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Research Application Summary

Influence of plant and seed characteristics on cowpea weevil infestation of *Vigna unguiculata*

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Abstract

Cowpea (*Vigna unguiculata*) is a self-pollinated popular legume that can be consumed as vegetable, grains stew or otherwise used for animal forage. It is only propagated by seed. Cowpea weevil (*Callosobruchus maculatus*) is a field and storage pest which results in poor quality seed upon infestation. It can cause up to 100 % loss when cowpea seed is stored without any form of seed treatment hence the study was carried out to determine how plant and seed characteristics influence weevil infestation of seed. The field and lab experiments were carried out in Jomo Kenyatta University of Agriculture and Technology (JKUAT) between October 2016 and August 2017. Plant and seed characteristics were measured. Seed biochemical composition (protein and tannin content) were evaluated following the AOAC methods. Data were subjected to Principal component analysis for classification, ANOVA (5% LSD) and correlation analysis to determine if treatment effects have an influence on weevil infestation. From the plant and seed characteristics evaluated, cowpea seed could be classified into various groups based on skin characteristics; grey mottled, white, light red, red, black, cream, brown mottled. The results also showed that there is significant variation in plant and seed characteristics and these eventually have an influence on cowpea weevil infestation. Large and brightly colored seeds were more infested compared to dark and small seeds. Days to flower and maturity and flower pigmentation had positive correlation with weevil infestation. Lines with high level of protein content were more infested than cowpea lines with low protein content. Lines with high tannin levels (above 0.25) had less infestation compared to those with low tannin content (below 0.05). There was variation with regard to plant and seed characteristics among the cowpea lines studied and these had an influence on the cowpea weevil infestation. This information is important for farmers and breeders selection for cowpea improvement.

Key words; *Callosobruchus maculatus*, morphological biochemical, seed infestation, *Vigna unguiculata*

Résumé

Le niébé (*Vigna unguiculata*) est une légumineuse populaire autogame qui peut être consommée comme légume, ragoût de céréales ou autrement utilisée comme fourrage pour les animaux. Il ne se propage que par graines. Le charançon du niébé (Callosobruchus maculatus) est un ravageur des champs et des entrepôts qui produit des semences de mauvaise qualité en cas d'infestation. Cela peut causer jusqu'à 100 % de perte lorsque les semences de niébé sont stockées sans aucune forme de traitement des semences. L'étude a donc été menée pour déterminer comment les caractéristiques des plantes et des semences influencent l'infestation des semences par le charançon. Les expériences sur le terrain et en laboratoire ont été menées à l'Université d'Agriculture et de Technologie de Jomo Kenyatta (JKUAT) entre Octobre 2016 et Août 2017. Les caractéristiques des plantes et des semences ont été mesurées. La composition biochimique des graines (teneur en protéines et en tanin) a été évaluée selon les méthodes AOAC. Les données ont été soumises à une analyse en composantes principales pour la classification, une ANOVA (5 % LSD) et une analyse de corrélation pour déterminer si les effets du traitement ont une influence sur l'infestation par les charançons. À partir des caractéristiques des plantes et des graines évaluées, les graines de niébé pourraient être classées en différents groupes en fonction des caractéristiques de la peau ; gris chiné, blanc, rouge clair, rouge, noir, crème, marron chiné. Les résultats ont également montré qu'il existe une variation significative dans les caractéristiques des plantes et des graines et que celles-ci ont finalement une influence sur l'infestation par le charançon du niébé. Les grosses graines aux couleurs vives étaient plus infestées que les graines foncées et petites. Le nombre de jours avant la floraison, la maturité et la pigmentation des fleurs avaient une corrélation positive avec l'infestation par les charançons. Les lignées à haute teneur en protéines étaient plus infestées que les lignées de niébé à faible teneur en protéines. Les lignées à forte teneur en tanin (supérieures à 0,25) étaient moins infestées que celles à faible teneur en tanin (inférieures à 0,05). Il y avait des variations en ce qui concerne les caractéristiques des plantes et des graines parmi les lignées de niébé étudiées et celles-ci ont eu une influence sur l'infestation par le charançon du niébé. Cette information est importante pour la sélection des agriculteurs et des sélectionneurs pour l'amélioration du niébé.

Mots clés: *Callosobruchus maculatus*, biochimie morphologique, infestation des graines, *Vigna unguiculata*

Introduction

Cowpea (*Vigna unguiculata*) is a self-pollinated popular legume (Coulibaly *et al*., 2008). It is a multipurpose crop which is consumed as a vegetable, grain and used as forage (Timko *et al*., 2007). Cowpea is a very nutritious crop; it is a rich source of dietary fiber necessary for digestion and a source of cheap protein. The leaves and grains of cowpea contain 34.2 g and 24 grams per 100 grams of protein respectively hence making cowpeas a cheap source of protein (Grubben *et al*., 2014). Agronomic benefits of cowpea include its ability to fix up to 30 kg/ha of nitrogen which results in increase in yield for intercrops like maize, and also control of Striga (Khan *et al*., 2007). Cowpea is solely propagated through seeds making seed quality an important aspect in its production. Among the major challenges facing production of this crop is poor seed quality, seed pest infestation and unavailability of quality seed (Biemond *et al*., 2012). Lack of quality seed at planting time is a common problem to cowpea farmers. This can be attributed to use of "farmer saved seeds" and damage caused by cowpea weevil (*Callosobruchus maculatus*). Poor seed quality results in poor stand establishment, reduced productivity, and hence low production leading to food insecurity.

Cowpea seed in storage is prone to *Callosobruchus maculatus* infestation which can cause up to 100% loss under no form of seed treatment (Cruz *et al*., 2015). The weevil infests the crop in the field during the later stage of pod development, through harvesting as well as during storage. The weevil penetrates through the seed testa causing holes within the seed resulting in reduced eating quality, low germinability low vigor of seedlings, hence reduced economic value to farmers (Umeozor, 2005). Cowpea weevil larvae feed on the protein part of the seed that is the food reserve thus depleting the embryo's food reserves. This eventually causes poor germination, low viability and vigor of the seedling after planting in the field (Cruz *et al*., 2015). The farmers in many cases are forced to dispose off their produce immediately after harvest to avoid storage losses hence minimal returns (Gomez, 2004).

The objective of this study was to determine the influence of plant and seed characteristics on cowpea weevil infestation of cowpea. Morphological characteristics including plant features and seed characteristics tend to differ depending on the cultivars. Amino acids, tannin, alpha amylase inhibitor, trypsin inhibitor, tannin, carbohydrate, seed protein are some of the components that determine preference of the pest on the different lines (Lattanzio *et al*., 2005; Maina *et al*., 2015; Nikolova, 2016). These biochemical components differ among cowpea genotypes. There is a wide variation in seed protein content and subsequently the specific proteins including; globulins, albulins and prolamins (Tchiagam *et al*., 2011). Lattanzio *et al*. (2005) established that the tannin content and the alpha amylase of cowpea lines differed and these components had an influence on cowpea weevil infestation. Nikolova (2016) observed that crude protein; crude fiber and phosphorus contents have an influence on resistance of peas to *Bruchus pisorum*. The ability of a certain cowpea lines to be resistant or susceptible is dependent on individual characteristics of the line.

There is variability among cowpea lines in Kenya. Farmers use their own saved seed in most cases for the next season propagation. In a bid to identify characteristics of resistant cowpea lines for management of cowpea weevil, an assessment was carried out to determine influence of plant and seed characteristics on cowpea weevil infestation. The relationship between cowpea seed characteristics and cowpea weevil infestation will inform research and farmers in the identification and selection of appropriate lines that are resistant to weevil infestation. These can be widely exploited by farmers in identifying lines for subsequent planting seasons.

Materials and Methods

The study was carried out in Jomo Kenyatta University of Agriculture and Technology (JKUAT) from October 2016. In order to determine the plant morphological characteristics, the cowpea lines were planted at JKUAT farm. The individual seed characteristics were determined at the plant physiology lab in JKUAT. Fifty cowpea seed lines were used for the experiment. 34 lines were obtained from gene bank of Kenya, 7 from farmer's collection, 8 lines from Hortinlea SP6 project, and 1 commercial line.

Table 1. Seed characteristics of the experimental material

Skin Characteristics							
Grey mottled	White	Light Red	Red	Black	Cream	Brown mottled	SP6 lines
GBK003780	sGBK003674	GBK 003703	GBK003660R	GBK026941	GBK003652	GBK003723	Ex Iseke
GBK005173	GBK003659	KOL5	LAM 4	GBK026958	GBK003721	KAB1	Dakawa
GBK003660	GBK003645	GBK034732	KAK 2	GBK003699	GBK003645	GBK003724	Acc 20
GBK003702	GBK003656	GBK003814	MAR 5	GBK003876	GBK003700	GBK003680	Acc 25
GBK003658	GBK003707	GBK026958	MAR 3	GBK003695	GBK003650	GBK003654	EAseed 9334
GBK003820	GBK003690	GBK0034722	KOL 1	GBK003697	GBK003702		GKKCP-2
			KEN KUNDE				ACC6

GBK-Gene Bank of Kenya which is the source of the lines. The SP6 lines are sourced from AVRDC and Kenyan Farmers

Assessment of plant and seed morphological characteristics. The 50 lines were planted at JKUAT in randomized complete block design with each line replicated three times. The 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. No pesticides were applied during cultivation. The seeds were harvested when pods were dry dried cleaned and kept for further lab analysis. Data were obtained for plant height, hairiness, pigmentation, leaf color, shape, texture, flower color, pigment, pod color, pigment, curvature, thickness, days to flowering, days to podding; number of pods per plant and yield according to IBPGRI (1983). Data on seed color, seed length, seed width, seed coat thickness, seed shape, seed texture, seed eye pattern and seed weight were determined as per IBPGRI (1983). The seed length, seed width, seed coat thickness and seed weight was obtained by getting the means for 10 seeds of each line. Biochemical composition of each line was carried out on the freshly harvested and dried seeds that were obtained at the end of the growing season.

Protein analysis. Crude protein level was determined using the semi Microkjeldahl method of Association of Official Agricultural Chemists (AOAC) (1995); the specification for AOAC is 950.46 method 20.87-37.1.22. During the test, 0.2 g of the cowpea ground sample was put in digestion flask, 0.5 g potassium sulphate, 0.5 g of copper sulphate and 15 ml of Sulphuric acid was added to act as a combined catalyst allowing digestion to take place in a fume chamber until the blue color was obtained. The digest was allowed to cool to room temperature and transferred to 100 ml volumetric flask and topped up to the mark with deionized water. A control comprising of blank digestion with the catalyst was prepared separately. 10ml of diluted digest was transferred into the distilling flask and washed with distilled water. Subsequently, 15 ml of 40% NaOH was added to the contents and washed with distilled water. Distillation was done to a volume of about 60 mL distillate. Titration of the distillate was done using 0.02 N HCL to an orange color of the mixed indicator, which signified the end point.
The amount of nitrogen in the sample was calculated as follows;

$$\%\,Nitrogen = \frac{(V1 - V2) \times N \times F \div (V \times 100)}{Sample\ wt} \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (1)$$

Where: V1 is the titre for sample in ml, V2 is titre for blank in ml; N= normality of standard HCL (0.02); f= factor of std HCL solution; V= volume of diluted digest taken for distillation (10 mL); S= weight of sample taken for distillation (1 g).

The protein content was calculated as follows;

$$\%Protein = Nitrogen \times Protein\ factor(6.25) \quad \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (2)$$

Tannin content analysis. The seed tannin content was determined using vanillin hydrochloride method involving extraction, shaking, centrifugation and reading at the UV spectrometer according to AOAC methods. The principle behind this method is that the vanillin reagent reacts with any phenol that has unsubstituted resorcinol or phloroglucinol nucleus and forms a colored substituted product which is measured at 500nm. The 8% HCl was dissolved in methanol, 4% vanillin in methanol and then the two were mixed making sure it is not colored. Catechin-Stock standard solution was prepared containing 1mg catechin/ml methanol. A working standard was prepared by diluting the stock solution 10 times from 10-100 ml (100mg/mL). 0.25 g of cowpea ground sample was extracted using acidic methanol. 10 ml of 4 % HCL –methanol was added, sealed with parafilm and shaken for 20 minutes followed by centrifugation at 4500 rpm for 10 min, 25’c. The supernatant was transferred into 2 ml flask. Second extraction was done with 1 % HCL methanol.1 ml of extracted sample was taken and in it 5 ml of freshly prepared vanillin –HCL was added. The color developed and was read at uv-visible spectrophotometer at 500 nm. A blank was prepared separately. A standard graph with 20-100mg catechin using the diluted stock solution was prepared. Calculation was done from the standard graph and the amount of catechin calculated. This is the tannin in the sample as per the absorbance values and the results expressed as catechin equivalents. The calculation is as shown below.

Tannin= % CE = CC. Vm *100/ Ve.Wt

Where; CE-Catekin equivalent, CC-catekin conc., Vm-volume 25 ml, Ve-volume taken, Wt-sample weight.

Data collection

Damage. Damage by weevil was assessed on the 50 cowpea lines. The experiment was set up in a completely randomized design in JKUAT entomology lab. The experiment was replicated three times. The seeds were kept in khaki papers. The damage was evaluated by assessing weight loss and damaged seed by observing weevil presence. The data on damage caused by the weevil were determined by randomly picking a number of seeds from the previously stored samples. The seeds that were damaged due to the weevil were counted. This was repeated three times to get average percentage. The percentage damaged seeds was calculated as follows;

%damaged seed=number of seeds with holes/total number of seeds picked) *100%.

The data were transformed using then subjected to ANOVA to determine variation among the lines.

Data analysis

Descriptive statistics was used to obtain means and performance of the cowpea lines. The quantitative data were subjected to analysis of variance (ANOVA) and means separated using Duncan Multiple Range Test to see if the cowpea lines were significantly different at 5% LSD. Principal component analysis was done to determine the parameters that appropriately contribute to the observed variation. Correlation analysis was also done to determine the relationship between plant and seed (morphological and biochemical) characteristics and weevil infestation.

Results

The cowpea lines showed variation at vegetative and reproductive stages. The leaf length ranged from 4 to 13.8 cm, the average leaf length being 8cm. Also, 16% of the lines had broad leaves (above 10cm). The leaf mean width was found to be 6.5 cm with a maximum of 7.7 cm and a minimum of 3 cm. The average plant height was 19.5 cm and the minimum 16 cm. Overall, 22% of the lines did not attain the average height and were shorter. The plants had a mean of 10 nodes each and the main branch mean was 6 while the maximum number of main branches recorded was 11. The frequent growth habit among the 50 lines was the acute erect whereby branches formed acute angles with the main stem and this comprised 44% of the lines and this was followed by semi erect and the least was the erect type at 4%. Overall, 90% of the lines displayed determinate growth pattern and the rest indeterminate. The plants with no twining tendency were above 50%. And none recorded pronounced twining tendency. The other 48% had either slight or intermediate twining. Also, 20% of the lines had full plant pigmentation, 14% were not pigmented and the rest had partial pigmentation. On the other hand, 40% of the cowpea lines displayed globose type of leaflet shape, while 2% of the lines had the hastate type of leaflet shape. All the lines had the same texture in terms of plant hairiness popularly known as glabrescent limited hairiness. Also, 32 % of the lines had dark green leaves, 34% intermediate green and the other 34 % pale green while 90% of the leaves portrayed intermediate leaf texture. The raceme position of the lines was found to be above and throughout the plant part contributing to 76%. Ten (10) percent of the pods had uniform pigmentation. None of the pods was straight or coiled. However, 50% of the lines had pods that were slightly curved. The flower pigment pattern was wing pigmented for 78% of the lines while 66% of the lines had violet flowers, 58% of the lines had the pod color perceived as pale tan. Also, 56% of the lines portrayed thick pods.

The 1st principal component which accounted for 74% of the total variation was contributed by pod thickness, pod curvature, and pod color. The Principal Component (PC2) accounted for 70.2 % of the variation and was associated with days to flowering and days to harvesting/maturity. Parameters associated with principal component 3 were pods per plant, number of seeds per plant and in some pod length, leaf number, and plant height.

Table 2. Cowpea plant characteristics variation within lines

Parameter	Mean	Maximum	Minimum	Median	Mode
Days flower	67	75	63	66	63
Pod/plant	16	28	7	16	16
Pod length	15.4	18 cm	13 cm	15.2 cm	15.3 cm
Plants/plot	24	30	14	27	29
Yield/plant	20.6g	27.4g	4g	17g	18g
D Maturity	105	113	92	110	112
Seed/pod	12	17	11	13	13

The results obtained showed that there were significant differences in seed length width, thickness, and weight. The seed length ranged from 5.9-8.7 mm, seed width ranged from 4.8-7.1mm, seed thickness 4-5.8mm, while for the 100 seed weight there was variation from small seeds weighing 8.6g to large seeds weighing 19.57 grams. Some of the lines that were small in size include; GBK026941, MAR 5, GBK026958, GBK003699 and GBK003702.

These accessions were less damaged compared to those that were large in size as shown in Table 3. The lines with significantly higher weight and large in size included ACC 20, (farmers collection), GBK003814, GBK003707, GBK003660R, and GBK046540.

Table 3. Variation in seed damage due to the cowpea weevil in cowpea lines

Cowpea line	% damaged seed	Cowpea line	% damaged seed	Cowpea line	% damaged seed
9334	0^{a}	GBK003690	4.27^{abcde}	GBK003703	18.67^{hij}
GBK003689	0.5^{ab}	GBK0034732	5.17^{abcdef}	GBK003659	18.8h^{ij}
GBK003699	0.8^{abc}	EASEED	6.1^{abcdef}	GBK026958LR	20.63^{ij}
DAKAWA	0.97^{abc}	GBK005173	6.23^{abcdef}	GBK046540	21.23^{jk}
GBK003702CB	1.53^{abc}	GBK003650	6.33^{abcdef}	ACC 6	23.73^{jkl}
MARI 3	1.67^{abc}	KOL 1	6.4^{abcdef}	GBK003723	27.97^{klm}
KOL 5	2.53^{abcd}	GBK003707	7.2^{bcdefg}	KAR 2	28.13^{klm}
ACC 25	2.57^{abcd}	EX ISEKE	7.33^{bcdefg}	GBK0034722	30.47^{lm}
ACC 20	2.6^{abcd}	GBK003721	7.9^{cdefg}	GBK003876	33.67^{mn}
GBK003674	2.7^{abcd}	LAM 4	9.23^{defg}	GBK003656	33.93^{mn}
GBK003697	2.8^{abcd}	GBK003645	9.63^{defg}	GBK003658	38.33^{no}
GBK003814	2.97^{abcd}	GBK003820	10.47^{efg}	GBK003724	41.77^{op}
GBK003780	3.13^{abcd}	GBK026958BL	11.7^{fgh}	GKKCP2	44.77^{opq}
GBK026941	3.57^{abcde}	GBK003695	11.83^{fgh}	MARI 5	48.2^{pqr}
KAB 1	3.8^{abcde}	GBK003654	12.13^{fgh}	GBK003660GM	51.03^{qr}
GBK003702	3.83^{abcde}	GBK003652	13.83^{ghi}	KENKUNDE	54.73^{r}
GBK003660R	4.1^{abcde}	GBK003700	13.93^{ghi}		

Lines with similar letters are not significantly differentg at P 0.05

Correlation analysis revealed that there exists a strong positive correlation between nitrogen and protein levels consequently positive correlation between protein content and the rate of weevil infestation. Within cowpea lines the protein variation was significantly different at 5% LSD as shown in table 4.

Table 4. Variation in protein level among the cowpea lines

Cowpea Lines	Mean	Cowpea Lines	Mean	Cowpea Lines	Mean
GBK003660R	0.1217[a]	GBK005173	0.5433[l]	GBK026941	0.76[stu]
MARI3	0.1733[ab]	Kenkunde	0.5453[l]	KOL 5	0.7623[stu]
GBK003702gm	0.1827[b]	ACC6	0.5623[l]	GBK034732	0.78[tuv]
EX Iseke	0.2063[bc]	ACC25	0.5767[lm]	GBK026958bl	0.7943[uvw]
Easeed	0.25[cd]	GBK003689	0.6157[mn]	Dakawa	0.8217[vw]
GBK003645	0.3d[e]	GBK003780	0.6283[mno]	GBK003699	0.8243[vw]
GBK003650	0.326[ef]	LAM 4	0.6543[nop]	GBK003652	0.839[w]
KAB1	0.3483[efg]	GBK003721	0.6667[nop]	GBK003876	0.907[x]
GBK003703	0.3667[fg]	GBK003814	0.67[opq]	MARI5	0.9267[xy]
GBK003723	0.382[gh]	GBK003659	0.681[opq]	GBK003674	0.9427[xy]
GBK003690	0.4217[hi]	GBK034722	0.691[pqr]	KAR2	0.9657[y]
GKKCP2	0.4237[hi]	GBK003697	0.7217[qrs]	GBK003660gm	1.0567[z]
GBK046540	0.4653[ij]	GBK003702cb	0.7407[rst]	GBK003654	1.0667[z]
GBK003700	0.4867[jk]	KOL 1	0.7407[rst]	GBK003656	1.08[z]
ACC20	0.525[kl]	9334	0.7467[stu]	GBK003724	1.084[z]
GBK003820	0.535[kl]	GBK026958LR	0.75[stu]	GBK003695	1.2567[A]
GBK003707	0.5373[kl]	GBK003658	0.7533[stu]		

Lines with similar letters are not significantly differentg at P≤0.05

Table 5. Seed tannin concentration in cowpea lines

Cowpea lines	tannin conc.	Cowpea lines	tannin conc.	Cowpea lines	tannin conc.
GBK 003645	0.032[A]	GBK005173	0.098[N]	MARI 5	0.15[x]
GBK 034722	0.0397[b]	MARI3	0.098[n]	ACC25	0.1507[x]
GBK- 003674	0.04[b]	GBK003723	0.1[o]	KABI Bm	0.1593[y]
ACC20	0.053[c]	LAM4	0.103[p]	GBK 003702 CB	0.1617[z]
GBK 003820	0.065[d]	GBK003700	0.112[q]	GBK003658	0.1647[A]
GBK 0034732	0.0657[d]	GBK003659	0.1123[q]	GBK003654	0.179[B]
GBK 003707	0.0677[e]	EA SEED	0.122[r]	KAR2	0.1813[C]
GBK003721	0.069[f]	GBK003689	0.1277s	GBK 003697	0.2087[D]
EKKCP-2	0.0703[g]	GBK 003656	0.1283[s]	KENKUNDE	0.2087[D]
GBK003690	0.0717[h]	GBK 3702 Gm	0.134[t]	KOL5	0.214[E]
GBK046540	0.08[i]	GBK 003660 RED	0.135[t]	KOLI	0.222[F]
GBK 003814	0.085[j]	GBK003652	0.135[t]	GBK 026941	0.2447[G]
9334	0.0853[jk]	GBK003660 gm	0.14[u]	GBK 003876	0.249[H]
DAKAWA	0.0863[k]	GBK 003650	0.1433[v]	GBK 003780	0.257[I]
EX-ISEKE	0.088[l]	026958LR	0.145[w]	GBK026958b	0.3397[J]
GBK003724	0.094[m]	GBK003703	0.146[w]	GBK 003695	0.3873[K]

Lines with similar letters are not significantly differentg at P≤0.05

Discussion

Plant characteristics differed among the various cowpea lines. The plant characteristics had an influence later during storage on the cowpea weevil infestation. Plant characteristics like leaf size and texture are important for selection of vegetable cowpea since there are both narrow leaved and broad leaved. Cowpea genotypes have different plant and seed characteristics (Kumar *et al*., 2015).

Cowpea lines showed variation in terms of maturity, production and overall leaf and grain yield. Genetic the variability is an important component for cowpea classification and selection. The variability among cowpea lines can be explored for breeding and crop improvement procedures (Gerrano *et al*., 2016).

The 1st principal component which accounts for 74% of the total variation was contributed by; pod thickness, pod curvature, pod color. The PC2 accounted for 70.2 % of the variation and this was associated with days to flowering and days to harvesting/maturity. The cowpea lines can thus be classified as early or late maturing as shown in Table 3 above. Parameter associated with principal component 3 are pods per plant, number of seeds per plant and in some pod length; hence the classification of high yielding lines like GBK005173, GBK003689 and GBK003814. The results are contrary to what was established that the 1st principal components contributed to 79% of total variability and parameters contributing to these were; pod length, leaf area, leaf area index and number of seeds per plan. Leaf number, plant height, dry biomass and fresh biomass contributed mainly to PC2 (Gerrano *et al*., 2016). The results are however similar to the findings whereby the number of pods per plant and grain yield in cowpea were the traits contributing to variation (Manggoel and Uguru, 2011). Farmers will select cowpea seed based on color, size, relative resistance to diseases and taste (Kumar *et al*., 2015).

Seed size and color have an influence on the weevil infestation; the seeds that were comparatively smaller and dark colored were less infested than those that were large and lightly colored. These results are in agreement with those of Duraimurugan *et al*. (2014) who found that physical seed characteristics of green gram and black gram had the same influence on *Callosobruchus chinensis* oviposition and infestation. Cowpea weevil tends to prefer shiny seeds and larger seeds since they have large mass for consumption. Similarly in a study on the resistance of grains to pulse beetle in chick pea it was found that small seeds were less infested compared to those that had high 100 seed weight (Chandel and Bhadauria, 2015). The relative resistance is based on the presence of components present in dark seeds that hinder oviposition. Kamble *et al*. (2016) reported that pulse beetle in chick pea preferred white to brown small seeds as opposed to the large colored yellow seeds. The preference of small seeds by pulse beetle is contrary to the current findings.

A positive correlation between flower color and flower pigment was registered; flower pigment and pod pigment; pod color and days to maturity where the colored pods took long to dry and consequently to mature. The longer it took to flower the longer it took for the lines to mature hence this can be classified as late maturing. Baidoo *et al*. (2010) found that the maturity of cowpea lines ranged from 60 to 80 days. Pods per plant were positively correlated (r =0.57) with yield in that lines that had high number of pods recorded high yield. Farmers select high yielding cowpea lines since it will translate to maximum return. Seed, thickness, seed length and seed width were all positively correlated. This translates to smaller seeds and large seeds and consequently high 100 seed weight. Days to harvest, flower color and flower pigment had positive correlation with the weevil infestation. Cowpea lines that took long to mature were more predisposed to the weevil infestation while in the field prior to storages (Baidoo *et al*., 2010). Cowpea lines that had colored or pigmented flowers were more infested eventually during storage. Pod thickness, color and pigmentation were negatively correlated to the weevil infestation. Thick and darker pods were less infested .Thick pods create a physical barrier that the weevil is not able to penetrate. The results are in agreement with what Sousa *et al*. (2015) observed.

There was positive correlation between the protein content and the level of infestation. The higher the protein content then the higher the rate of infestation. These results are in tandem with what Kamle *et al*. (2016) obtained. The chick pea seeds that had protein level above 20% were more infested than those with less protein content. On the contrary, studies on nutritional and anti nutritional content of soybean; revealed that seeds with high protein content were more resistant than those with less protein like for cowpea and chick pea ((Sharma and Thakur, 2014).

Tannin level in cowpea lines in Gizan region was found to be 0.23% ((Abdelatief and El-asser, 2011). The results also agree with what Alwala *et al*. (2014) found in tannin composition of indigenous vegetables and grains. Asante *et al*. (2004) reported that protein and tannin content range from 16.4 to 27.3% and 0.12 to 2.38%, respectively. Seeds that had the high tannin levels were brown mottled, dark mottled, brown or flesh-colored. There was a significant negative correlation between 100-seed weight and protein content. The tannin content was higher in dark colored seed compared to brightly colored seed. Further, there was a negative correlation between the tannin content and the cowpea weevil infestation with lines that had high tannin content were being less infested compared to lines with low tannin content. In addition, seeds that were black experienced less egg oviposition compared to white ones. Dark color in seeds is associated with high tannin content (Oyeyinka *et al*., 2013) .

Conclusion

Plant and seed characteristics of cowpea lines differ and they have an influence on cowpea weevil infestation. Protein level has positive correlation with weevil infestation while tannin content had negative correlation with cowpea weevil infestation.

Recommendation

The information on seed characteristics should be incorporated in plant breeding and it is important for farmer seeds selection. The following lines can be considered for production by farmers since they are high yielding and are relatively resistant to the cowpea weevil attack: GBK003699, MAR 3, GBK003689, DAKAWA ACC 20 and GBK005173.

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