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Agro-morphological and molecular evaluations of local cowpea varieties (*Vigna unguiculata* (L.) Walp) at the Saria research station in Burkina Faso

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Abstract

Local varieties could be a source of genetic variability. For this reason, six varieties were evaluated for agro-morphological and molecular variability. A randomized Fisher's complement block with three replications was used. Pod pigmentation, seed size, stem habit and hilum color showed a difference in at least one of the 5 varieties. Statistical analysis was highly significant for the 50% flowering date parameter at 54.00±3.00 days after sowing (DAS) and highly significant for the 95% maturity parameter at 81.33±3.17 DAS compared with the control, which were around 43.00±0.00 DAS and 68.67±1.67 DAS. The best pod weights of around 3087.33±498.49 g and 3042.83±338.96 g with seed weights of around 18.00±0.32 g and 19.43±0.31 g were obtained by the Bengringa flat and round brown hilum varieties respectively, while the seed weights of Komcallé were 1733.46±526.54 g and 16.13±0.31 g respectively. 2.02±0.19 t/ha of haylage was obtained by the Bengraga B variety, while Komcallé obtained 1.06±0.33 t/ha. The molecular markers used were unable to discriminate between the varieties.

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Introduction

Cowpea [*Vigna unguiculata* (L.) Walp.] is one of the main legumes grown and consumed in tropical and subtropical areas of Africa, Asia, Europe and America (Taffouo, 2008). It is an important source of a protein-rich plant belonging to the genus Vigna in the Fabaceae family and is native to Africa (Padulosi and NG, 1997). In Burkina Faso, national cowpea production has been estimated at 829,204.072 tonnes for the 2022-2024 crop year, an exceptional 36.80% increase on the variation over the last five years (MARAH, 2024). Cowpea fodder is a valuable feed for livestock and processed products, notably steamed doughnuts and cakes, which are highly prized snacks. It is nicknamed "poor man's meat" due to its high protein content of between 23 and 32% (Quin, 1997). The leaves and immature pods are eaten as a vegetable (Pasquet, 1998).

Thanks to its capacity for symbiotic fixation of atmospheric nitrogen, cowpea can be used to meet nitrogen fertilizer requirements in crop rotations (Carsky, 2002; Tarawali, 2002). However, cowpea cultivation comes up against numerous biotic and abiotic constraints that affect yield, seed quality and forage. The corollary of these constraints is the abandonment of long-cycle and unsuitable varieties, resulting in the disappearance of certain genes of interest. In order to create genetic variability, the use of local varieties is of the utmost importance.

The aim is to (i) assess the agro-morphological variability of local cowpea varieties; ii) assess the molecular diversity of local cowpea varieties.

Materials and methods

Plant material

The plant material consisted of seeds of five local varieties (Bengraga N; Bengringa rond hile brun; Bengraga B; Bengringa plat hile brun; Bengringa hile noir) and one improved variety (Komcallé) of cowpea (Vigna unguiculata L. Walp). The Komcallé variety was used as a control. These varieties differ in their development cycle (Table 1), and the shape of the seed hilum (Figure 1).

Study site

The present study took place at the Institut de l'Environnement et de Recherches Agricoles (INERA) station in Saria. The station is located in the village of Saria, 80 km north-west of Ouagadougou, capital of Burkina Faso, and 25 km east of Koudougou, capital of the Centre-Ouest region (Figure 2).

According to the geographical coordinates, the village of Saria is located at latitude 12°16' North, longitude 2°09' West, and at an altitude of around 300 m above sea level. The Saria station covers an area of 400 ha.

Conduct of the study

The study was conducted over two crop years (2019 and 2020). The experimental set-up is a Fisher block design with three replications and one factor, the variety, covering an area of 434 m2 (31 m x 14 m). The spacing between replicates was 2 m, with 1 m between each elementary plot. Each replication comprised a total of six elementary plots, each measuring 4 m x 4 m, i.e. a surface area of 16 m².

Sowing consisted of placing two seeds in each poquet, followed by removal of the plants 15 days after sowing (JAS). Plot maintenance consisted of two weeding operations spaced two weeks apart from 15 days after sowing, a base fertilization with NPK 14-23-14 at the time of the first weeding operation, weeding 21 days after the second weeding operation and a phytosanitary treatment by spraying with DELTACAL 12.5 EC 22 days after sowing (at the vegetative stage) with the active ingredient Lambda Cyhalothrin 15 g/L +Acetamipride 20 g/L EC.

PACHA 25 EC 41 JAS (at flower bud formation) with active ingredient Acetamiprid 10 g/L + Lambda-Cyhalothrin 15 g/L and K-OPTIMAL 25 EC 55 JAS (at pod formation).

Data collection

Data were collected on the central lines of each elementary plot, eliminating the border lines to avoid the border effect. The following agro-morphological parameters were collected:

● 50% flowering, expressed in JAS, the period between sowing and the date when 50% of the plants on an elementary plot have flowered.
● stem habit determined by the position of branches in relation to the main stem (erect; semi-erect; intermediate; semi-prostrate; creeping).
● plant span characterized by the distance from the longest leaf on one side to the longest leaf on the opposite side of the main stem, from the start of pod formation
● flower color noted at flowering (White; White-Violet; Violet).
● leaf shape observed from first flowering (1=Globular; 2=Sub - Globular; 3=Sub -hastate; 4=Hastate) leaf coloration (light green, intermediate green and dark green).
● 95% maturity expressed in JAS, corresponding to the period between sowing and the date when 95% of pods have dried on an elementary plot.
● number of plants harvested, corresponding to the total number of plants from which seeds and pods were removed at the end of the trial.
● pod pigmentation or pod color at maturity for each elementary plot.
● Pod weight
● Weight of 100 seeds obtained by counting one hundred (100) then weighing for each elementary plot after weighing the seeds.
● Hull yield (t/ha) was calculated on the basis of hull weight for each individual plot.
● Hull yield (t/ha) = (hull weight x 10000)/(area of elementary plot)
● Seed hilum color (Black, Brown, Brown)
● Seed size (small; medium; large)
● Seed texture (rough; smooth; wrinkled).

Molecular characterization

This consisted of DNA extraction, PCR of SSR markers followed by agarose gel electrophoresis. DNA extraction was performed on an immaculate FTA card of fresh young leaf shreds. Discs of 5 mm diameter made from the FTA card were washed twice with FTA solution (200 µL/disc for 5 min) then solubilized with Tris EDTA solution (200 µL/disc for 5 min) twice successively before being spread out and allowed to dry at room temperature for 24 h in FTA tubes.

The PCR polymerase chain reaction was prepared using a positive control (C) consisting of a lyophilized premix (DNTP; Taq polymerase; MgCl2); 2 µL of primer (Forward and Reverse) plus 18 µL of ultrapure water and a disc containing DNA. Reaction volume was 25 µL. The marker primers used are listed in Table 2.

The PCR program consisted of 35 cycles, each with an initial denaturation phase lasting 2 min at 94°C, followed by a final denaturation phase lasting 15 s at 94°C, then a hybridization phase lasting 15 s at 52°C and finally an elongation phase lasting 30 s at 72°C.

Electrophoresis

Electrophoresis was performed on a 2% agarose gel, with 10 µL of each sample containing PCR product deposited in each well. The cell was then connected to an 80 v; 50 mA voltage generator, and the migration time was 1h30mn. Molecular parameter data were collected on the basis of differences between varieties characterized by the presence of bands on the agarose gel.

Statistical data analysis

The quantitative agro-morphological data obtained were entered using Microsoft office Excel 2016 software and processed using Minitab 16 software (Minitab Inc., USA). An analysis of variance (ANOVA) was performed using minitab18 software as a function of variety factor. Means were compared using the Fisher test at the 5% threshold.

Results

Qualitative agro-morphological parameters of local cowpea varieties

The results of the analysis of the various qualitative agro-morphological parameters are presented in Tables 3 and 4. Flower color, leaf coloration, seed hilum color, seed texture and pod pigmentation are shown in Table 3. The white color of the flowers and the wrinkled texture of the seeds were characteristics common to both local varieties and the control variety, Komcallé. The local varieties differ from the control variety Komcallé in terms of leaf color and stem habit. A creeping habit with dark green leaves was observed in the local varieties, whereas in Komcallé, the habit is semi-erect with intermediate green leaves.

Table 1. Origin and development cycle of cowpea varieties.

Number	Varieties	Orign	Cycle in days
1	Bengraga N	Burkina Faso	90
2	Bengringa rond brown hilum	Burkina Faso	90
3	Bengraga B	Burkina Faso	96
4	Bengringa plat brown hilum	Burkina Faso	96
5	Komcallé	Burkina Faso	60
6	Bengringa black hilum	Burkina Faso	96

Pod pigmentation was brown in the Bengringa flat brown hilum variety and yellow in the other varieties. Seed hilum color varied by variety. Brown in Bengringa B, Bengringa plat hile brun and Bengringa rond hile brun, and black in Bengraga N and Bengringa hile noire. The color of the hilum ring of the seeds of the local varieties was different from that of the seeds of the improved control variety, Komcalle.

Quantitative agro-morphological parameters of local cowpea varieties

The results of the analysis of variance of some quantitative agro-morphological parameters are presented in Table 5. No significant differences were noted between varieties for the number of plants harvested parameter, compared with the control, Komcallé ($P = 0.757$). However, there were significant differences between varieties for the parameters 50% flowering date ($P=0.000$), spread ($P=0.003$) and 95% maturity ($P=0.001$), compared with the Komcallé control. The local varieties had later 50% flowering and 95% ripening dates (over 50 JAS and 80 JAS respectively) than the Komcallé control variety (43 and 68 JAS). On the other hand, they had a smaller spread (under 50 cm) than the control (over 50 cm). Stem habit, leaf shape and seed size are recorded in Table 6. The stems of local varieties had a creeping habit, while the control variety, Komcallé, had a semi-erect habit.

Table 2. SSR molecular marker sequences.

Primers	Séquences F – R (5' -3')
VM22	GCGGGTAGTGTATACAATTTG
	GTACTGTTCCATGGAAGATCT
VM32	AGCTCCCCTAACCTGAAT
	TAACCCAATAATAAGACACATA
VM35	GGTCAATAGAATAATGGAAAGTGT
	ATGGCTGAAATAGGTGTCTGA
VM73	CGG CGT GAT TTG GGG AAG AAG
	CTA GTA ACG GCC GCC AGT GTC CTG
VM39	GAT GGT TGT AAT GGG AGA GTC
	AAA AGG ATG AAA TTA GGA GAG CA

Subglobular leaf shape was observed for all varieties. Small-seeded varieties such as Bengaraga B and N were observed, followed by medium-seeded varieties such as Bengringa plat hile brun, Bengringa rond hile brun and the Komcallé control.

Quantitative agro-morphological parameters of local cowpea varieties

The results of the analysis of variance of some quantitative agro-morphological parameters are presented in Table 5. No significant difference was noted between the different varieties for the parameter number of plants harvested, compared with the control, Komcallé (P = 0.757). However, there were significant differences between varieties for the parameters 50% flowering date (P=0.000), spread (P=0.003) and 95% maturity (P=0.001), compared with the Komcallé control.

The local varieties had later 50% flowering and 95% ripening dates (over 50 JAS and 80 JAS respectively) than the Komcallé control variety (43 and 68 JAS). On the other hand, they had a smaller spread (under 50 cm) than the control (over 50 cm).

Table 3. Color of flowers, leaves, hilum of seeds, pigmentation of pods and texture of seeds of varieties.

Variieties	Coul. Fl	Col. F	Pig. Gs	Coul. CHG	Text. gn
Bengraga B	1	2	4	7	8
Bengraga N	1	2	4	6	8
Bengringa dark hilum	1	2	4	6	8
Bengringa plat brown hilum	1	2	5	7	8
Bengringa rond brown hilum	1	2	4	7	8
Komcalle	1	3	4	5	8

Legend: Coul. Fl: flower color; Col F: leaf color; Coul. CHG: seed hilum color; Text. gn: seed texture; Pig.Gs: pod pigmentation. Meaning code: 1: white color; 2: dark green color; 3: intermediate green color; 4: yellow color; 5: brown color; 6: black color; 7: brown color; 8: wrinkled texture.

Table 4. Stem habit, leaf shape and seed size of varieties.

Varieties	P.t	F. f	T. gr
Bengraga B	1	3	5
Bengringa plat hile brun	1	3	4
Bengringa flat brown hilum	1	3	4
Bengraga N	1	3	5
Bengringa dark hilium	1	3	4
Komcallé	2	3	4

Legend: P.t: stem habit; F.f: leaf shape; T.gr: seed size. Meaning code: 1: creeping; 2: erect; 3: subglobose; 4: medium; 5: small.

Figure 2 shows the total weight of harvested pods by variety. For this parameter, no significant difference was observed between varieties (P= 0.101, Figure 2).Analysis of variance for the hundred-seed weight parameter (Figure 4) revealed a highly significant difference between varieties (P=0.000). The Bengringa round brown hilum and Bengringa flat brown hilum varieties respectively recorded mean values of around 19.43±0.31 g and 18±0.32 g, compared with the Komcallé control whose mean value was around 16.13±0.31 g. However, the average values of the Bengraga B, Bengraga N and Bengringa hile noire varieties were similar to and lower than those of the Komcallé variety.

Varietal haulm production, shown in Figure 5, is a very important criterion in the choice of varieties by growers. The Bengraga B variety recorded the highest mean value (2.02±0.19 t/ha) compared with the Komcallé control (1.06±0.33 t/ha) (P=0.0349; Figure 5). The other four varieties showed intermediate values.

Table 5. Some quantitative agro-morphological parameters of varieties.

Varieties	Nb pl harvested	50% fl. (JAS)	Env. (cm)	95% mat. (JAS)
Bengraga B	81,00±20,25^{a}	59,00±2,64^{a}	44,42±0,30^{b}	83,00±2,31^{a}
Bengraga N	59,67±8,95^{a}	59,00±1,15^{a}	47,00±0,85^{b}	83,33±1,76^{a}
Bengringa black hilum	72,67±3,75^{a}	54,00±3,00^{a}	44,37±0,81^{b}	81,33±3,17^{a}
Bengringa flat brown hilum	71,33±4,48^{a}	62,00±0,57^{a}	48,13±1,49^{b}	85,00±0,00^{a}
Bengringa round brown hilum	73,67±6,12^{a}	60,00±0,57^{a}	47,04±0,42^{b}	84,33±0,88^{a}
Komcalle	77,33±6,69^{a}	43,00±0,00^{b}	52,46±1,95^{a}	68,67±1,67^{b}
Probability(P)	0,757	0,000	0,003	0,001

Legend: 50% fl: date of 50% flowering; Env: plant span; 95% mat: date of 95% maturity.

Molecular parameters

DNA from the various samples tested was amplified and different types of bands were noted for all five molecular markers used. Monomorphic bands were revealed ranging from one band to four types per marker. Markers VM22 and VM35 showed two band types each: 50 Pb and 150 Pb for VM22; 50 Pb and 100 Pb for VM35. Four band types for markers VM32 and VM73. The bands for marker VM32 are at 50 Pb, 150 Pb, 250 Pb and 300 Pb, while those for marker VM35 are at 50 Pb, 200 Pb, 250 Pb and 350 Pb. As for marker VM39, it presented one type of band for all samples. Figure 5 shows the electrophoretic profile of cowpea samples with markers VM22, VM32, VM35, VM39 and VM73. All samples were amplified.(figure 6).

Fig. 1. Seeds of six local cowpea varieties.

Discussion

Analysis of qualitative traits such as pod pigmentation, stem habit, seed size, leaf color and seed hilum color showed variability within the six varieties studied. Similar results were obtained by Gbaguidi *et al.* (2015) and Stoilova et Pereira(2013).

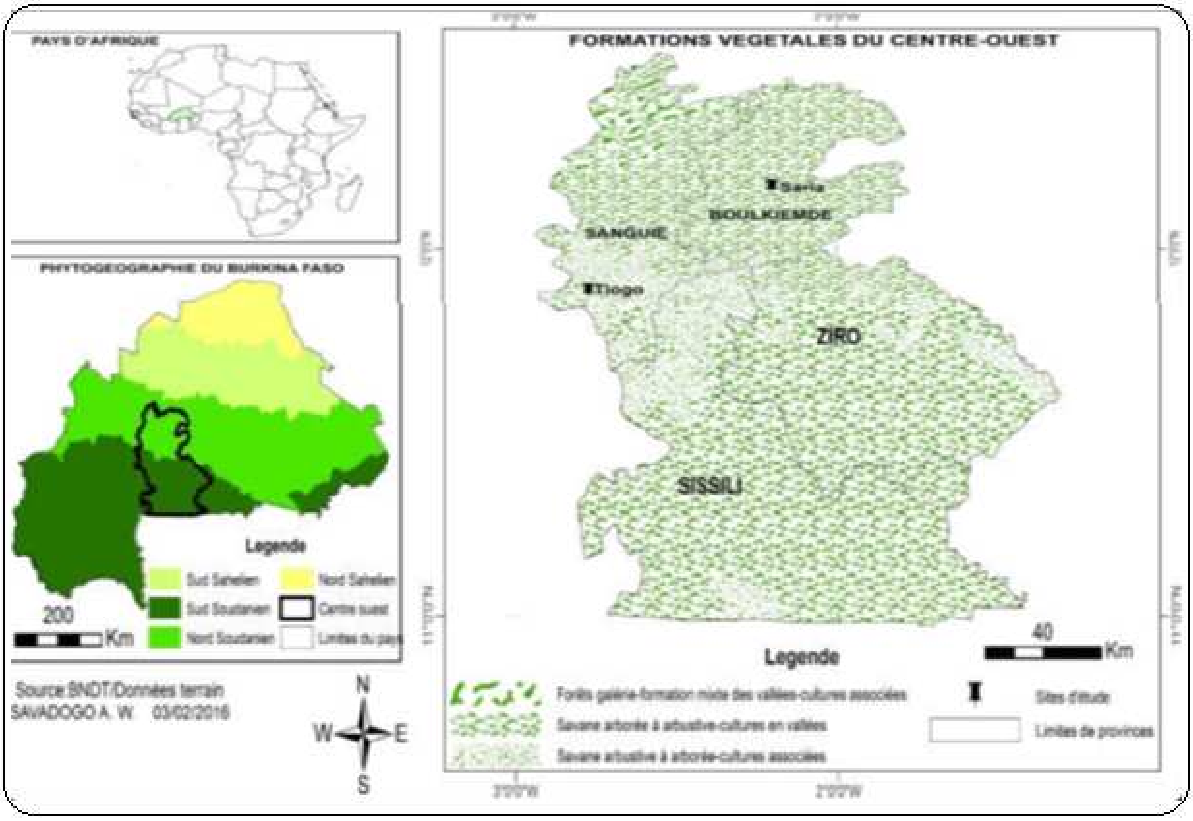

Fig. 2.

The study made it possible to discriminate between varieties. As did Ouédraogo *et al.* (2010), who characterized 122 cultivars and showed that several agro-morphological traits enable judicious choices to be made for production and research uses. No significant differences were observed between varieties for quantitative traits such as number of plants harvested and pod weight per plant. These results are contrary to those obtained by Nadjiam *et al.* (2015) and could be explained by soil fertility, photoperiodic and climatic factors. However, the results for 50% flowering date, hundred-seed weight, haulm yield and 95% maturity date showed a significant difference compared with the control.

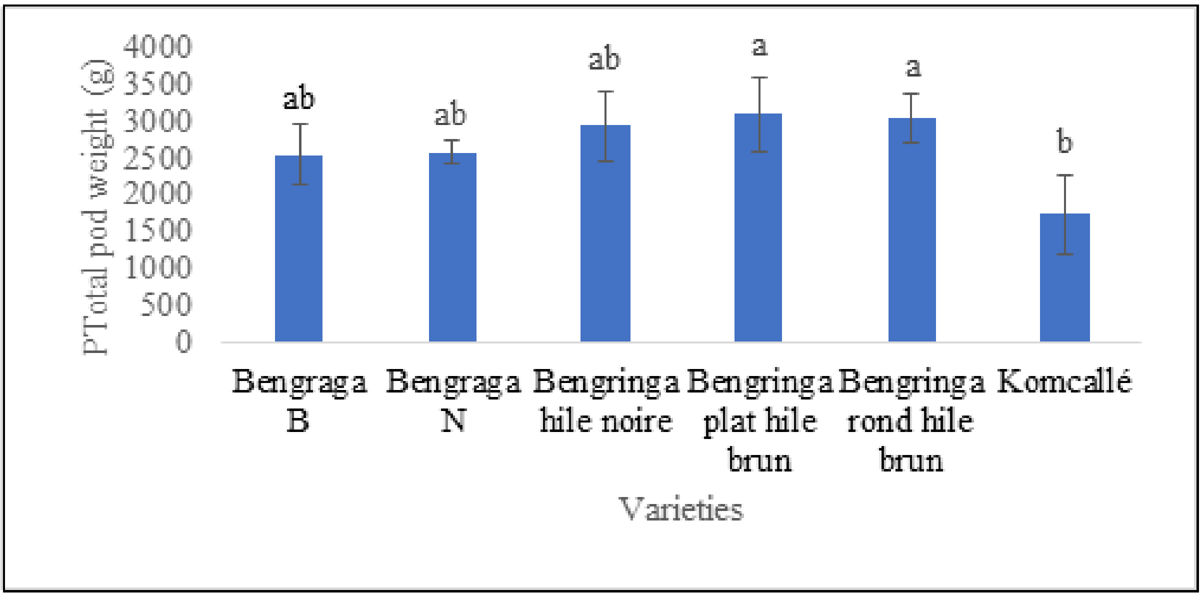

Fig. 3. Total pod weight of varieties.

Local varieties reached 50% flowering with an average of between 50 and 60 JAS, results similar to those of Gbaguidi *et al.* (2015) in Benin and Makanur *et al.* (2013). The average flowering time obtained by Gbaguidi *et al.* (2015) was 52J AS on 124 traditional varieties evaluated in Benin and in India by Makanur *et al.* (2013) with 35 varieties. However, these results are contrary to those of Nadjiam *et al.* (2015, who evaluated cultivars from the Sudanian zone of Chad, reporting an average 50% flowering equivalent to 75.00 JAS. Climatic factors such as temperature and photoperiod could explain this difference in results.

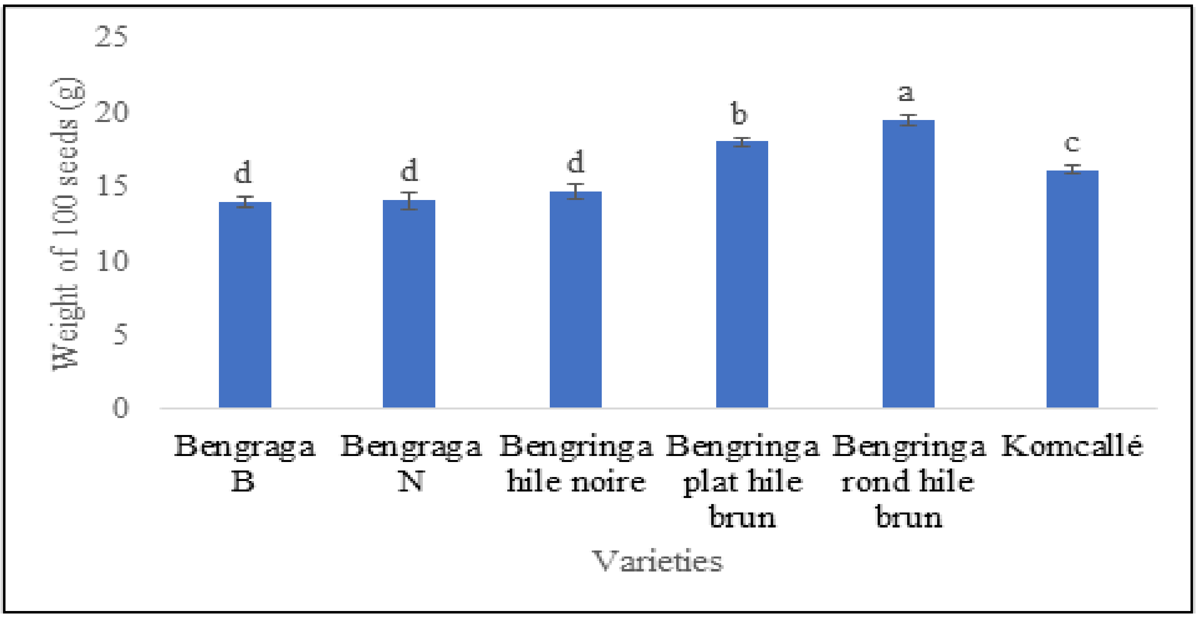

Fig. 4. Weight of one hundred seeds per plant.

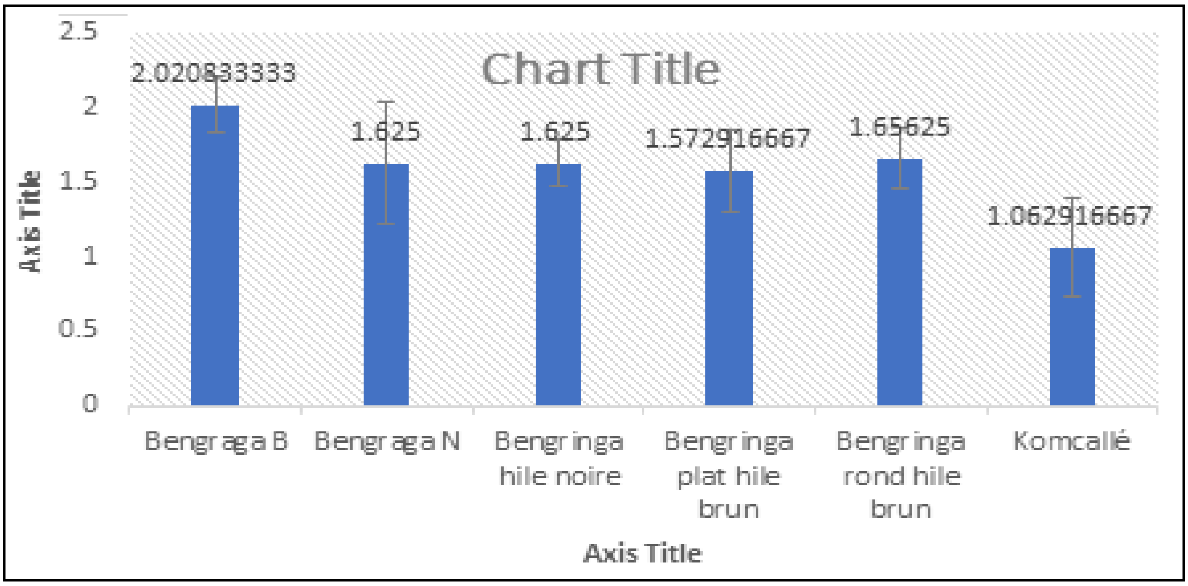

Fig. 5. The haulm yield of varieties.

Indeed, numerous studies have shown that daylength has variable effects on the vegetative and physiological development of cowpeas, depending on the author: Mukhtar and Singh (2006), Gonne *et al.* (2013); Nuhu and Mukhtar (2013).

All the local varieties evaluated were late with more than 85 JAS as 95% maturity, only the control variety Komcallé was early with an average of 68.67±1.67 JAS. However, the local varieties Bengringa plat and rond hile brun gave the best hundred-seed weights, and the variety Bengraga B was the best in terms of haulm production. This result is similar to those obtained by Gbaguidi *et al.* (2015) for the local Benin varieties Ewaoloy, Kaki and Yanbodo and Doumbia *et al.* (2013) on cowpea accessions from Ghana and Mali. Khan *et al.* (2010) explain the difference in 100-seed weight by the accumulation of reserves in the seeds as a function of genotype type and climatic factors.The markers used in the present study are specific to cowpea. All markers were monomorphic. This result can be explained by the fact that self-pollinating species such as cowpea often have low intra-ecotype variability, according to Nkongolo *et al.* (2003).

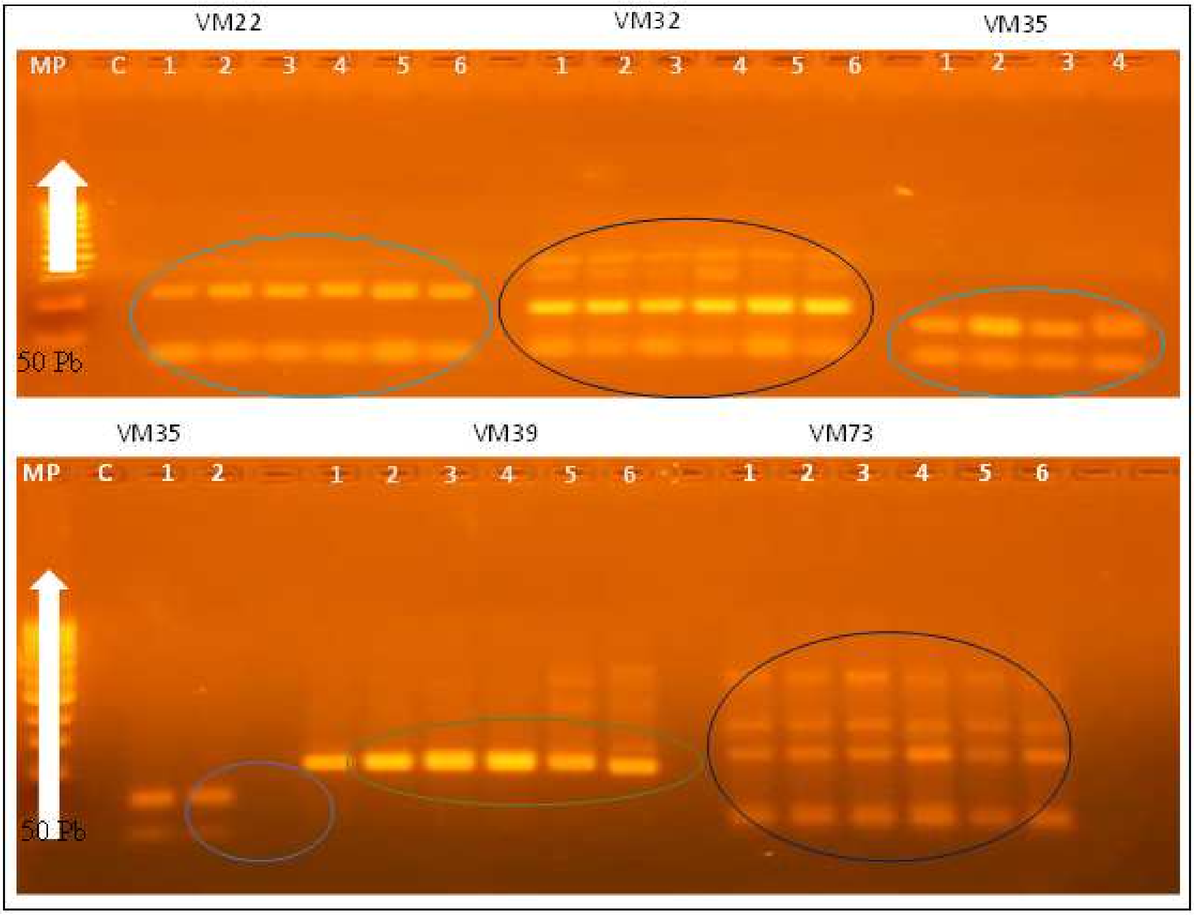

Fig. 6. Electrophoretic profile of the varieties used.

This low polymorphism was also highlighted by Doumbia *et al.*,2012. These authors evaluated 47 accessions from Mali and 47 from Ghana using 20 SSR molecular markers, and six markers were able to amplify the DNA. The use of more primers and in greater numbers could enrich the characterizations.

Conclusion

This agro-morphological and molecular evaluation revealed a certain variability in the six varieties used in this study. Local cowpea varieties were differentiated on the basis of agro-morphological parameters. The best yields in terms of seed weight were obtained by the varieties Bengringa plat hile brun and Bengringa rond hile brun, but the variety Bengraga B gave the best yield in terms of haulm production. Molecular evaluation failed to distinguish between the varieties, probably due to the small number of molecular markers used, but also to the low polymorphism rate present in most legumes, including cowpea. This work will serve as a basis for the improvement of legumes for the production of quality seeds in terms of nutrient reserve content (Bengringa flat brown hilum and Bengringa round brown hilum varieties) and the production of fodder for animal feed (Bengraga B).

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