Potential for Cowpea Improvement of a Collection of Fifty Varieties Based on Morphological Characters

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ABSTRACT

Cowpea is a nutritious legume consumed as vegetable; grain stew or fed to animals as forage. Being a rich protein source, it's used as an alternative to animal protein for resource-poor farmers. Due to a wide range of uses, morphological diversity and individual crop preferences, farmers face a challenge in variety selection. Their choice seed is saved using traditional methods such as pots, sacks and silos. This results in limited grower-collected varieties from which to select. Seed from local vendors also are of poor quality. Limited research has been carried out on farmers' varieties and what is available for utilization. This study was conducted to determine variation in morphological plant and seed characteristics among 50 cowpea varieties as available germplasm for the farmers to evaluate. Cowpea varieties were selected based on availability and utilization by farmers. The cowpea germplasm collection was field-grown at Jomo Kenyatta University of Agriculture field station in Juja, Kenya, in a randomized complete block design and evaluated for variation in morphological characteristics based on IBPGRI cowpea descriptors. Upon harvesting, seed morphological parameters were assessed for variation. Descriptive statistics were used to assess variation in cowpea (plant height (cm), hairiness, pigmentation; leaf color, shape and texture; flower color, and pigment; pod color, pigment, curvature and thickness; days to flowering and pod formation; number of pods per plant. Seed coat coloration, length, width, coat thickness, and the 100 seed weight). Variation in quantitative traits among the cowpea varieties was determined using Analysis of variance. ANOVA of each of the quantitative characteristics; days to maturity, pod size, pods per plant and 100 seed weight, revealed significant differences ($p \leq 0.05$) among the cowpeas. Seed coat characteristics; grey mottled, white, light red, red, black, cream, brown mottled and SP6 mixtures were used to classify the cowpea varieties. A wide range of variation exists in each of the characters across the cowpea varieties and germplasm that satisfy the diverse needs of individual farmers' that can also be used for breeding and selection of improved lines.

INTRODUCTION

Cowpea *(Vigna unguiculata.* (L). Walp*)* is a popular legume that is self-fertilizing.

Depending on its utilization, cowpea is classified into three categories namely vegetable cowpea, grain cowpea, and dual-purpose cowpea. Vegetable cowpea varieties are mainly consumed as vegetables (leaves and immature pods) whereas grain cowpea is used for preparing grain stew and dual-purpose cowpea is utilized leaves, immature pods and grains. Foliage from cowpea is also used for animal consumption. Cowpea is produced in Europe, Asia, America and Africa majorly parts of East and West Africa (Coulibaly et al., 2008). Production of cowpea in the world has been on the increase from 2014 to 2018; Africa accounts for 96%, Asia 2.4% Americas 1.1% and Europe 0.4%. Kenya is ranked 7th worldwide in production of cowpea and Nigeria is the leading producer as reported by Food and Agriculture Organization in 2019.

Cowpea is a very nutritious crop; a source of dietary fiber and inexpensive protein. The leaves and grains of cowpea contain 34.2g and 24 grams per 100 grams of protein respectively when fresh (Grubben et al., 2014). It is also the second most important legume behind beans in Kenya (Wambugu and Muthamia, 2009). Besides high protein content cowpea foliage is used as animal forage (Timko et al., 2007). Agronomic benefits of cowpea include the ability to fix up to 30kg ha^{-1} of Nitrogen which results in an increase in yield for the intercrops like maize and sorghum (Khan et al., 2017). Similarly, recent studies in Zambia on Biological Nitrogen Fixation and contribution to maize yield revealed an increase in maize grain yield by 12 ton ha^{-1} during maize cowpea rotations (Simunji et al., 2019). Compared to ground nut and bean, cowpea has been found to significantly reduce Striga, a noxious weed in sorghum hence beneficial in weed management (Khan et al., 2007). Use of cowpea as green manure reduces use of synthetic Nitrogen fertilizers thus contributing to 'clean' production, environmental conservation and maintenance of soil health is also achieved in the process. In Nigeria, use of cowpea green manure besides realizing increased output, small scale farmers get a net profit of 877 dollars compared to 685 dollars realized from use of synthetic fertilizers (Fabunmi and Agbonlahor, 2012).

Morphological attributes are important for characterization of cowpea and the selection of cowpea preferred by farmers for example could include growth habit, yield components, maturity time, seed color and texture. Cowpea production is frequently impacted by weed competitions, pests and diseases, as well as occurrence of mixed types due to cross-pollination (Thooyavathy et al., 2013). A collection of eight cowpea mutant genotypes varied in morphological characteristics including plant height, leaf characteristics, days to maturity, pod size and 100 seed weight (Porbeni et al., 2016). In addition, the major challenges facing production of cowpea is unavailability of quality seeds and suitable varieties for specific sites (Biemond et al., 2012). Farmers save their own seed because of limited good quality commercial lines, less cost, and sometimes a farmer's inability to select better varieties due to lack of knowledge and experience. Field trials carried out to evaluate agronomic performance of improved varieties have shown significant differences in number of branches, pods per plant and seed yield compared to the local cowpea accessions (Kamai et al., 2014). Significant variation has been observed among cowpea lines collected in East Africa for days to 50% flowering ranging from 65 to 82. Number of pods per plant positively correlates with yield of cowpea; an important attribute when analyzing morphology in relation to cowpea variety selection and production (Menssena, et al., 2017). Earliness in maturity, growth habit, resistance to diseases drought tolerance, high and stable seed yield output, harvest index and good seed quality are important cowpea morphological traits in tropics. These are important characters whose traits are considered in breeding programs (Abadassi, 2015). This study therefore focused on determining variation in plant and seed morphological traits of 50 cowpea collections

obtained from different areas in Kenya and advanced accessions from World Vegetable Centre. The results will inform Kenyan farmers and breeders on the potential for improvement of cowpea adapted to the local needs and in the identification of those with the field performance growers may find of interest.

MATERIALS AND METHODS

The study was carried out at the Jomo Kenyatta University of Agriculture and Technology (JKUAT) at Juja from November 2016 to March 2017. Juja is located 13 kilometers from Thika town and approximately 35 kilometers from Nairobi in Kiambu County at 1°11' 0'' S, 37° 7' 0'' E. The site is in Agro-ecological Zone Four (Foeken, 1994). Annual minimum and maximum temperature experienced is 10.4 and 22.7^{0}C respectively. Mean annual rainfall is 856 mm.

Fifty cowpea accessions were used in the experiment; thirty-four accessions were obtained from National Gene bank of Kenya, eleven from farmers' collection, four lines from World Vegetable Centre (AVRDC) and one commercial line (Table 1). SP6 seed mixtures were obtained through the HORTINLEA SP6 project (Horticultural Innovation and Learning for Improved Nutrition and Livelihood in East Africa), an interdisciplinary research project addressing food security in East Africa, particularly in Kenya. The varieties were planted at JKUAT farm in a randomized complete block design, with three replications. Plants were spaced at 60 cm by 30 cm inter-row and intra row respectively. Two seeds per hole were planted and subsequently gapping and thinning to one plant per hole was done at 21 days after planting (Fig. 1). Each plot therefore contained 30 plants, 10 on each row and the plot size was 1.2m by 2.7m translating to a potential plant density of 9846 plants per ha. Recommended agronomic practices were carried out according to the guidelines of production of cowpea outlined (Hutchinson et al, 2017). Fertilizers and pesticides were not used during the experiment. The crop relied on rainfall and sprinkler irrigation was applied once a week whenever there was need.

Morphological data was collected according to IBPGRI (1983) cowpea descriptors; Vegetative data at 42 days after planting (Fig. 2), inflorescence data when 50% of plants in a plot had flowered. Data were obtained for; plant height (cm), hairiness, pigmentation; leaf color, shape and texture; flower color, and pigment; pod color, pigment, curvature and thickness; days to flowering and pod formation; number of pods per plant and grain yield. Seeds for each variety were harvested when 50% of pods were dry. The pods were threshed, and the seed was cleaned and kept for further laboratory evaluation. Seed coat coloration, length, width**,** coat thickness, and 100 seed weight were determined post-harvest in the plant physiology laboratory. Data on seed length, width, and thickness were obtained by getting the mean for 10 healthy seeds of each variety and 100 seed weight was obtained by getting an average weight of 100 mature, healthy seeds for each variety. Descriptive statistics were used to determine the distribution of above attributes/traits among cowpea accessions used. Quantitative Data (for days to flowering, days to maturity, pod length, pods per plant and 100 seed weight) were analyzed using GenStat software. Analysis of Variance (ANOVA) was carried out to determine variation among cowpea varieties. The means were separated using Least Significant Differences at P= 0.05

RESULTS

Cowpea varieties were classified into eight groups based on seed coat coloration. Seed coat colors were; grey mottled, white, light red, red, black, cream, brown mottled and SP6 mixtures (Table 1 and Fig. 2). Farmers' collections were mainly the red and black type, whereas the Gene bank accessions were distributed across the different colors. The varieties were distributed across the traits of each of the evaluated plant

characters (Table 1).

At the vegetative and inflorescence stages (Table 2), variation was noted for; leaf size, shape color and texture (Fig. 3). Different positions of the raceme were observed across the cowpea varieties (Fig. 1). Plants also experienced differences in growth patterns, growth habits and hairiness (Table 2). Varieties had flowers and pods with different colors (Fig. 5) and pigmentation (Figs. 6 and 7). Relative to growth, 22% of the cowpeas had an average height above 19.5 cm whereas the rest of the varieties had shorter plants. 76% of the cowpea had the raceme distributed throughout the plant canopy whereas 24 % of the plants had the raceme above the canopy. All the plants evaluated were hairy (glabrescent) on stems, leaves, and pods. For flower pigment pattern 78% comprised of wing pigmented whereas 22% were not pigmented. 66% of the cowpea varieties had violet flowers and 34% had white flowers (Table 2, Fig. 5). The cowpea varieties had seed of different sizes; small sized and large sized (Table 2). Leaf yield was not calculated but it was observed that 34% of the varieties had leaf persistence and low grain yield while 50% had both leaf persistence and grain production. Potentially high grain yield was observed to range from 2973 Kg/ha to 4527 Kg/ha. The lowest potential grain yielding varieties (GBK003659, GBK003723, GBK026941, GBK003652, and GBK0036602) ranged from 421.9 Kg/ha to 1071Kg/ha

The cowpea varieties had different characteristics at vegetative and reproductive stages (Table 3). Significant variation ($p \leq 0.05$) among varieties was observed in days to flowering, days to maturity, pod length, pods per plant and 100 seed weight. Early maturing varieties (<85 days to maturity) at this site included ACC25, GBK003689, GBK003695, DAKAWA, and MARI3. Late maturing varieties (>95 days to maturity) included ACC6, GBK003652, GBK003707, GBK026941, and 9334. Cowpea lines also differed in pod sizes. Small-seeded cowpea lines (< 14g 100 seed weight) were observed with GBK003659, GBK003724, GBK003658, 9334, and EASEED; whereas large-seeded cowpea lines (>16g 100 seed weight) were from KOL1, MAR3, LAM4, GBK003814, GBK003689, and GBK005173. From cowpea varieties analyzed 34% were observed to be persistent vegetables, 16% grain cowpeas and 50% were both persistent in leaf production and seed production (GBK003645, DAKAWA, GBK003674, GBK003702, GBK003703, GBK003721, KENKUNDE and KAR 2).

DISCUSSION

The varieties of evaluated cowpea were variable in plant and seed characteristics. Selection and plant improvement are dependent on variability in available germplasm. Farmer's preference is also dependent on plant morphological and agronomic characteristics of the variety. Cowpea varieties were classified based on seed coat color. Variation in seed size and color are some of the attributes that determine farmers' preferences and selection of cowpea (Ndiso et al., 2016), Farmers in Kilifi County (coastal part of Kenya) prefer white, light red and the large-sized seed as they produce mostly grain cowpea for consumption. Seed color varied with 24% of the varieties had white and light red seed coat such as GBK 003656, GBK003674, GBK 046540 GBK 0034722, GBK003814 and GBK026958. Leaf color, shape and texture are also important traits for vegetable cowpea production; leaf color also denotes chlorophyll concentration within the plant. Globose leaf shape and intermediate texture are preferred attributes for vegetable cowpea production as the intermediate texture is more palatable (Hutchinson et al., 2017). Within this germplasm collection 40% of the cowpea lines had globose leaf shape and 90 % had intermediate leaf texture, thus having a sizeable proportion of varieties suitable for production preferred among communities that eat vegetable cowpea. Indeterminate growth pattern is associated with continuous production and non-uniform grain maturity within the planting

season. Determinate pattern is associated with uniform grain maturity hence allows for adoption of mechanized harvesting especially large scale production (Kumar et al., 2015). The variations in cowpea characteristics provide a basis for selection in cowpea varieties.

Variation among cowpea lines in leaf shape, growth pattern, habits, pigmentation, maturity and seed characteristics are indicators of potential value of a germplasm collection for use in breeding and crop improvement (Gerrano et al., 2016). Morphological characteristics especially plant features and seed characteristics differ depending on cowpea variety. Variation in these plant and seed characteristics is important to farmers for identification, selection and naming of preferred cultivars. Among preferred properties of cowpea for tropical zones are erect growth habit, resistance to diseases and early maturity (Abadassi, 2015). Varieties ACC25, GBK003689, GBK003695, DAKAWA and MAR 3 could be incorporated in cowpea improvement project for early maturity; varieties suitable for dry areas have the ability to escape drought. Growth pattern, flower color and seed coat characteristics have been considered in development of new and improved varieties of cowpea (Aysun & Erkut 2013). Kamai et al., (2014) established that branches, peduncles, and number of pods per plant are important morphological characteristics considered in cowpea crop improvement because they determine vegetative and grain yield. Highly branched cowpea has more foliage and those with fully formed pods will translate to high grain yield. In this study on morphological diversity of cowpea, variation was observed among cowpea lines for days to 50% flowering, 100 seed weight and number of pods per plant. Results in the current study are similar to Manggoel and Uguru (2011) who established that days to 50% flowering and 50% maturity in cowpea were significantly different in the varieties. Early maturity and ease of harvesting is a preferred trait by grain cowpea farmers, because they escape drought and make large scale production of cowpea efficient through mechanization (Ndiso et al., 2016).

Seed size (length width and thickness) in the current study were not significantly different among the varieties except for 100 seed weight. Varieties GBK 003659, GBK003658 and EASEED recorded low 100 seed weight (less than average 12g) in contrast to MAR 3, LAM 4, and GBK005173 which each had high 100 seed weight (above 16g) grams. Similar results have been reported by Menssena et al., (2017) where the hundred seed weight ranged from 7.67 to 15.12g. Duraimurugan et al., (2014) reported that physical seed characteristics of green gram and black gram legumes differ and are linked to pest infestation. The size of the grain is an important component in pest infestation for example resistance of grains to pulse beetle in chickpea is associated with seed size and 100 seed weight. Low seed weight is negatively correlated with pest infestation (Chandel & Bhadauria, 2015). Hutchinson, et al., (2017) also established variation among Kenyan accessions collected in the coastal region; when planted out had different seed coat characteristics. Kamble et al., (2016) demonstrated that seed color influences preference by e.g. pulse beetle in chickpea where white to brown seeds are more infested as opposed to yellow seeds. Cowpea varieties that take longer to mature are more predisposed to weevil infestation while in the field and during storage (Baidoo et al., 2010). Pod length and pods per plant are traits of cowpea directly related to cowpea seed yield (Kamai et al., 2014). The cowpea varieties with the highest number of pods have potential for higher grain yields. In this study DAKAWA, GKKCP-2, GBK003656, MAR3, KOL 1 and GBK003689 have high potential seed yield ranging from 2973kg/ha to 4527Kg/ha.

CONCLUSION

The set of cowpea varieties evaluated in this study differed in a wide range of plant and seed characteristics. This demonstrates the potential for these varieties to be exploited in

plant breeding programs especially since they were collections obtained mainly from the farmers. The farmers will benefit from the diverse range in specific characteristics that can be selected to suit their needs and preferences. DAKAWA, GKKCP-2, GBK003656, MAR3, KOL 1 and GBK003689 are highly recommended for cowpea grain farmers.

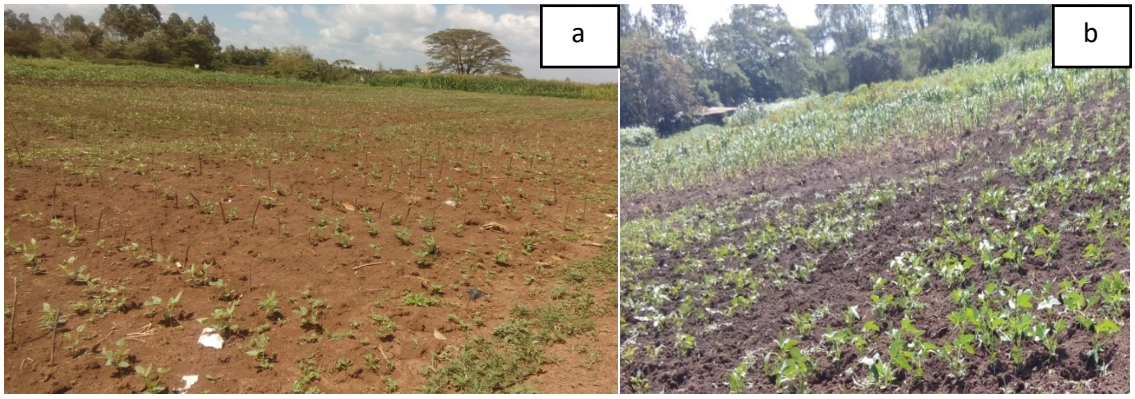

Figure 1: Field layout of cowpea lines at (a) 21 days after planting; and (b) 42 days after planting.
This study included the evaluation of 50 cowpea lines planted at Jomo Kenyatta University of Agriculture and Technology as observed at after 21 days after planting and 42 days after planting.

Figure 2: Characteristics of the classes of the cowpea lines. Cowpea lines a-grey mottled, b-white, c-light red, d-red, e-black, f-cream, g-brown mottled, h-purple and other mixtures (SP6).

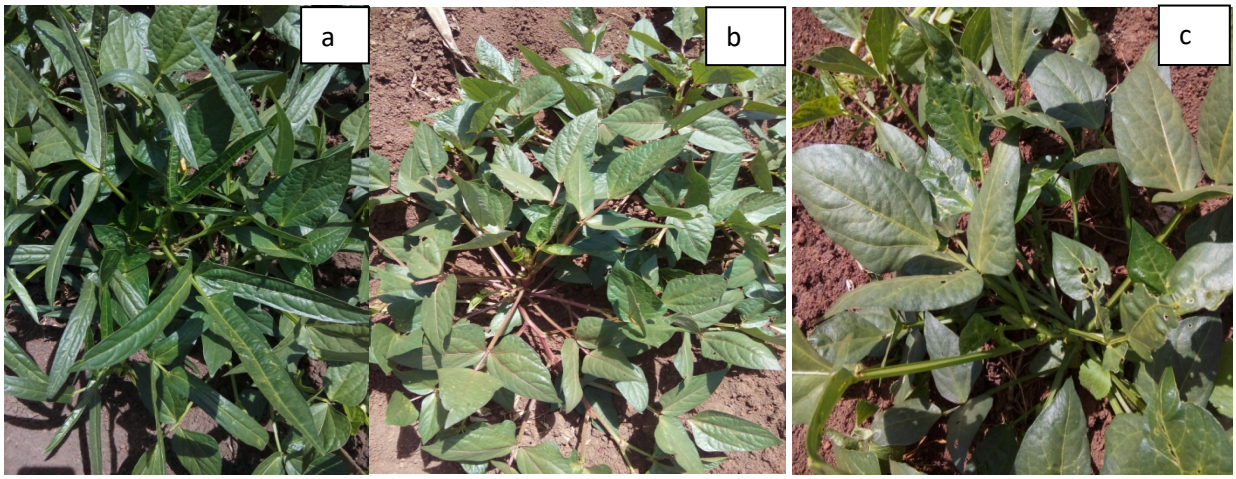

Figure 3: Leaf characteristics of the cowpea lines used. Variation in leaf characteristics among cowpea lines planted in the field a-narrow leaved and b-broad leaved pigmented, c-broad leaved non-pigmented.

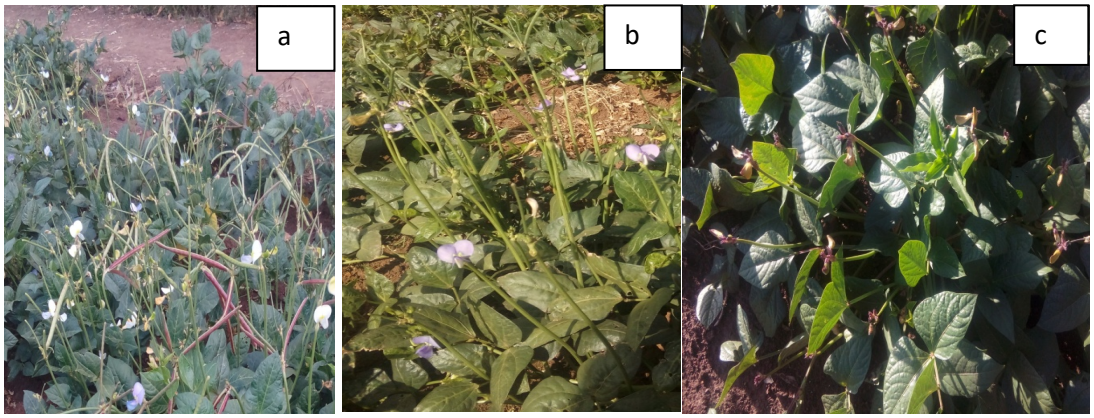

Figure 4: Raceme position for cowpea lines planted in the field. Variation in the positioning of the raceme in the cowpea lines planted a- mostly above canopy b-in upper canopy, c- throughout canopy.

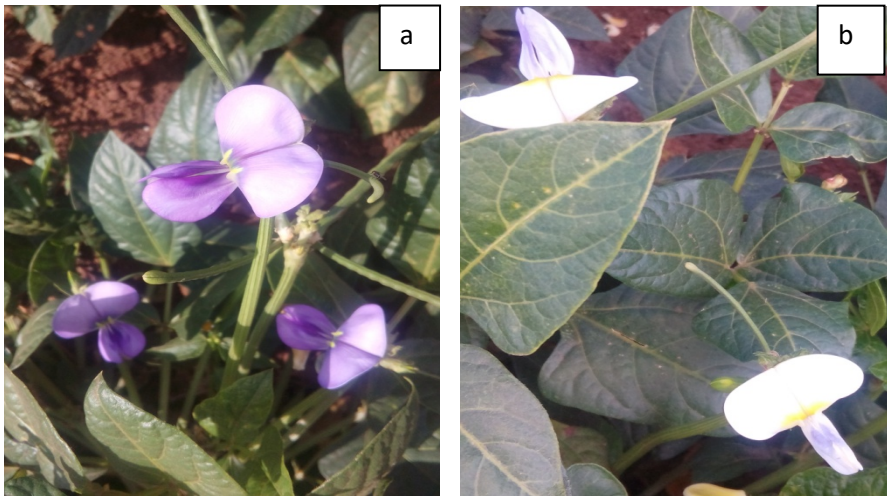

Figure 5: Dominant flower color and wing pigmentation for various cowpea lines. Variation in flower color and wing pigmentation a) violet and pigmented b) white and not pigmented.

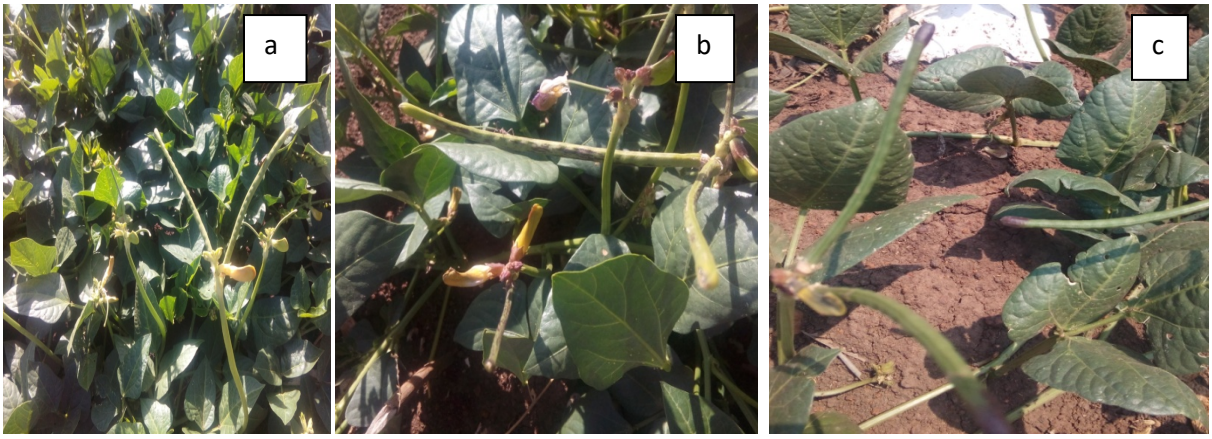

Figure 6: Pod pigmentation in the cowpea lines. Variation in pattern of pigment distribution on full-grown immature pod a-none b- splashes of pigment, c- pigmented tip.

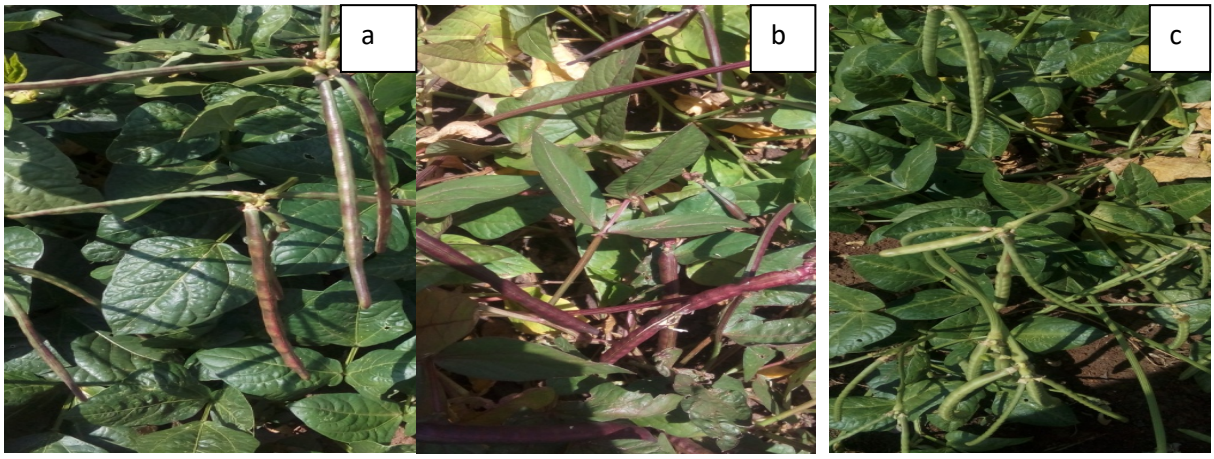

Figure 7: Pod coloration in different cowpea lines. Characteristics of cowpea lines for pod color at maturity a-pale tan/straw b-dark tan c- green.

Table 1: Cowpea seed coat color characteristics of 50 accessions.

+	Grey Mottled	White	Light Red	Red	Black	Cream	Brown mottle	SP6 LINES variable
LINES	GBK003780	GBK003674	GBK003703	GBK003660B	GBK026941	GBK003652	GBK003723	EXISEKE
	GBK005173	GBK003659	KOL5	LAM4	GBK026958B	GBK003721	KAB1	DAKAWA
	GBK003660A	GBK046540	GBK034732	KAK2	GBK003645	GBK003724	AC C 20	
	GBK003702A	GBK003656	GBK003814	MAR5	GBK003699	GBK003650	AC C 25	
	GBK003658	GBK003707	GBK026958A	MAR3	GBK003876	GBK003689	EA SEED 9334	
	GBK003820	GBK003690	GBK0034722	KOL1	GBK003695	GBK003702B	GBK003654	GKKCP-2
				[1]KENKUNDE	GBK003697			AC C6
Source	Gene Bank of Kenya							World Veg and Kenyan Farmers

Classification of cowpea lines used in the experiment based on seed coat color and source of the lines. SP6 comprised of improved lines from World Vegetable Centre and farmers collections from Western Kenya. GBK- Gene Bank of Kenya [1] commercial line widely grown in Kenya.

Table 2: The means, range and distribution of various characters of traits evaluated for the cowpea lines.

Characteristic	% distribution	Range	Mean	Characteristic	% distribution
Leaf length cm	Broad leaf 16	4-13.8	8	Leaflet shape	Globose 40
	Narrow leaf 84				Hastate 40
Leaf width cm		3-7.7	6.5		Sub globose 20
Plant height cm	Above average 22		19.5	Plant hairiness	Glabrescent 100
	Below average 78			Leaf color	Dark green 32
Nodes			10		Intermediate green 34
Main branches			6		Pale green 34
Seed length mm		5.9-8.7	7.6	Leaf texture	Intermediate 90
Seed width mm		4.8-7.1	6.2		Membranous 10
Seed thickness mm		4-5.8		Raceme position	Above and throughout 76
100 seed weight g		8.6-19.57			Upper canopy 24
Growth habit	Acute erect 44				
	Semi-erect 30			Flower pigment pattern	Wing pigmented 78
	Prostrate 22				Not pigmented 22
	Erect 4			Flower color	Violet 66
Growth pattern	Determinate 90				White 34
	Indeterminate 10			Immature pod pigmentation	Uniform 10
Twining tendency	No twining 52				Pigmented tip & splashes 90
	Slight/intermediate 48			Pod curvature	Slightly curved 50
Plant pigmentation	Extensive 20				Curved 50
	None 14			Pod color	Pale tan 58
	Intermediate 66				tan 21
					Green 21
				Pod thickness	Thick pods 56
					Thin pods 44

Distribution of cowpea lines among the respective traits of the evaluated characters at vegetative and inflorescence stage.

Table 3: Variation in plant characteristics of the cowpea germplasm for key quantitative parameters ($p \leq 0.05$).

Plant Character	P value	Attributes/Cowpea line;	Attributes/Cowpea lines
Days to flowering (64-82)	P≤0.001	**Early flowering (<65)** Dakawa, GBK 3674, GBK3723, GKKCP, ACC20	**Late anthesis (>70)** GBK3660GM, GBK3700, GBK026958LR, 9334, GBK3656
Days to harvest (72-113)	P≤0.011	**Early maturing** (<85) ACC25, GBK003689, GBK003695, DAKAWA, MARI3	**Late maturing (>95)** ACC6, GBK003652, GBK003707, GBK026941, 9334
Pod Length cm (8.67-18.37)	P≤0.010	**short pods (<14)** GBK003659, GBK026941, GBK003697, GBK003645, EASEED	**long pods (>16)** 9334, MARI5, GBK003689, GBK003660R, DAKAWA
Pods per plant (7-27)	P≤0.047	**least pod number (<14)** GBK003659, GBK003658, GBK003652,9334, GBK003700	**highest pod number (>17)** MAR3, GBK3699, DAKAWA, 3656, KAB1
100 seed weight (8.60-19.57g)	P≤0.001	**Low seed yield (<12.0g)** GBK003659, GBK003724, GBK003658, 9334, EASEED	**best seed yield (>16g)** KOL1, MAR3, LAM4, GBK003814, GBK003689, GBK005173

Cowpea line classification based on days to flowering and maturity, size of pods cm, number of pods and 100 seed weight in grams.

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