried out mainly concerned watering if necessary, manual weeding and pitching of the pots. Seedlings were single planted per pot 8 days after sowing.

Adult aphids were carefully removed from the aphid bank using small brushes and kept in Petri dishes. They were then transferred to growing APAGBALA plants in 8-day-old pots. Regular checks were carried out to remove parasitoids and predators (ants, spiders ...) All adult aphids were removed and destroyed after the first generation of nymphs were produced one day after the infestation. These nymphs were left to develop for a period of 6 days until becoming adults. The latter, in turn, were removed and destroyed after the production of the second generation of nymphs. These nymphs, all aged 4 days, were used to infest the different varieties growing in the greenhouse at the 2 leaf stage, that is to say, 4 days after emergence.

Each seedling was artificially infested with 5 aphid nymphs. Using small brushes, 5 puce nymphs were deposited on the top of one leaf of each seedling. Two days after the infestation, a check was carried out to ensure that each seedling contained its 5 Aphids.

Collection of data: The first observations and measurements were made before the infestation on each of the five plants in each variety. The parameters of interest were: the height of the stems, the number of leaves, the length and the width of the primary leaves in order to calculate their surface area by the following formula:

$Sf = 2/3 [(L_1 \times l_1) + (L_2 \times l_2) + (L_3 \times l_3) + \ldots (L_n \times l_n)]$

With Sf : leaf area (cm^2); L: length of each leaflet (1; 2; 3; ... 20); L: width of each leaflet (1; 2; 3; ... 20).

Subsequently, they were made every 7 days after infestation for 21 days. In addition to the parameters mentioned, the number and damage

of the aphids were evaluated. The scale (from 1 to 5) defined by Singh et al. (1996) evaluated the damage of aphids (Table 2) and categorized the different varieties. This scale stipulates that at the end of the assessment, the varieties with average damage between 0 and 2 are sound and considered to be resistant; Between 2.1 and 3 are moderately healthy and considered tolerant; Between 3.1 and 5 are widely infested and considered to be susceptible to aphids. The main symptoms of damage were: stunting of the plant, leaf deformation, early defoliation and seedling dieback. Based on these observations, categorization of varieties was obtained (Table 3).

Concerning the identification of SSR markers, a susceptible variety (VYA) and a resistant variety (SARC-1-57-2) to aphids were selected for the identification of microsatellite markers related to resistance genes in the molecular biology laboratory. The activity involved two major phases, which took place in two separate compartments to limit contamination as much as possible, notably: DNA extraction and Chain Polymerization/Electrophoresis Reaction. Regarding DNA extraction, young leaves of both varieties (VYA and SARC) aged 15 days were cut in half with the help of sterilized scissors. Using a plastic pestle, these were pressed onto FTA Plant Card to extract the juices containing the genomic DNA. Once the leaf tissue and the cell walls were removed, the whole was left to dry for 3 Hours and the DNA disks of each variety were separately picked up using specialized slides and held in numbered tubes at the rate of 4 discs per tube. Subsequently, a series of two washes of these disks were performed by pipetting 100 µl of FTA Purification into each tube and subjecting them to the Vortex Genie for 4 to 5 minutes. Twin rinsing of these disks was also carried out by pipetting 100 µl of the TE-1 buffer into each tube and subjecting them to the Vortex Engineering for 4 to 5 minutes. The obtained DNA disks (white) were dried for 1 hour in an open air on absorbent paper at room temperature. Using a healthy needle, dry DNA discs were stored separately in labeled tubes and stored in a cool place. To carry out the PPR (Polymerise chain reaction) and the electrophoresis, we had previously reconstituted the pairs of primers. A series of 5 primer pairs was tested each day. For this purpose, we pipet 50 µl of buffer solution (1 x TE) into each of the tubes containing the lyophilized primer pairs for dissolution. This stage took place on the ice to avoid the denaturation of the primers by the heat. The whole thing was kept in the refrigeration for two hours. During this time, the PCR mixture was primed. The PCR was made from the Ready-To-GoTM PCRBeads kit (from the University of Virginia in the USA) in a microplate of 15 wells previously numbered from 1 to 15. The numbering was as follows: in the first row that corresponded to the first primer pair tested, number 1 represented control, number 2, susceptible variety (VYA) and number, 3 resistant variety (SARC-1-57-2). This order was retained throughout the test. Each kit in the 0.2 ml volume contained 1.5 U lyophilized Taq polymerase, 200 µM dNTPs, 1.5 mM MgCl2, 10 mM Tris-HCl, 50 mMKCl. With the aid of a needle, we scrupulously introduced into these tubes, with the exception of the control tubes (which only verified the absence of contamination), respecting the numbers assigned to the different varieties. 1 DNA disk of each variety .Afterwards, all the tubes received 23 µl of sterilized water and the whole was also kept in the cool. After two hours, 2 µl of each pair of primers to be tested (combining Forward and Reverse) were pipetted and added to the 23 µl of each tube in order to obtain a maximum reaction of 25 µl in each PCR tube. The PCR tubes were loaded into the Applied Biosystems 2720 thermocycler having a capacity of 96 wells. The thermocycler has been carefully closed and programmed. The PCR program used in this study is the same as follows:

94° ——— 1min
94° ——— 15 min
60,5° ——— 15 min } 35 cycles
72° ——— 10 min
4° ——— ∞

The thermocycle was stopped at the end of the reaction. Amplicons in abundant quantities were migrated into an agarose gel (2%).

In this study, the mean electrophoresis cell (70 ml) was used and the gel preparation proceeded as follows:

3 g of Agarose salt were weighed, to which 150 ml of the 1 × TBE solution were added;

N.B: To prepare the 1 x TBE solution, 100 ml of the 10 x TBE solution were pipetted and diluted with 900 ml of watermolecularbiology to obtain 1000 ml of 1 x TBE.

The solution obtained was heated in a microwave for 3 to 4 minutes until a homogeneous solution was obtained. 3 µl of ethidium bromide was added to the solution and the gel was allowed to cool for an instant. Thereafter, the combs were placed in the electrophoresis tank and the gel flew. After hardening (35-45 min. Later), the combs were removed and PCR buffer added; Amplicons supplemented with Bromophenol blue were loaded into the wells by means of a micropipette. The first well, reserved for the standard marker (high Ranger 1kb DNA ladderready to use) received 10µl of the latter and the tank was connected to the electrophoresis apparatus;

-A current generator was used to control the voltage and current intensity during electrophoresis. A timer was included to stop electrophoresis at the end of the migration. In our case, it lasted 45 min and the voltage was 120V.

At the end of the migration, the gel was removed with great care and placed in an ultraviolet ray camera for viewing the obtained profiles and capturing the images.

Data analysis: The data collected were entered using the Excel 2007 spreadsheet. They were then analyzed with the GenStatTwelfth Edition software which performed the analysis of variance (ANOVA) to establish the differences between treatments. The probability threshold used was 5% to evaluate the significant differences and 1% for the highly significant differences. The use of Newman-Keuls multiple comparison (SNK) was used to group the averages in case of significant differences. The link between the different parameters were tested using the Bravais Pearson coefficient of simple correlations. As for the molecular analysis of the results, it was based on the visual interpretation of the different profiles obtained.

Results

Evaluation of the average number of aphids in the varieties of cowpea tested: Table 4 shows the average number of aphids recorded in the different varieties of cowpea tested. The results obtained show that the number of aphids recorded varies from 3 ± 0.45 (in varieties B-301, NGT115 and LORI) to 4 ± 0.17 (in the other varieties tested). The analysis of variance had on the values of the average number of aphids on the ten varieties tested shows that there are no significant differences.

Evaluation of the average damage of the aphids in the varieties of cowpea tested: Table 5 shows the damage caused by the aphids on the different varieties of cowpea tested. The results obtained show that the damage caused by the aphids on the varieties of cowpea tested varied from 2.7 ± 0.36 (in the NGT115 variety) to 3.41 ± 0.09 (VYA). The analysis of variance, carried out on the means of the damage caused by the aphids on the varieties of cowpea tested, shows that there are no significant differences.

Effect of aphids on the height of seedlings of cowpea varieties tested: Table 6 shows the effect of aphids on the height of seedlings of the varieties of cowpea tested. The results obtained show that the stem height of the different varieties of cowpea tested varied from 8.1 ± 0.45 cm (in variety B-301) to 14.51 ± 0.55 (in variety IT97K-556- 6). The variance analysis performed on the average root height values of the different varieties tested shows that there are significant

differences (P = 0.022< 0.05) at the α = 5% threshold between the varieties tested.

Effect of aphids on the number of leaves of varieties of cowpea tested: Table 7 shows the effect of aphids on the number of leaves of cowpea varieties tested. The results obtained show that the number of leaves of the varieties of cowpea tested varied from 3 ± 0.37 (in BR1 and LORI varieties) to 7 ± 1.47 (in NGT115 and SARC1-57-2 varieties). The variance analysis carried out on the values of mean leaf number of the cowpea varieties tested shows that there are significant differences (P = 0.021< 0.05) at the threshold α = 5% between the varieties tested.

Effect of aphids on the surface of primary leaves of cowpea varieties tested: Table 8 shows the effect of aphids on the surface of the primary leaves of the cowpea varieties tested. The results obtained show that the surface of the primary leaves of the cowpea varieties tested varies from 11.07 ± 5.02 cm 2 (in the LORI variety) to 38.89 ± 1.35 cm 2 (in the variety IT97K-556-6). The variance analysis carried out on the mean values of the surface area of the primary leaves of the cowpea varieties tested shows that there is a high significance difference (P<0 .001) at the threshold α = 1% between the varieties tested.

Classification of tested varieties: Table 9 presents the categorization and classification of the ten varieties of cowpea tested for aphid resistance according to the scale defined by Singh et al. (1996). The results show that the KVX-295-2-124-99, BR1 and VYA varieties are between 3.1≤ average damage ≤5 with damage between 61-80% and 81-100% on the other hand are classified as sensitive. On the other hand, the varieties NGT115, KVX-165-14-1, LORI, SARC-1-57-2, IT97K-556-6, B-301 and APAGBALA which are between 2.11 average damage ≤ 3 with damage Rated between 41-60% are classified tolerant. No variety was classified as resistant (0 ≤ average damage ≤ 2).

Correlations between the parameters evaluated: Table 10 presents the correlations between the various parameters evaluated. It is found that apart from the stem height and aphid damage at date 4 (r = -0.0645) on the one hand and aphid damage and damage on the fourth date (r = -0.066) which respectively obtained a negative correlation, the other combinations of parameters studied obtained a positive correlation (r >0).

Molecular analysis results: Figure 1, 2 and 3 illustrate the band profiles obtained with primer pairs CP51 / CP52, CP53 / CP54 and the other primers respectively. The 31 other primer pairs failed to reveal amplification and presented a profile without bands.

Discussion: Cowpea is a food product that contributes to food security in Cameroon (Ndjouenkeu *et al.*, 2010, Kouebou *et al.*, 2013, Kosma *et al.*, 2014, Djile *et al.*, 2016). However, the aphids *Aphis craccivora*, constitute one of the main constraints to their productions. The present work has made it possible to determine the varieties of cowpea resistant to *Aphis craccivora* aphids on the one hand and on the other hand has identified the polymorphic markers that can be used in the development of the cowpea resistant varieties and to test these markers for progeny. At the end of the working season, the minimum number of aphids was observed in varieties B-301, NGT115 and LORI. These three varieties would have acquired certain characteristics that would prevent aphids from tolerating the damage of aphids, in particular and an accumulation of toxic substances in their sap (Kumar, 1999). The highly sensitive variety VYA, which had the highest aphid number just like the other sensible variety would also have in addition morphological characteristics, other advantages such as the succulent nature of the stems and easy penetration in case non-toxic substances is removed from their sap which would attract more aphids (Kumar, 1999). Note that the variety VYA is one of the elite varieties used by the IRAD in its improvement program because it is much sought after by the farmers.

The minimal damage of aphids (2.7 ± 0.36) observed in NGT115 may be due to morphological characteristics (small leaves) and early accumulation of toxic substances in the sap of this variety which would remove the aphids. The values (2.7 ± 0.36) of NGT115 and (2.83 ± 0.29) of SARC1-57-2 were not significantly different. This allows us to confirm that SARC1-57-2 is tolerant to aphids. This result is consistent with the varietal screening work for aphid resistance conducted at SARI in Ghana, which used SARC1-57-2 as a source of aphid resistance genes in its work. On the other hand, VYA, which exhibited the maximum damage (3.41±0.09), would have morphological characteristics, other advantages (succulent stem, easy penetration of non-toxic sap) which would attract more the aphids. This further confirms the sensitivity of the latter to aphids.

The highest mean height is observed in variety IT97K-556-6 (14.51 ± 0.55 cm). This could be explained first of all by the fact that all the plants of variety IT97K-556-6 (4JAS) have been successfully raised and, above all, by the fact that this variety tolerated the presence of the aphids. This result is consistent with the work of IITA (1984) in Nigeria.

The highest average leaf number was observed in SARC1-57-2 (7 ± 1.47) and NGT115 (7 ± 1.61). This could be explained by the fact that these varieties exhibited a tolerance of the presence of aphids.

The mean primary surface of the primary leaves is obtained with the variety IT97K-556-6 (38.89 ± 1.35 cm2). This value was followed by that of SARC1-57-2 (27.66 ± 1.75 cm2), KVX-165-14-1 (21.5 ± 0.83 cm2) and NGT115 (20.41 ± 0.83 Cm2). The average damage of the aphids on these varieties is respectively (2.9 ± 0.29), (2.83 ± 0.29), (2.73 ± 0.51) and (2.7 ± 0.36). This result allows us to observe that the plants which possess the broad leaves attract more the aphids than those which possess the narrow leaves. This result is similar to that obtained by Laamari et al. (2008)

According to the scale defined by Singh et al. (1996). At the end of this study, no variety was found to be resistant to seedling aphids ($0 \leq$ Mean damage ≤ 2). Seven varieties were tolerant to aphids. This is based on the observed decreasing tolerance level of NGT115, KVX-165-14-1, LORI, SARC-1-57-2, IT97K-556-6, B-301 and APAGBALA. The three remaining varieties showed a very high degree of sensitivity to aphids. This is based on the increasing sensitivity of KVX-295-2-124-99, BR1 and VYA. This result allows us to note that true resistance occurs at low percentages and, where appropriate, does not exist in the plant material evaluated (Smith, 2006, Hill *et al.*, 2004, Mensah *et al.*, 2005; Diaz-Montano, 2006). The lack of resistance observed in the varieties tested could be explained by the development over time of new biotypes of insects capable of bypassing the resistance, rendering the genes of resistance contained in the genome of certain varieties ineffective. Smith, 2006). Kamau et al. (2008) obtained similar results by testing the resistance of some Lablabpurpureus accessions to cowpea *Aphis craccivora* Koch aphids in Kenya at different stages of growth.

The correlation between mean stem height and aphid damage at date 4 is negative (r = -0.0645). Also, the correlation between number and damage of aphids at the same date is negative (r = -0.066). These results show that aphid damage is higher in the juvenile stage and decreases with seedling development. Resistance to insects in general and aphids in particular therefore increases with the age of the plants. This result corroborates with those obtained by Nair et al. (2003). Of the 40 pairs of primers tested in this study to reveal the microsatellite markers (length polymorphism) linked to the aphid resistance gene, 2 pairs of primers namely CP51 / CP52 and CP53 / CP54 could reveal amplification. The amplified band corresponds to 400 Pb and 100 Pb respectively. Being on the same band on both parents, this means that we have to do with mono-morphic markers. They can therefore not

be used for the detection of the aphid resistance gene. These two primers have certainly identified the microsatellite marker closely linked to the aphid resistance gene in cowpea but the latter is not polymorphic since it makes no distinction between the two parents. Unlike us, Benchasri et al. (2007) had better phenotypic resistance to aphids (station) with resistant parent (IT82E-16), which were confirmed by molecular assays using the SSR primer (VM37). This result obtained within the framework of our study, which is not the least, could be justified by a few flaws during manipulations.

Conclusion: The main objective of this work was to determine the aphid-resistant of cowpea varieties on the one hand and to identify the polymorphic markers that could be used in the development of aphid-resistant cowpea varieties and to test these markers for progeny. For this purpose, 10 varieties of cowpea and 40 pairs of primers were tested. In view of the results obtained, we can conclude that the varieties NGT115, SARC-1-57-2, KVX-165-14-1, LORI, IT97K -556-6 IITA, B-301 and APAGBALA of Sari are tolerant to *A. craccivora*. On the other hand, the varieties KVX-295-2-124-99 and BR1 are sensitive to *A. craccivora*. The variety VYA very much demanded by producers appears very sensitive to *A. Craccivora*. Two primer pairs CP51 / CP52 and CP53 / CP54 identified mono-morphic markers which cannot therefore be used for the detection of the aphid resistance gene. The other primers did not identify anything. These results demonstrate the need to search for sources of resistance in indigenous material. They demonstrate the need to further investigate polymorphic markers closely related to aphid resistance genes to integrate them into marker-assisted breeding programs. These findings are particularly important for breeders and small producers.

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Table 1. Morphological characteristics of the seeds of the varieties used

Varieties	Origin	Colours	Aspects of tegument	Colours of the eye
KVX-165-14-1	Burkina Faso	Brown	Smooth	Milky
APAGBALA	Sari (Ghana)	White	Fluffy	Milky
KVX-295-2-124-99	Burkina Faso	White	Fluffy	Milky
LORI-NIEBE	Cameroun (IRAD)	White	Fluffy	Yellowish
SARC1-57-2	Sari (Ghana)	White	Fluffy	Yellowish
IT97K-556-6	IITA (Nigeria)	Marron	Fluffy	Milky
VYA	Cameroun (IRAD)	White	Fluffy	Yellowish
BR1	Cameroun (IRAD)	White	Fluffy	Yellowish
B-301	Botswana (South Africa)	Gray	Smooth	Yellowish
NGT115	IITA (Nigeria)	Dyed gray	Smooth	Yellowish

Table 2. Rating Scale of Aphid Damage Symptoms

Level of rating	Description of symptoms

0	Visual damage < 10%
1	Visual damage of 10 – 20%
2	Visual damage of 21 – 40%
3	Visual damage of 41 – 60%
4	Visual damage of 61 – 80%
5	Visual damage of 81 – 100%

Table 3. Categorization scale of varieties

Level of classification	Categories
0 ≤ Average Damage ≤ 2	Resistant
2.1 ≤ Average Damage 3	Tolerant
3.1 ≤ Average Damage ≤ 5	Sensible

Table 4. Evaluation of the average number of aphids on the varieties of cowpea tested

Varieties	Average Number of aphid	Coefficient of variation
B-301	3 ± 0.45 a	33.19
NGT115	3 ± 0.46 a	33.63
LORI	3 ± 0.35 a	24.06
IT97K-556-6	4 ± 0.17 a	11.17
BR1	4 ± 0.36 a	22.80
SARC1-57-2	4 ± 0.48 a	29.04
APAGBALA	4 ± 0.20 a	11.94
KVX-165-14-1	4 ± 0.47 a	26.02
KVX-295-2-124-99	4 ± 0.22 a	12.23
VYA	4 ± 0.17 a	8.99

Table 5. Assessment of Average Aphid Damage on Cowpea Varieties Tested

Varieties	Average Damages	Coefficient of variation
NGT115	2.7 ± 0.36 a	30.37
KVX-165-14-1	2.73 ± 0.51 a	41.94
LORI	2.73 ± 0.32 a	26.44
SARC1-57-2	2.83 ± 0.29 a	23.53
IT97K-556-6	2.9 ± 0.29 a	22.48
B-301	2.9 ± 0.43 a	33.16
APAGBALA	2.9 ± 0.15 a	11.92
KVX-295-2-124-99	3.3 ± 0.4 a	27.29
BR1	3.33 ± 0.43 a	28.94
VYA	3.41 ± 0.09 a	6.45

Table 6. Effect of aphids on mean height of seedlings of cowpea varieties tested

Varieties	Average Height (cm)	Coefficient of variation
B-301	8.1 ± 0.45 a	12.41
APAGBALA	10.04 ± 1.13ab	25.31
LORI	10.06 ± 1.74ab	38.72
NGT115	11.16 ± 0.83ab	16.77
BR1	11.7 ± 1.41 ab	27.12
SARC1-57-2	12.08 ± 0.64ab	11.99
KVX-165-14-1	12.23 ± 0.8 ab	14.65
VYA	12.37 ± 1.92ab	34.71
KVX-295-2-124-99	13 ± 1.54 ab	26.61

IT97K-556-6	14.51 ± 0.55 b	8.54

The average stem heights with the same letter are not statistically different (threshold 5%); ±: standard deviations

Table 7. Effect of aphids on the mean number of leaves of varieties of cowpea tested

Varieties	Average number of Leaves	Coefficient of variation
BR1	3 ± 0.37 a	30.15
LORI	3 ± 1.08 a	79.34
KVX-295-2-124-99	4 ± 0.15 ab	87.12
B-301	4 ± 0.43 ab	24.21
IT97K-556-6	4 ± 0.51 ab	28.14
APAGBALA	4 ± 0.81 abc	42.21
VYA	5 ± 0.93 abc	40.95
KVX-165-14-1	5 ± 0.62 bc	25.83
NGT115	7 ± 1.61 c	55.21
SARC1-57-2	7 ± 1.47 c	49.94

The average number of sheets assigned to the same letter is not statistically different (threshold 5%); ±: standard deviations;

Table 8. Effect of aphids on the leaf area of the varieties of cowpea tested

Varieties	Surface of average primary leaves (cm^2)	Coefficient of variation
LORI	11.07 ± 5.02a	101.5
KVX-295-2-124-99	11.63 ± 3.57a	68.69
B-301	12.85 ± 1.37a	23.95
BR1	13.87 ± 4.07a	65.74
VYA	17.30 ± 5.33ab	68.88
APAGBALA	18.89 ± 2.44ab	28.97
NGT115	20.41 ± 0.83ab	9.18
KVX-165-14-1	21.5 ± 0.83ab	8.71
SARC1-57-2	27.66 ± 1.75bc	14.20
IT97K-556-6	38.89 ± 1.35c	7.77

The leaf areas affected by the same letter are not statistically different (threshold 5%); ±: standard deviations;

Table 9. Categorization of cowpea varieties tested for aphid resistance

Classification	**Categories**	**Varieties**
0 ≤ average Damage≤ 2	Resistant	**-**
2.1≤ average Damage ≤ 3	Tolerant	NGT115, KVX-165-14-1, LORI, SARC-1-57-2, IT97K-556-6, B-301and APAGBALA
3.1≤ average Damage ≤ 5	Sensibles	KVX-295-2-124-99, BR1 and VYA.
Varietties	**Observed damages**	**Class or Categories**
NGT115, KVX-165-14-1, LORI, SARC-1-57-2, IT97K-556-6, B-301 and APAGBALA	41 – 60%	Tolerant
KVX-295-2-124-99 and BR1	61 – 80%	
VYA	81 – 100%	Sensibles

Table 10. Correlation of Bravais Pearson

Vbles	Hteur_Plt	Nbre_Feu	Ppuc2	Ppuc3	Ppuc4	Surf_feu	D_g_2	D_g_3	D_g_4
Hteur_Plt	-								
Nbre_Feu	0,4303	-							
Ppuc2	0,1345	0,2645	-						
Ppuc3	0,4388	0,5327	0,488	-					
Ppuc4	0,5131	0,5081	0,216	0,4383	-				
Surf_feu	0,6152	0,2514	-0,153	0,0924	0,3045	-			
D_g_2	0,114	0,298	0,9023	0,4702	0,273	-0,0841	-		
D_g_3	0,4322	0,3205	0,4171	0,7561	0,1575	0,0999	0,4753	-	
D_g_4	-0,0645	0,1504	0,1444	0,462	-0,066	-0,2897	0,1749	0,6194	-

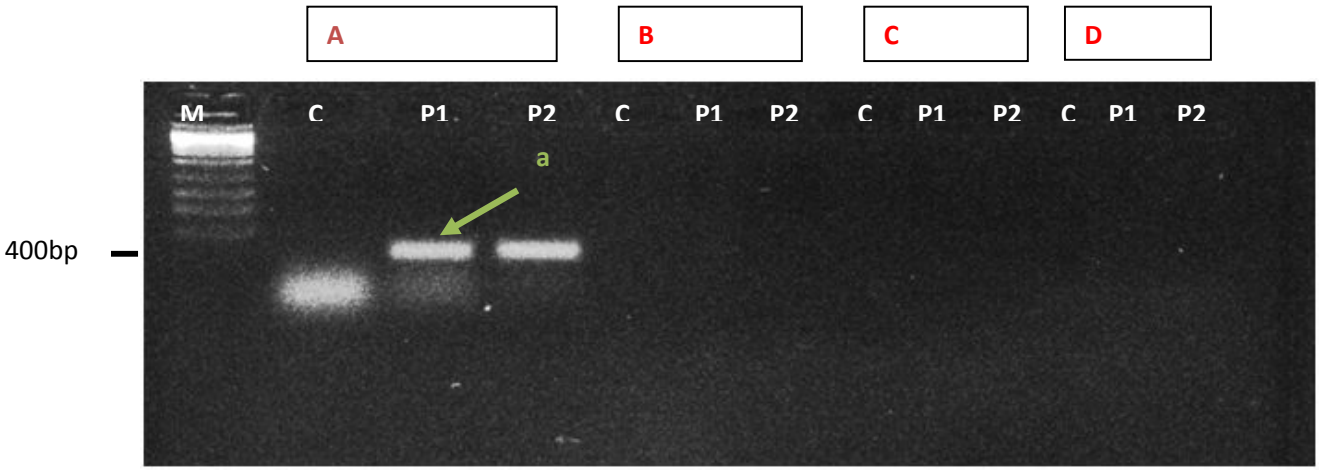

Figure 1. Microsatellite profile obtained from sensitive and resistant parents.
M is the standard marker, **C** is the control, **P1** is a sensitive parent (VYA), **P2** is a resistant parent (SARC1-57-2), **A, B, C** and **D** are CP51 / CP52, CP169 / CP170, CP313 / CP314 and CP399 / CP400. The arrow (a) on the image indicates the position of the monomorphic strip.

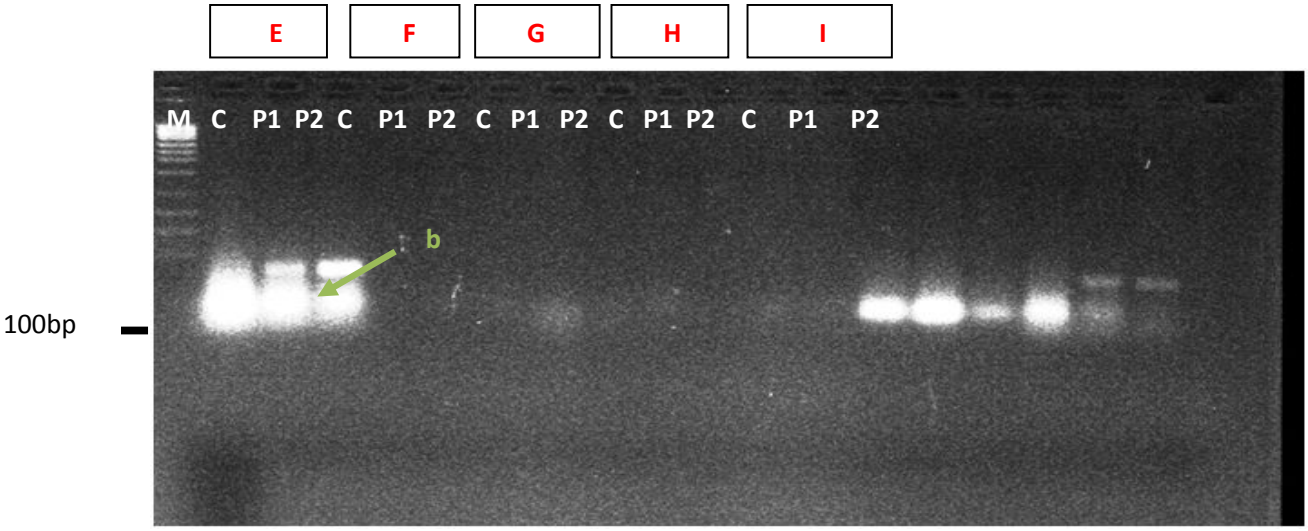

Figure 2. Microsatellite profile obtained from sensitive and resistant parents.
M is the standard marker, **C** is the control, **P1** is a sensitive parent (VYA), **P2** is a resistant parent (SARC1-57-2), **E, F, G, H** and **I** are primer pairs CP53 / CP54, CP171 / CP172, CP315 / CP316, CP403 / CP404 and CP605 / CP606. The arrow (b) on the image indicates the position of the monomorphic strip.

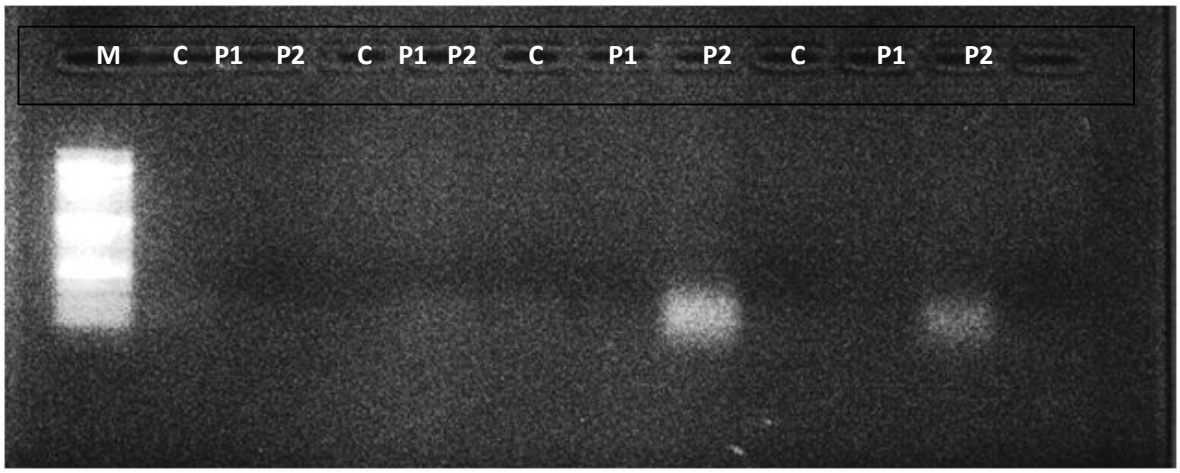

Figure 3. Microsatellite profile obtained with the other primer pairs