



Field Performance Evaluation and Multiplex SNP Marker Application of Selected Varieties of Mungbean [*Vigna radiata* (L.) R.Wilczek]

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Abstract:

Mungbean [*Vigna radiata* (L.) R.Wilczek] is a drought tolerant orphan legume crop. The aim of the present study was to evaluate fifteen (15) varieties of mungbean using phenological data, yield attributes and SNP marker assisted tools. Field trial was established following standard agronomic practices. Molecular characterization was carried using a multiplex of SNP primers. Data analysis was done using Minitab 17.0 for data description, test of dependence, correlation and clustering options. Polymorphism Information Content (PIC) was calculated using standard the formula. Results confirmed a successful establishment and adaptability of mungbean varieties in the study area. Growth, flowering and yield attributes were excellent. RMG-344 was the best variety in phonological (flowering) and pod yield. Average grain yield was 779 kg/ha where COGG-912 produced the highest grain yield of 995.31kg/ha followed by, MH-421 (984.33kg/ha) and ML-818 (933.57kg/ha). Plant biomass had the highest variability among all traits. SNP markers employed were highly polymorphic (average PIC was 0.849). A multiplex of CLM0115, CLM0118 and CLM0119 markers were the most polymorphic in terms of number of polymorphic bands produced, percentage polymorphism and PIC value. ML818 variety was the most divergent variety based on morphological attributes while molecular data showed five different groups of varieties that differ in their genetic constitution for an unknown trait that may be of interest to breeders. IC-39298 variety was ungrouped in the molecular data. The crop is suitable for adoption as a resilient, early maturing and high yielding legume crop. Selected varieties with quality agronomic traits such as flowering time, pod and seed yield should be included in breeding program. The highly polymorphic markers should be included in mungbean breeding work

Keywords: Mungbean, Evaluation, Performance, Markers.

INTRODUCTION

Mungbean [*Vigna radiata* (L.) R.Wilczek], a member of the family Fabacea, is a drought tolerant legume also known as the green gram. It was domesticated in Persia (Iran) where its progenitor *Vigna radiata* subspecies *sublobata* occurred wild (Belay *et al.*, 2019). Cultivated mungbean later spread to China and Southeast Asia. Today, it is mainly cultivated as a food crop and major ingredients of different food products in East Asia, Southeast Asia and India as an annual crop (Zhu *et al.*, 2018; Baraki *et al.*, 2020). India is the largest producer of mungbean which accounts for 54% of world production (Baraki *et al.*, 2020). Farmers in some African countries notably Kenya, Tanzania, Ethiopia, Mozambique and Uganda have keyed into the production of

mungbean as a staple protein source (Nair *et al.*, 2019; Baraki *et al.*, 2020). It is cultivated to supplement the protein needs in seasons of insufficient rainfall. Apart from drought, studies have shown high tolerance of mungbean varieties to other stress conditions such as salinity (Sehrawat and Jaiwal, 2014; HanumanthaRao *et al.*, 2016).

Productivity of mungbean is reportedly higher than many legume crops (Sehrawat and Jaiwal, 2014; Kumar *et al.*, 2017; Sehgal *et al.*, 2018). The seeds are loaded with quality nutrients sufficient enough to address the challenges of malnutrition. High adaptability to different environments, low input costs, and high yield make it highly suitable for cultivation in countries with protein deficiency (Baraki *et al.*, 2020). The earlier introduction of some mungbean varieties into the humid and dry agro-ecosystems of Nigeria have failed to yield success due to lack of robust evaluations of a broad germplasm needed for varietal selections and adoption by farmers (Asari *et al.*, 2019). Mungbean was among the legumes evaluated for potential use in dryland farming systems as a drought-tolerant crop that can provide a resilient response to the changing climate. From the previous evaluations done so far (2019-2021), some varieties were selected based on their potential agronomic and breeding values of interests to both the researchers and the growers.

The present study became necessary at this crucial moment when food security is the target of the United Nations in the present decade. Hunger eradication in African countries is possible when integrated approaches are adopted. Part of these strategies is to seek alternative legume crops that are highly stress tolerant, disease resistant and high yielding that can be produced at minimal costs. There is need to evaluate mungbean in terms of adaptability for possible adoption in African countries. Previous projects established at the Joseph Sarwuan University Makurdi has resulted in the identification and selection of some varieties with excellent breeding qualities. In the current work, the phenotypic and genotypic studies are necessary to further characterize these selections using molecular markers and identify those that can serve as templates in future breeding work for possible release of quality seeds that can be adopted by the Nigerian farmers. The aim of the present study was to evaluate fifteen (15) selected varieties of mungbean using phenological, yield attributes and marker assisted tools.

MATERIALS AND METHODS

Study Site

The experimental site was an expansive land along Gbajimba Road allocated to the College of Agronomy, JOSTUM for legume trials, Makurdi town is the headquarter of Makurdi LGA. (Latitude 7°38'N-7°50'N; longitude 8°24'E-8°38'E) with a landmass of about 16 kilometres (km) in radius and a population of 300,377 (Abah, 2013). The rainy season lasts from April to October with 5 months of dry season. The mean annual temperature of the area is 1173mm but the temperature is high throughout the year ranging between 22.5°C and 40°C. The vegetation is of the Guinea Savannah type (Abah, 2013). Benue State is popularly known as the food basket of the nation. Agricultural activities are high in the study area especially among the rural dwellers who cultivate diverse types of fruit and vegetable crops, pulses and cereals at both subsistence and commercial level.

Planting Season

The study was carried out in the 2022 planting season. Field was established on 21st August, 2022 alongside other legume trials evaluated by the Department of Plant Breeding, College of Agronomy, Joseph Sarwuan Tarka Makurdi, Benue State, Nigeria (JOSTUM).

Seed Source and Management

Fifteen (15) varieties of mungbean seeds were sourced from the germplasm of the Molecular Biology Laboratory, Department of Plant Breeding, College of Agronomy, JOSTUM. These varieties selected from previous trials for continuous evaluations based on the unique agronomic values of interest to the breeders. Seeds were packaged in sealed envelopes labeled with names of each variety written on them. The varieties are: GAM-5; Ganga-8; MH-421; SML-668; RMG-344; RMG-492; COGG-912; ML818; MUM-2; IC-39298; IC-39300; IC-39368; IC-39375; IC-39500 and IC103245

Field Preparation, Experimental Design and Planting

Land clearing, ploughing and ridging were done using mechanized farming system established by the STOL project team. A total of 15 varieties were planted in a RCBD (Randomized Complete Block Design) of 3 blocks and 3 replications to determine varietal performances. Four (4) seeds of each variety were sown in 4 rows of 4 m length spaced 0.60 m apart and within -row plant to plant distance of 0.15 m to give a gross plot size of 4 x 3 m (12 m²) (IBPGR/IPGRI, 2019). A mixture of pendilin and gramoxone at a rate of 2.5 L ha⁻¹ gramoxone and 1 L ha⁻¹ pendilin was applied immediately after planting using Knapsack sprayer. Hoe weeding was used just before flowering to remove subsequent weeds. Fertilizer rate 15:15:15 was applied using a compound fertilizer (NPK 15; 15; 15) to provide 15 kg each of N, P, and K ha⁻¹ as starter dose.

Varietal Characterization

Phonological and yield related data were collected using the IBPGR/IPGRI descriptors of 2019. Data entry was done in the field log book aided by the use of visual observation, meter rule, portable digital weighing balance, pencil, erasers and pen. The following morphological traits were characterized in the field: number of primary branches, days to flowering, days to 50% flowering, number of pods per plant, days to maturity, pod length (cm), pod weight (g), seed weight per plot (g), 1000 seed weight (g) number of seeds/pods, average biomass (kg) and yield (kg/ha)

Molecular Characterization

DNA extraction was done using the CTAB method (Omoigui *et al.*, 2015). A suitable protocol was established after the optimization stage. A total of ten (10) legume-specific SNP primers was used for DNA amplification stage as listed in *Table 1*. A Multiplex PCR (Polymerase chain reaction) was performed on a heated lid thermal cycle using the thermal cycle operated as follows: 35 cycles of denaturation at 94 °C for 30s, followed by annealing temperature at 57.5 °C for 30s and extension at 72 °C for 2 min. A final extension cycle of 10 min at 72 °C was added to ensure completion of the final amplification products as described by Omoigui *et al.* (2015). Amplicons were resolved in an agarose-based gel electrophoresis (0.8% agarose powder in 1xTAE buffer; 50L of ethidium bromide) Galileo Bioscience tank connected to Consort EV243 electrophoresis power supply. The gel ran at 120v for 45 minutes. DNA purity and quality was checked using UV spectrometer light (Omoigui *et al.*, 2015). The ethidium bromide-stained gel was visualized on an UV transilluminator and photographed using a digital camera

Table 1: SNP Primers and their sequence

Primer	Forward sequence (3-5')	Reverse sequence (5-3')
CLM0110	CAGGCTGAAAGTGCCATTAT	GGAAGTTTCACCACCCTTTC
CLM0111	ATAGGAATTGGACTGGGCTT	TGCAAAGGGTTGATTTGAA
CLM0112	GCTGCCATGTCCATTAGAGT	GAGAGGACTTGGGTTTCGAGT
CLM0113	TCAAGAATCTTTAGACCGAA	CCTGAGTTTAATTATTTGTTTCA
CLM0114	TTCCTTAGCCAAAGTGTTCC	TCAACGACAGCGTTATCAAA
CLM0115	TTTCATTGCATTTATTCCAC	TCAGGAGACAGAATGGAAGG
CLM0116	GACTGCTACATGGCCTCAAA	CCTGCATTTACACATTGTCTC
CLM0117	AAGACAAGCTCCCTGGAAGT	GTCATTATGCATTGGGCATT
CLM0118	AAATTCTGCAACGACTACGC	TGCATTCTCCGTAGTGTGA
CLM0119	GAGATGTTGAGATGGTGGCT	CCTGGTCATTGAACCTCTC

Scoring of Gel Images and Analysis

Only clear and unambiguous bands were scored. Markers were scored for the presence and absence of the corresponding band among the genotypes. The scores '1' and '0' indicate the presence and absence of bands, respectively to generate a binary matrix. Data analysis was done using the Microsoft Excel Workbook and Minitab 17.0. Statistical operations carried out on field data were: data description with graphical summaries for each trait, test of dependence using the Chi-square tool, correlation analysis using the Pearson Product Moment method. Polymorphic bands were analyzed for each primer used. Polymorphism Information Content (PIC) was calculated using the formula adopted by Weir (1990) and Xu (2010).

$$PIC = 1 - \sum p_{i2}$$

Where p_i is the frequency of the i th allele for each SSR marker i th summed across all alleles for the loci. Cluster analysis was performed on morphological and molecular data each resulting in the construction of dendrogram using the Average Linkage method measured on Euclidean Distance.

RESULTS

Table 2 gives a description of mungbean plants in the field. The varieties produced between 0.6 and 2.6 branches in the field. They flowered from day 25 to 32 after planting while the mean flowering day was 29.2 ± 1.86 . Varieties produced 50% flowering within 32-36 days. Mean number of pods produced per plant was 36.78 ± 12.0 ranging from 17 to 55.8 pods among the plants. The 1000 seed weight varied between 36.5g and 49.3g. Seed weight per plot measured between 387.50g and 658.30g. Total yield grain varied from 504.30kg/ha to 995.30kg/ha among the varieties while average yield produced was 778.5 ± 160.2 kg/ha. Plant biomass had the highest coefficient of variability (45.7%) followed by the number of primary branches (32.75%), number of pods per plant (32.6%), pod weight (20.8%) and yield (20.6%). Distribution of grain yield was not normal (A -squared = 0.54, $p > 0.05$) using the Anderson-Darling normality test (skewness = -0.36) while 95% confidence interval for the mean was between 646.9kg/ha to 867.2kg/ha (Figure 1). Top three high yielding varieties were COGG-912 (995.31kg/ha), MH-421 (984.33kg/ha) and ML-818 (933.57kg/ha) (Figure 2). The overall yield measurement in kg/ha was dependent on the type of varieties ($\chi^2 = 461.30$, $P < 0.05$). Chi-square distribution showed that the observed values in yield were higher than the expected values in MH-421, COGG-912, ML-818, RMG-344, SML-688, RMG-492, MUM-2, IC-39298 and IC-39300 (Figure 3). Table 3 gives the correlation coefficients of the quantitative characters in a matrix table. Moderate positive correlation ($r = +0.40-0.69$) exist

between: pod weight and number of pods per plant ($r=0.574$); number of seed per pod and number of pods per plant ($r=0.521$); seed weight per pod and pod weight ($r=0.651$), biomass and number of seed per pod ($r=0.673$). Moderate negative correlation was found between pod length and day to maturity ($r= -0.551$). Yield in kg/ha positively correlated with both pod weight ($r= 0.790$) and seed weight per plant ($r= 0.701$). Cluster analysis done using morphological data showed that similarity coefficient level was between 73.3 and 93.7 resulting in a distance of between 168.9 and 39.8 respectively among the varieties. The resulting dendrogram of phenotypic data constructed using the single linkage method (*Figure 4*) gave 3 clusters where varieties displayed some levels of similarity in phenological and yield attributes. The most related varieties were RMG-492 and IC-39300. Others such MUM-2 and IC-39298 were both similar but were separated from other varieties thus forming the third cluster. The most divergent variety was the ML818 (similarity coefficient = 76) being the highest in pod weight (937.55g), seed weight per plot (658.32g). It had a very high pod yield (54.1) and the second highest producer of total grain yield (933.57kg/ha).

Phenotypic assessment has revealed a successful crop establishment as shown from the luxuriantly growth, appealing vigour and good performances of the varieties although with some levels of variability. The above observation could be an indication of high ecological adaptation and tolerance to the biotic and abiotic environment including the climatic and edaphic related factors as suggested in other studies (Yashvir and Rex, 2018; Nair *et al.*, 2019). This report is also in tandem with other findings that mungbean is a resilient crop that tolerates different environment (Yashvir and Rex, 2018; Nair *et al.*, 2019). There appears to be a strong indication of high influence of the environment on phenological and yield related traits of the fifteen mungbean varieties in the field. The impact of main effects of environment (E) and the interactions on yield of genotypes (G) was earlier established (Yashvir and Rex, 201) some of which were related to physiological traits including time to flowering and maturity. Average flowering time was 29 days while maturity time was 75 days. Time to flowering in mungbean was subject to both genetic and environmental control (Miko, 2018). The photo-thermal control on flowering time has been extensively modeled (Kaur *et al.*, 2015; Sharma *et al.*, 2016; Fathy *et al.*, 2018; Nair *et al.*, 2019; Baraki *et al.*, 2020). The reported flowering time of mungbean appeared similar to cowpea, an indication that mungbean completes its lifecycle quickly. Early flowering is a good characteristic as harvesting could be achieved before the severity of drought in semi-arid regions. This agrees with the findings of Kaur *et al.* (2015) who studied the inheritance of time to first flowering in photo-insensitive mungbean. According to the authors, early maturity as determined by early flowering is an important agronomic trait that is crucial in the adaptation of annual crops. In the present work, RMG-344, RMG-492 and IC-393638 have been selected in this study as the best mungbean varieties with earliest flowering time. Pod characteristics in terms of number of pods per plant, pod sizes, pod weight and number of seeds present in each pod of the evaluated mungbean varieties are excellent attributes that may attract the attention of growers. RMG-344 (the best variety in number of pods) produced as high as 46 pods per plant and closely followed by SML-668 that produced 44 pods per plant. These two varieties have been selected for inclusion in breeding work. However, number of these two varieties was the best in terms of number of seeds per pod. SML-668 and MUM-2 were selected for this trait.

The average grain yield of 779 kg/ha established in this work was higher than 750kg/ha reported by Olson *et al.* (2011) but lower than the values reported by Belay *et al.* (2019) and Baraki *et al.* (2020) who worked on mungbean. Due to several challenges, the average cowpea production in West Africa was reported to be as low as 358kg/ha (FAO, 2000), although some improved cowpea varieties had slightly higher yield with improved resistance (Omoigui *et al.*, 2017). Thus, farmers

can take advantage of high yield attributes of mungbean as an excellent plant-based protein source to supplement cowpea production. Top high yielding varieties including COGG-912 (995.31kg/ha), MH-421 (984.33kg/ha) and ML-818 (933.57kg/ha) should be considered in breeding program to improve other low yielding varieties. Baraki *et al.* (2020) who worked on the genotype x environment interaction and yield stability analysis of mungbean genotypes reported a significant variation in grain yield among the genotypes, environment and genotype by environment interaction. In the present study, it was established that yield is positively influenced by pod and seed weight. Plant biomass had the highest variability among the evaluated varieties and it was moderately influenced by number of seed per pod. As a weight related parameter, plant biomass has been extensively reported to be affected by the amount of water present in a living system (Sharma *et al.*, 2016; Yashvir and Rex, 2018; Baraki *et al.*, 2020). It may be inferred that the fifteen varieties differ in the amount of water present as a function of water absorption and utilization. It should be noted that mungbean is a drought tolerant legume crop. In other studies, biomass (dry matter) was the most important yield determinant of with over 90% of the total variation in yield being accounted by this trait (Sharma *et al.*, 2016; Fathy *et al.*, 2018). Developing mungbean plants with a root system that is able to draw more moisture from the soil could help reduce this extreme phenological and morphological sensitivity to water stress situations (Baraki *et al.*, 2020).

Table 2: Description of quantitative characters

Traits	Mean±SD	CV%	Min	Max
Number of primary branches	1.81±0.59	32.68	0.60	2.60
Days to First flowering	29.2±1.86	6.37	25.00	32.00
Days to 50% Flowering	34.93±1.16	3.33	32.00	36.00
Number of pods per plant	36.78±12.00	32.62	17.60	55.80
Days to Maturity	75.27±1.10	1.46	73.00	76.00
Pod length (cm)	8.05±0.33	4.06	7.60	8.80
Pod weight (g)	693.7±144.5	20.83	444.40	937.50
Seed weight per plot (g)	495.7±88.4	17.83	387.50	658.30
1000 seed weight (g)	40.39±3.22	7.96	36.50	49.30
Number of seeds per pod	10.75±0.82	7.65	9.50	12.50
Average Biomass (kg)	0.43±0.20	45.74	0.15	0.94
Yield kg/ha	778.5±160.2	20.57	504.30	995.30

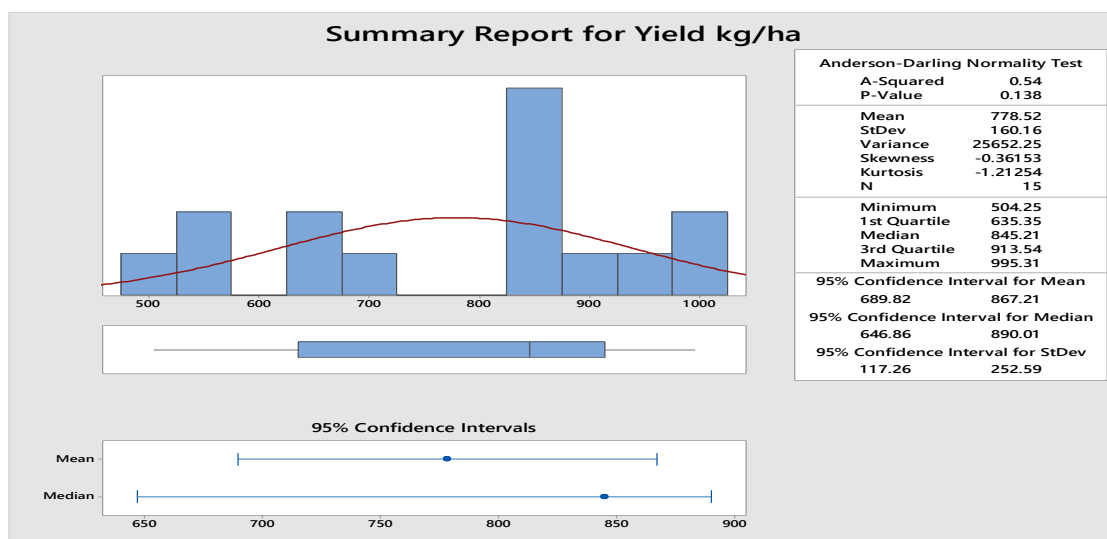


Figure 1: Graphical summary for Yield (kg/ha)

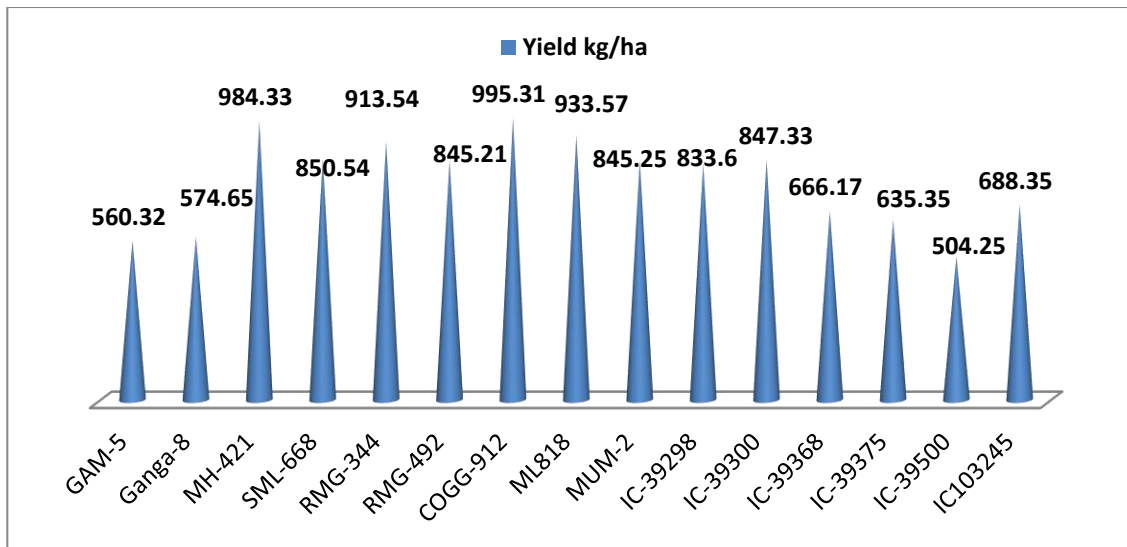
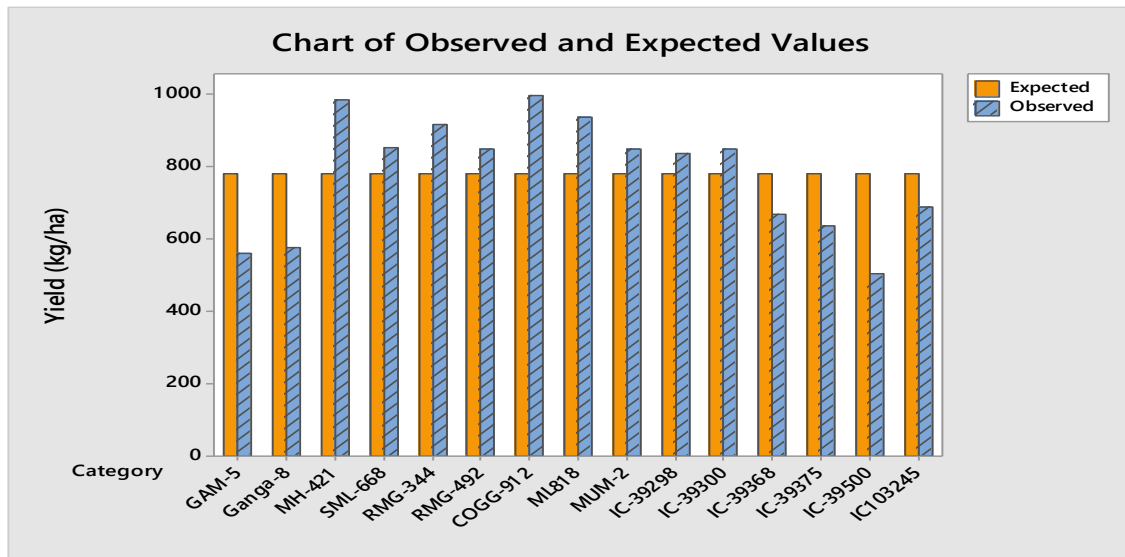


Figure 2: Performances in grain yield among mungbean varieties



χ^2 (Yield) = 461.302, P=0.000 (P<0.05)

Figure 3: Test of dependency between seed weight and mungbean varieties

Table 3: Pearson Correlation Matrix of Quantitative Traits

Traits	NPB	DFF	D50F	NPP	DM	PL	PW	SWP	1000SW	NSP	AB
DFF	-0.016										
D50F	-0.258	0.667									
NPP	0.330	0.188	-0.168								
DM	0.027	-0.028	0.127	-0.368							
PL	0.056	0.371	0.046	0.361	-0.514						
PW	-0.190	-0.246	-0.235	0.574	-0.290	0.097					
SWP	-0.321	-0.174	-0.311	0.464	-0.210	0.242	0.651				
1000SW	0.024	0.107	0.072	-0.335	0.023	0.436	-0.479	-0.192			
NSP	0.214	0.288	0.220	0.521	-0.339	0.199	0.120	-0.094	-0.108		
AB	0.188	0.052	0.069	0.344	-0.499	0.202	0.460	-0.075	-0.181	0.673	
Yd	-0.341	-0.221	-0.296	0.488	-0.350	0.194	0.790	0.701	-0.377	0.162	0.337

Number of primary branches = **NPB**; Days to First flowering = **DFF**; Days to 50% Flowering = **D50F**; Number of pods per plant = **NPP**; Days to Maturity = **DM**; Pod length (cm) = **PL**; Pod weight (g) = **PW**; Seed weight per plot (g) = **SWP**; 1000 seed weight (g) = **1000SW**; Number of seeds per pod = **NSP**; Average Biomass (kg) = **AB**; Yield = **Yd**

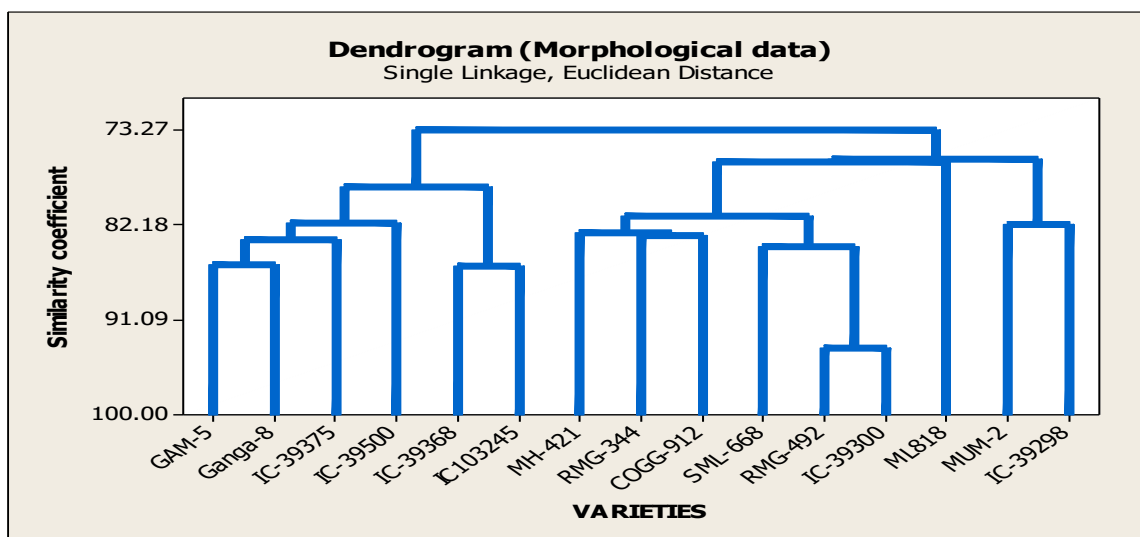


Figure 4: Dendrogram showing clustering pattern of mungbean accessions using morphological data

All the varieties possessed a specific trait (500bp) but different heritability pattern as some varieties showed double bands while others showed single bands at the same locus (*Plate 1*). CLMo110, CLMo112 and CLMo113 multiplex primers produced double bands in all except in three varieties resulting in 5 polymorphic bands where % Polymorphism was 33.3% and Polymorphic information content (PIC) was 0.845. CLMo114 and CLMo116 multiplex primers produced double bands in 8 out of 15 varieties resulting in 4 polymorphic bands where % Polymorphism was 26.7% and PIC was 0.779. CLMo115, CLMo118 and CLMo119 multiplex primers produced 6 double bands resulting in 6 polymorphic bands where % Polymorphism was 40.0% and PIC was 0.922, thus the most polymorphic primer set. All primers gave an average of 5 polymorphic bands and 0.849 PIC (*Table 5*). Similarity coefficient among the varieties ranged from 22.06 to 100.0 while genetic distance was between 1.35 and 0.00. Intermediate similarity level (42.3) was observed. Average distance from centroid was 0.801 while the maximum distance from centroid was 1.069. The resulting dendrogram (*Figure 5*) gave 2 clusters and 5 groups of varieties that are similar in banding pattern (5 polymorphic groups). Groups containing two clustered varieties were GAM-5 and IC-39375 as well as IC-39368 and IC-103245. Other groups comprised 3-4 varieties. The only ungrouped variety among them was IC-39298.

The use of SNP (single nucleotide polymorphism) in this study has proven to be a good marker candidate in showcasing the relatedness and divergence among the mungbean varieties. As the name implies, each SNP reveals the polymorphism that exists among varieties differing by a single nucleotide at a particular locus. The primer combinations were polymorphic with high PIC values. According to Nelson *et al.* (2016), PIC values of 0.5, 0.4 and 0.2 are classified as highly informative, moderately informative and little informative respectively. The average PIC of 0.849 obtained in this study was higher than values obtained in many studies (Dhaliwal *et al.*, 2014; Kumar *et al.*, 2016). A marker is said to be polymorphic if it has at least two alleles (Mason, 2015). Here, we reported a multiplex of CLMo115, CLMo118 and CLMo119 as the most polymorphic in terms of number of polymorphic bands produced, percentage polymorphism and PIC value. These markers may be useful in future breeding work of mungbean. This outcome is consistent with the report given by Omoigui *et al.* (2015) who determined the suitability and use of two molecular markers to track race-specific resistance to *Striga gesnerioides* in cowpea. Information obtained from cluster analysis reveals ML818 as the most divergent based on morphological

attributes while molecular data showed five different groups of varieties that differ in their genetic constitution for an unknown trait that may be of interest to breeders. IC-39298 variety should be investigated further to ascertain why it was unrelated genetically.

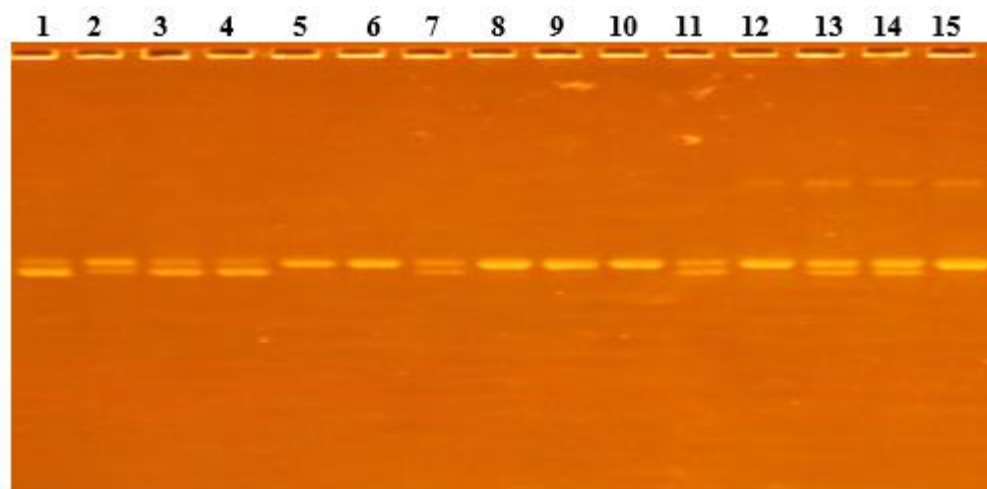


Plate 1: DNA band profile of CLMo114 and CLMo116 Multiplex Primers

Table 5: Multiplex Primers and their Polymorphism Information Content (PIC)

Primer name	Forward Sequence	∑ Polymorphic bands	% Polymorphism	PIC 1 - $\sum pi^2$
CLM0110	CAGGCTGAAAGTGCCATTAT	5	33.33%	0.845
CLM0112	GCTGCCATGTCCATTAGAGT			
CLM0113	TCAAGAATCTTTAGACCGAA			
CLM0114	TTCCTTAGCCAAAGTGTTCC	4	26.67	0.779
CLM0116	GACTGCTACATGGCCTCAAA			
CLM0115	TTTCATTGCATTTATTCCAC	6	40.00%	0.922
CLM0118	AAATTCTGCAACGACTACGC			
CLM0119	GAGATGTTGAGATGGTGGCT			
Total		15		2.546
Mean		5.0		0.849

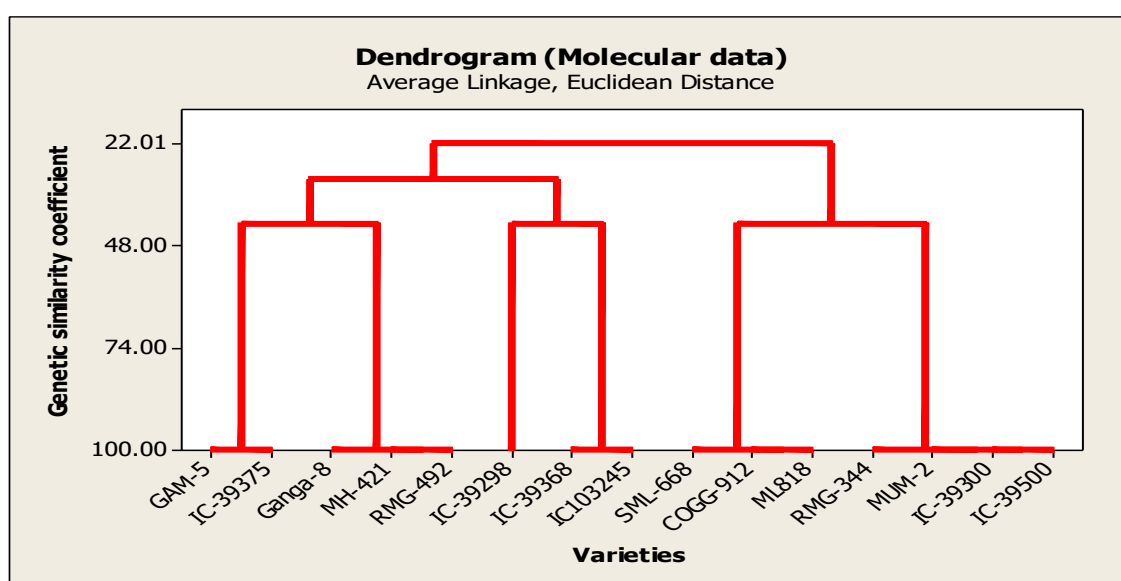


Figure 5: Dendrogram showing clustering pattern of mungbean accessions using molecular data

CONCLUSION

This study confirmed a successful establishment and adaptability of mungbean varieties in the study area. RMG-344 was the best variety in phenological (flowering) and pod yield. Average grain yield was 779 kg/ha where COGG-912 produced the highest grain yield of 995.31 kg/ha). ML818 variety was the most divergent variety based on morphological attributes while molecular data showed five different groups of varieties that differ in their genetic constitution. The crop is therefore suitable for adoption and cultivation among Nigerian farmers being a resilient, early maturing and high yielding legume crop. Selected varieties with quality agronomic traits such as flowering time, pod and seed yield should be included in breeding program. The highly polymorphic markers selected (CLM0115, CLM0118 and CLM0119) should be included in mungbean breeding work.

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