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RESISTANCE OF UGANDA SOYBEAN GERMPLASM TO ADZUKI BEAN BRUCHID

U.M. MSISKA, T.L. ODONG, M. HAILAY, B. MIESHO, S. KYAMANYWA, P.R. RUBAIHAYO and P. TUKAMUHABWA

Department of Agricultural Production, College of Agricultural and Environmental Sciences, Makerere University, P. O. Box 7062, Kampala, Uganda
Corresponding author: ulemsiska@gmail.com

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ABSTRACT

Soybean (*Glycine max* (L.) Merrill) is among the major food and industrial crops grown globally for its high protein and oil content. Lately, in Uganda, soybean reportedly faces challenges with a storage pest, *Callosobruchus chinensis*. This study was carried out to quantify the damage caused by the pest and identify the sources of resistance in the germplasm in Uganda. The study was conducted at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) in Uganda, during 2015 and 2016. *Callosobruchus chinensis* was used to challenge 498 soybean lines under no choice condition, in the laboratory. Results showed no significant differences in eggs laid amongst the different genotypes; however the genotypes performed significantly different ($P< 0.05$) for adult insect emergence, median development period (MDP), Dobie susceptibility index (DSI), growth index (GI), insect percent emergence (% IE) and seed weight loss (%WL). Genotype AVRDC G8527 had the lowest % IE (6.31), DSI (0.7), % WL (0.02) and GI (0.07), suggesting high resistance. Weight loss of up to 27% was recorded in genotype USA 7. There was a strong positive correlation between number of adults that emerged with DSI ($r=0.87$), eggs ($r=0.88$), % weight loss ($r=0.73$), and growth index ($r=0.996$). Cluster analysis revealed that AVRDC G8527, a resistant genotype was closely related to S-line 13.2A, a moderate resistant genotype. Regression analysis, revealed that adult bruchid emergence explain seed weight loss with 62% coefficient of determination; while seed colour could be used to determine genotype DSI with up to 74% coefficient of determination. Genotypes AVRDC G8527 and G89 were identified as the most resistant genotypes based on levels of DSI.

Key Words: *Callosobruchus chinensis*, Dobie susceptibility index, seed weight loss

RÉSUMÉ

Le soja (*Glycine max* (L.) Merrill) est parmi les cultures alimentaires majeures et industrielles cultivées généralement pour sa grande teneur en protéine et en huile. Récemment, en Ouganda, le soja fait face à des défis avec une peste de stockage, *Callosobruchus chinensis*. Cette étude a été conduite pour quantifier les dommages causés par la peste et identifier les sources de résistance dans le germplasm en Ouganda. L'étude a été conduite à l'Institut de Recherche Agricole de l'Université de Makerere, Kabanyolo (MUARIK) en Ouganda, en 2015 et 2016. *Callosobruchus chinensis* a été utilisé pour évaluer 498 lignées du soja sous une condition sans choix, dans le laboratoire. Les résultats ont montré qu'il n'y a pas de différences significatives dans la ponte des œufs parmi les différents génotypes; toutefois, les génotypes ont montré des performances significativement différentes ($P<0,05$) pour l'émergence des insectes adultes, la période moyenne de développement (MDP) l'indice de sensibilité de Dobie (DSI), l'indice de croissance (GI) ; le pourcentage d'émergence de l'insecte (%IE) et la perte du poids

(%WL). Le génotype AVRDC G8527 a eu le plus faible %IE (6,31), DSI (0,7), %WL (0.02) et (0.07) suggérant une grande résistance. Une perte de poids jusqu'à 27% a été observée sur le génotype USA 7. Il y a une forte et positive corrélation entre le nombre d'adultes qui ont émergé avec DSI (r=0,88), la perte du poids (r=0.73), et l'indice de croissance (r=0,996). L'analyse hiérarchique a révélé que AVRDC G8527, un génotype résistant a été lié à la lignée S-line 13.2A, un génotype modérément résistant. L'analyse en régression linéaire a révélé que l'émergence du bruche adulte explique la perte du poids avec un coefficient de détermination de 62% ; alors que la couleur des graines pourrait être utilisée pour déterminer le DSI jusqu'à 74% de coefficient de détermination. Les génotypes AVRDC G8527 et G89 ont été identifiés comme génotypes les plus résistants sur la base des niveaux de DSI.

Mots Clés: *Callosobruchus chinensis*, indice de sensibilité de Dobie, perte du poids de la graine

INTRODUCTION

Bruchids are the most important insect pests of stored grain legumes, because their damage starts in the field and continues along the value chain. Damage by bruchids is irreversible and direct on the grain (Kananji, 2007). Due to the bruchids' high fertility, ability to re-infest and short generation times, even low initial infestation rates can lead to tremendous damage (Yamane, 2013). Bruchids cause overall seed weight loss, loss of seed viability and altered nutritional quality due to the presence of insect frass, excrement and dead insects in and on the seed. A single beetle is able to cause 3.5% weight loss in cowpea seeds (Tembo *et al*., 2016).

In cowpea (Onyido *et al*., 2011) and beans (Credland, 2000), losses of up to 100% have been reported after 3-6 months of storage. However, it is important to note that losses due to bruchids vary from crop to crop (Kananji, 2007; Swella and Mushobozy, 2009). Although bruchids are known to attack many legume species, literature indicates that there is lack of information regarding damage caused to soybean by bruchids. Most previous reports have been done on other legumes such as cowpea, chickpea (Sharma and Thakur, 2014a) and common beans (Kananji, 2007); but little is documented for soybean, suggesting that damage on soybean by bruchids has previously been considered negligible. Nevertheless, Tukamuhabwa (2015, soybean breeder, Makerere University Agricultural Research Institute personal communication) indicated that soybean was being seriously damaged by bruchids in some parts of Uganda.

One of the major bruchid attacking stored legumes is *Callosobruchus chinensis,* commonly called adzuki bean weevil or chinese bruchid (Spradbery, 2013). A record of *C. chinensis* in Uganda was first published in 1978 by Nyira, then in 1995 by Nahdy on pigeon peas (Nahdy, 1995).

Realising how damaging bruchids can be on legumes, different control methods have been undertaken by farmers; of which pesticides have been the principal means (Dent, 2000). However, pesticides have drawbacks associated with their use such as pest resistance, destruction of beneficial insects, environmental contamination and hazards to the user; in addition to them being expensive for subsistence farmers (Dent, 2000). Resistant varieties, therefore, would provide a sustainable environment friendly method to reduce soybean pre- and post-harvest losses due to *C. chinensis,* and assist farmers as well as processors in storing their soybeans for long periods. In Uganda, soybean germplasm has not been explored for resistance to storage pests. No cultivar of soybean showing resistance to storage pests has been released so far in the world (Bansal *et al*., 2013).

The existence of genotypic variations in response to bruchid infestation reported in some legume species, such as cowpea (Deshpande, 2011), pigeon peas (Affognon *et al*. 2016), rice beans (Somta *et al*., 2006), chickpeas (Kar and Ganguli, 2016) and mungbean (War *et al*., 2017) was associated with physical and chemical signals by the adult female to detect diets that will provide better larva development and higher nutritive value (War *et al.*, 2017). Unfortunately, such studies have not been extensively done on soybean.

The objective of the study was to assess damage caused and identify sources of resistance to bruchids (*Callosobruchus chinensis)* in soybeans germplasm available in Uganda.

MATERIALS AND METHODS

Study area. The study was carried out in 2015 and 2016 at Makerere University Agricultural Research Institute, Kabanyolo (MUARIK) in Central Uganda. MUARIK is located between longitude 32^o 37E, Latitude 0^o 28 N at an altitude of 1200 m above sea level (Sserumaga *et al.*, 2015). The area receives mean annual rainfall of 1150 mm and has mean temperature of 21.5^{o}C (Fungo *et al.*, 2011).

Bruchid rearing. Adult bruchids used in this study were from a culture established in a laboratory at MUARIK. The bruchids which initiated the culture were collected from the National Crop Resources Research Institute (NaCCRI) soybean stores in Namulonge in Uganda. The laboratory culture was established at MUARIK by allowing the collected samples of insects to oviposit on three susceptible commercially grown varieties (Maksoy 2N, Maksoy 3N and Maksoy 4N). The insects were reared on 1 and 5 kg of seed placed in 1 L Kilner glass jars and 10 L plastic buckets, respectively. The jars and buckets were capped with muslin cloth to allow ventilation, but prevent insects from escaping. The populations were maintained by regularly transferring the bruchids to new grains. A sample from the reared bruchid population was confirmed to be *Callosobruchus chinensis* by the National Agricultural Research Laboratories (NARL), Kawanda in Uganda using diagnostic protocols by Farrell *et al.* (2015).

Soybean germplasm collection. A total of four hundred and ninety eight lines from Uganda (321), introductions from USA (56), AVRDC-Taiwan (110) and Zimbabwe (11) available in the germplasm collection of Makerere University Soybean Breeding and Seed Systems Programme were used in the study. The seed samples were oven-dried at 30 ^{o}C for 24 hours, to ensure that any eggs or adult insects from the field were killed (Amusa *et al.,* 2014). The samples were then removed from the oven and placed in laboratory shelves under room conditions for 7 days (Kananji, 2007).

Research design. A sample of 100 soybean seeds was drawn from each of the 498 genotypes and weighed to give baseline information of 100 seed weight. Subsequently, samples of 50 seeds each were placed in different plastic petri dishes and weighed to determine the initial seed weight. The soybean seeds in each petri dish were artificially infested with 20 randomly selected adult bruchids of 1-3 day old, from the bruchid colony under the no-choice test method as described by Somta *et al.* (2008). Petri dishes were laid out in a randomised complete block design, with insect infestation days as blocks; and were replicated thrice. Bruchids were removed from the soybean samples after 10 days (Kananji, 2007).

Data collection. Eggs laid on each of the 50 seeds were counted on day 11 (Kananji, 2007) and emerging adult insects were counted and removed daily until there was no new insects emergence for 5 consecutive days (Lephale *et al.,* 2012). Then final weight of seed samples in each petri dish was taken. Total number of eggs laid was taken as an indicator for oviposition (Amusa *et al.* 2014); while the number of bruchid emergence was taken as an indicator for magnitude of infestation (Emeka, 2010). From these data, the following variables were derived:

(i) Grain weight loss %, which is an economic loss indicator (Amusa *et al*., 2014), was calculated as follows:

$$\text{Grain weight loss } (\%) = \frac{100 \text{ x } (IGW - FGW)}{1GW}$$

Where:

FGW = final grain weight, IGW = initial grain weight for the sample.

(ii) Growth Index (GI), which is an indicator of genotype suitability for development of insects (Régnière *et al*., 2012) was calculated as:

$$GI = \frac{\%IE}{MDP}$$

Where:

%IE = Percent Insect Emergence and MDP = Median development period

The median development period (MDP) was calculated as the number of days from the middle of oviposition (d 5) to the first progeny emergence (Kananji, 2007).

(iii) Dobie susceptibility Index (DSI)
The data on the number of adult bruchid that emerged and the median development period were used to calculate the Dobie susceptibility index (Dobie, 1974) for each genotype using the formula:

$$DSI = \frac{\text{Log}_e Y \text{ x } 100}{t}$$

Where:

Y = total number of adult bruchid emerged, and t = median development period.

If no insect emerged over the test period, the Dobie susceptibility index value was equal to zero (DSI=0) (Derera *et al.,* 2001). The modified susceptibility index ranging from 0-9 was used to classify the soybean genotypes; where, 0 -1 =resistant; 2 - 3 = moderate resistant; 4 - 5 = susceptible and 3 - 6 highly susceptible. This is a modification from Dobie (1974), which has a range of 0 - 11. Dobie susceptibility Index was also modified by Kananji (2007) and Radha and Susheela (2014) so as to fit with the crops they were working on. The genotypes with high susceptibility indices (DSI) were considered susceptible and those with low susceptibility indices as resistant. This was based on the assumption that a few insect progenies would emerge out of a resistant genotype and insect progeny development would take a longer time in a resistant than in a susceptible genotype (Kananji, 2007).

Seed size determination. Seed size was categorised basing on Tukamuhabwa and Oloka (2016). Basing on this information, any genotype with 100 seed weight less than genotype Maksoy 1 was considered as small (<12000 mg), genotype same as Maksoy 1 (12000-14000 mg) was categorised as medium; genotype of the same size as Maksoy 2 (14000-20000 mg) was considered as large and genotype with higher 100 seed weight than Maksoy 5 (>20000 mg) was considered very large. Four soybean seed size categories were determined.

Data analysis. Data were subjected to One-Way ANOVA, using GenStat Statistical Package 12th Edition. Where assumptions of Analysis of Variance were found to have been violated, data transformations were performed. Genotypes were then grouped into four categories, namely: resistant, moderate/

intermediate resistant, susceptible and highly susceptible using the ratings described above basing on the means of DSI. Frequency distributions and correlation coefficients (r) were calculated for the parameters to determine relationships (Amusa *et al*., 2013). Hierarchical cluster analysis was carried out to determine relationships among genotypes. Cluster analysis grouped genotypes into classes according to their similarities basing on the morphological parameters. Similarities were calculated from each parameter using Euclidean test (Harding and Payne, 2012).

RESULTS

General observation on studied variables. The analysis of variance results for the parameters used to assess soybean resistance to *Callosobruchus chinensis* are presented in Table 1. Significant differences were observed (P<0.05) in 100 seed weight, initial weight, final weight, percent weight loss, adult emergence, Dobie susceptibility index, percent insect emergence, growth index and median development period; indicating genetic variability in the studied germplasm. However, genotypes did not show significant differences (P>0.05) on number of eggs laid on them.

Descriptive statistics of the studied parameters for 498 genotypes are presented in Table 2. The studied germplasm was genetically diverse and showed greatest variability on percent seed weight loss (CV=56.99%), followed by growth index (CV=50.52%); while median development period showed least variability (CV=11.67%). The calculated single variable percent coefficients of variation indicated that percent seed weight loss in soybean germplasm was more dispersed than DSI.

Relative susceptibility. Figure 1 presents DSI ranges of the studied 498 genotypes. Less than 1% of the genotypes were resistant, 19.08% of the genotypes showed moderate resistance, 54.82% were susceptible and 25.5% were highly susceptible indicating genetic variability

TABLE 1. Mean squares for variables used to assess soybean resistance to *Callosobruchus chinensis* in Uganda

Source of variation	d.f	Mean square									
		100 seed weight	Initial weight	Final weight	% weight loss	Eggs	Adults	DSI	% IE	GI	MDP
Genotypes	499	37590000	4304000	4631000	108.31	17.04	13.08	4.2	5.901	0.1011	0.002737
Blocks	2	1224000	4302	10910	4.47	2.34	2.38	0.999	0.3	0.4603	0.001529
Residual	998	12260000	276200	407400	74.97	17.38	10.39	3.26	3.815	0.3565	0.001662
Probability		<.001	<.001	<.001	<.001	0.595	0.001	<.001	<.001	<.001	<.001
CV (%)		25.4	7.6	10.3	82	38.3	44.3	35.6	30.2	45.9	2.7

d.f = degrees of freedom, DSI = Dobie susceptibility Index, IE = Insect Emergence, GI = Growth Index, MDP = Median development Period in the studied germplasm. Predominant DSI was 4. Genotype AVRDC G8527 had the lowest DSI (0.704), followed by Introduction G89 (1.67), whereas AVRDC G 2043 had the highest DSI (8.14) (Table 4).

Seed size and seed coat colour. The results on seed size of the studied genotypes based on 100 seed weights are presented on Table 3. From the studied genotypes, 56.24% had their 100 seed weights above the experimental mean of 13,898 mg. Most of the genotypes (43.17%) were large seeded; followed by medium seeded (39.76%) genotypes. The largest seeded genotype was AGS 329 (26481 mg); followed by AGS 292 (22367 mg); whereas the smallest

TABLE 2. Descriptive statistics of studied parameters for 498 soybean genotypes in Uganda

Parameter	Mean	Minimum	Maximum	CV (%)
100 seed weight (mg)	13865	5225	26481	18.00
Initial seed weight (mg)	6942	2713	13538	17.29
Final seed weight (mg)	6220	2309	12322	20.00
Number of eggs laid	136.10	24	312.30	37.88
Adult emergence	64.53	2.67	172.30	47.91
Insect emergence (%)	45.36	6.31	90.67	33.82
Seed weight loss (%)	10.47	0.02	27.18	56.99
DSI	5.07	0.70	8.14	23.35
Growth index	2.09	0.07	5.75	50.52
MDP	31.58	18.67	43.33	11.67

DSI = Dobie Susceptibility Index; MDP = Median Development Period

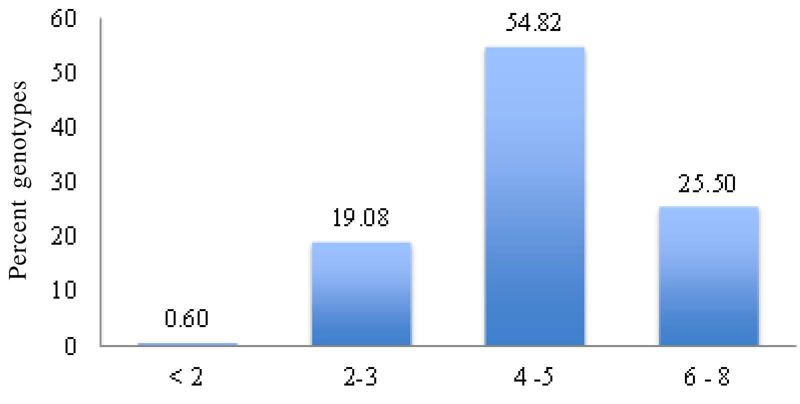


Figure 1. Percent genotypes for the 4 Dobie Susceptibility Indexes (DSI) categories in a soybean study in Uganda.

TABLE 3. Seed size categories of the studied soybean genotypes in Uganda

Category	—— Range (mg) ——		Genotypes (%)
Small	5000	< 12000	15.66
Medium	12000	< 14000	39.76
Large	14000	< 20000	43.17
Very large	20000	< 27000	1.41

TABLE 4. The 10 most resistant and 10 most susceptible genotypes of the 498 Evaluated in Uganda

Genotype	Source	Seed coat colour	Seed size	100 seed weight	Initial weight (mg)	Final weight (mg)	% weight loss	Number of eggs	Number of adults	% IE	MDP (days)	GI	DSI	Status
AVRDC G 8527	AVRDC (Taiwan)	Green	Small	8335	4198	4197	0.02	24.00	2.67	6.31	27.00	0.07	0.70	R
PI G89	AVRDC (Taiwan)	Cream	Medium	12137	6402	6081	4.46	26.33	4.67	23.86	43.33	0.12	1.67	R
G 7955	AVRDC (Taiwan)	Cream	Medium	13031	6293	5839	7.08	131.00	35.33	44.27	25.67	0.95	1.82	R
Elite Lines 4.11-11	Uganda	Cream	Large	14407	6824	6623	3.10	42.67	20.33	32.66	26.67	0.63	2.05	MR
S-Lines 13.2A	Uganda	Cream/black	Small	8455	3977	3842	3.44	140.00	12.00	7.18	23.67	0.36	2.12	MR
S-Lines 9.2	Uganda	Cream	Small	9566	6411	4926	15.94	52.00	14.00	17.58	24.33	0.38	2.12	MR
AVRDC SRE-B-15C	AVRDC (Taiwan)	Cream/black	Large	16495	8248	8219	0.36	77.00	7.67	9.21	36.67	0.24	2.14	MR
PI G49	AVRDC (Taiwan)	Green	Small	11105	6928	5759	13.86	76.33	11.00	11.03	38.67	0.34	2.15	MR
AVRDC 8586	AVRDC (Taiwan)	Green	Small	11756	5866	5591	4.60	74.67	19.33	15.13	24.67	0.58	2.31	MR
PI G43	AVRDC (Taiwan)	Cream/black	Medium	12563	5881	5833	0.78	34.00	9.00	29.64	36.00	0.25	2.34	MR
BSPS 52 C-1	Uganda	Cream	Large	14324	7356	6041	17.26	187.00	125.33	69.50	29.33	4.43	7.01	VS
BSPS 75B	Uganda	Yellow	Medium	13426	6869	5134	25.05	184.30	112.33	61.02	29.00	3.87	7.12	VS
Bulindi 56	Uganda	Cream	Medium	12422	6129	4702	23.21	229.00	151.67	66.19	30.33	5.05	7.15	VS
Bulindi 31	Uganda	Cream	Medium	12639	6496	4947	23.90	239.30	160.00	66.82	30.67	5.21	7.19	VS
S-Lines 3.17	Uganda	Cream/black	Small	10368	7526	5974	20.66	272.70	149.67	69.45	30.00	5.00	7.24	VS
Obs 116	Uganda	Yellow	Large	14757	7381	5848	20.78	312.30	146.33	47.08	29.67	4.95	7.31	VS
USA 7	USA	Yellow	Medium	13662	6708	4896	27.18	290.70	172.33	60.47	30.33	5.75	7.31	VS
Bulindi 4B	Uganda	Yellow/green	Medium	13162	7549	5712	24.37	282.30	157.67	56.22	31.00	5.37	7.33	VS
Bulindi 77B-1	Uganda	Yellow	Medium	13666	7033	5761	18.06	225.00	119.00	50.34	27.67	4.68	7.51	VS
AVRDC G 2043	AVRDC (Taiwan)	Yellow	Large	16726	8227	7342	10.82	216.70	93.33	40.73	24.00	4.18	8.14	VS
F.pr				<0.001	<0.001	<0.001	<0.001	0.595	0.001	<0.001	<0.001	<0.001	<0.001	
LSD (0.05)				6272	941.4	1143.5	15.51	7.468	5.775	3.4987	0.07305	1.0695	3.2345	

* Data was analysed for 498 genotypes but only the most resistant and very susceptible are presented for clarity. The Statistics is for the entire experiment (498 genotypes). R = Resistant, MR = Moderate Resistant, S = Susceptible, VS = Very Susceptible, IE = Insect Emergence, MDP = Median Development Period, GI = Growth Index, DSI = Dobie Susceptibility Index

seed size was observed on USA 33 (5225 mg); (Table 4).

Bruchid injury and soybean seed weight loss. Genotype AVRDC G8527 had the least seed weight loss (0.02%), followed by AVRDC SRE-B-15C (0.36%); whereas USA 7 recorded 27.18% the highest seed weight loss ($P<0.01$), (Table 4). Out of the studied genotypes 45.38% had percent weight loss above the experimental mean (10.47) within 3 months of storage. The resistant genotypes had a mean weight loss of 3.85%, the moderate resistant genotypes 5.44% and the susceptible genotypes 9.85%; while the highly susceptible genotypes had a mean weight loss of 15.68%.

Bruchid population dynamics

Oviposition. *Callosobruchus chinensis* laid eggs on all 498 studied genotypes. Over fifty percent of the studied genotypes had eggs less than experimental mean (136.1) (Table 4). Genotype OBS 116 had the highest number of egg counts (312), followed by NGDT 1.33-2 with 306 eggs; while AVRDC G8527 had the least number of eggs (24) (Table 4). However, there was no significant difference amongst genotypes on the number of eggs laid on them.

Magnitude of infestation. Genotype AVRDC G8527 had the least mean of adult emergence (2.67), while the highest mean was observed on USA 7 (172) (Table 4). The mean adult insect emergence for the resistant genotypes was 14.22; the moderate resistant 29.10, susceptible genotype 45.47; while the highly susceptible genotypes had a mean of 100.25 adults. Of the 498 studied genotypes, 52.41% had the number of adult emergence below the experimental mean value of 64.53.

Insect median development period. Results on median development periods (MDP) of the 498 studied genotypes are presented on Figure 2. Eighty-six percent of the genotypes had MDP range between 30-39 days, 13% had MDP range of 16-29 days, and 1% had MDP range of 40-45 days. No genotype had MDP below 15 days; while 57.23% of the genotypes had MDP above the mean experimental mean (31.58 days). The predominant MDP was 31 days. Genotype G89 had the longest MDP of 43.33 days; followed by AVRDC G84051-31-

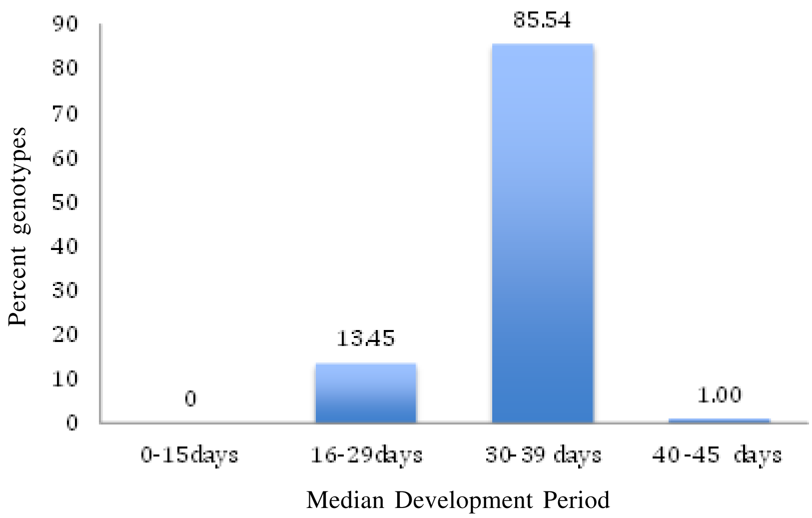


Figure 2. Median Development Periods for the 498 studied soybean genotypes in Uganda.

2 (2) with 41 days; while Bulindi 12 had the least MDP of 18.67 days (Table 4).

Insect growth index. Out of the 498 genotypes, AVRDC G8527 had the least growth index (GI=0.066), followed by G89 with GI=0.124; whereas the highest GI was observed from USA 7 (GI=5.75) (Table 4). Fifty-three percent of the genotypes had GI below the experimental mean of 2.09.

Correlations between variables. Results of correlation analysis are presented in Table 5. There were significant and strong positive correlations between adults that emerged with DSI (0.87), eggs (0.88), % weight loss (0.73) and growth index (0.996). The same trait (adult emergence) also showed a significant positive correlation with insect emergence (0.59); and a weak but significant correlation (0.17) with median development period. DSI had a significant strong correlation with eggs (0.77) and growth Index (0.86). Seed size (100 seed weight) had no correlation with DSI and MDP, but had very weak correlation with eggs, adults and GI.

The results of a simple linear regression analysis of percent seed weight loss against adult insect emergence are presented in Figure 3. The results revealed that adult insect emergence would predict 62% (R^2=0.624) of percent weight loss of seed. Regressing DSI against seed coat colour gave a significant relationship (P<0.001) and a strong coefficient of determination (R^2 =71%) (Fig. 4).

Results from cluster analysis of the 27 most resistant and 14 most susceptible genotypes are presented by the dendrogram in Figure 5. Cluster analysis placed the genotypes into two major groups designated cluster I and cluster II, marked by vertical bars. Five sub-clusters are highlighted with brown dots. Genotypes from the same susceptibility category (DSI basis) are presented with the same font colour. Genotypes in red font are resistant to bruchids, black are moderate resistant and the genotypes in blue are the very susceptible. Cluster analysis revealed that the resistant genotype AVRDC G8527 was closely related to a moderate resistant genotype S-line 13.2A (Fig. 5). In cluster I, genotype AVRDC G4890-21-13-13 was very dissimilar from the entire group. Analysing its characteristics, AVRDC G4890-21-13-13 had a very high growth index (GI) as compared to other genotypes in the same group. Group II consisted only of susceptible genotypes, with Bulindi 14A and AVRDC G2043 being very similar to each other.

DISCUSSION

The results in this study demonstrated that soybean genotypes responded differently to *C. chinensis* infestation (Table 1), indicating that there is variability in genotypes resistance

TABLE 5. Correlation coefficients (r) for experimental parameters, under *Callosobruchus chinensis* no-choice artificial-infestation on the 498 genotype samples

	100 seed weight	% weight loss	Number of eggs	Number of adults	% IE	DSI	MDP
% weight loss	0.0738*	-					
Eggs	0.0527*	0.6458**	-				
Adults	0.0559*	0.7316**	0.875**	-			
% IE	0.019ns	0.3573**	0.2614**	0.589**	-		
DSI	0.0419ns	0.5474**	0.7711**	0.8659**	0.669**	-	
MDP	-0.014ns	0.0551*	0.2024**	0.1741**	0.3865**	0.41**	-
GI	0.0603*	0.7293**	0.8669**	0.9962**	0.5828**	0.86**	0.14**

** Significant at P<0.01; * Significant at P<0.05; ns = Not significant. %IE = Percent Insect Emergence; DSI = Dobie susceptibility Index; MDP = Median Development Period

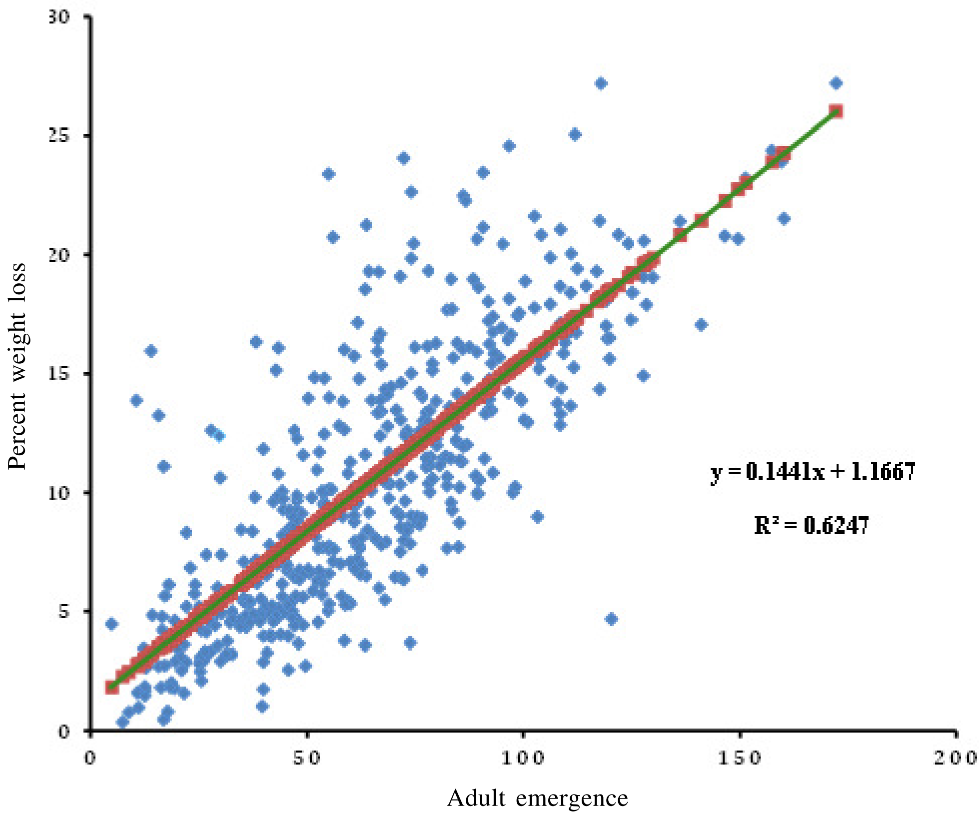
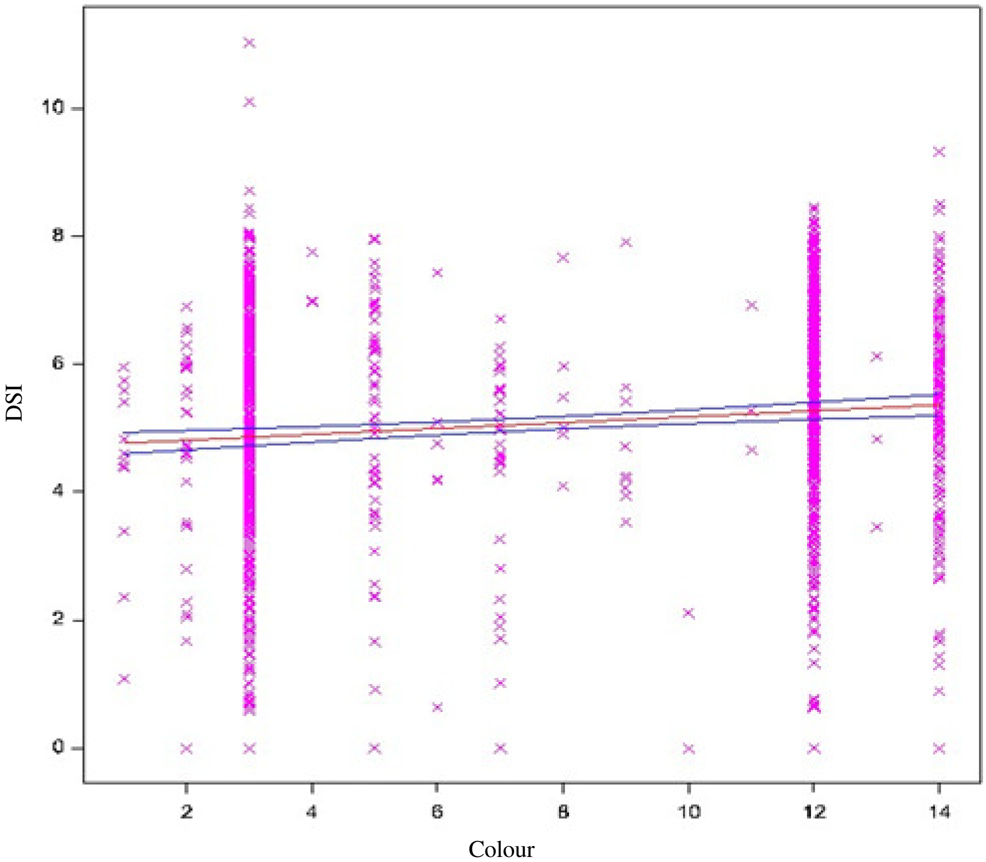


Figure 3. Regression of percent seed weight loss of soybean against adult insect emergence for the 498 genotypes.

levels. The variation in genotype resistance was basically due to variations in adult emergence, percent weight loss, median development period and growth index. The study identified three genotypes as resistant to *C. chinensis,* which therefore can confer the resistant gene for soybean breeding programmes. Since less than 1% of the genotypes was resistant, it implied that the search for resistance gene sources might have to go further. This is in agreement with Dong *et al.* (2001), who reported that sources of resistance to bruchids from cultivated legumes are low.

Relative susceptibility. The DSI indicated existence of genetic diversity among tested genotypes and thus the germplasm collection could provide parent materials for genetic studies. Mechanisms of resistance were beyond the scope of this study, but with the present findings, it can be speculated that the genotypes possess different intrinsic and extrinsic factors of different levels, which conferred different resistance levels either through antibiosis, antixenosis or both. Osman *et al.* (1991) reported that resistance to bruchids in soybean was due to presence of saponins, anti-nutritional factors, high fat and protein content which inhibit larval development. Osman *et al.* (1991) further reported that both antixenosis and antibiosis mechanisms were important in the soybean resistance to bruchids. Similar findings were reported by Lephale *et al*. (2012) in beans and Amusa *et al.* (2013) in cowpea.

Bruchid injury and soybean seed weight loss. The postulate of variability in soybean



Colour key:

1 = Black, 2 = Brown, 3 = Cream, 4 = Imperfect black, 5 = Cream/green, 6 = Cream/yellow, 7 = Green, 8 = Green/cream, 9 = Green/yellow, 10 = Green brown, 11 = Variegated, 12 = Yellow, 13 = Yellow brown, 14 = Yellow/green

Figure 4. Regression of DSI against seed coat colour for the 498 studied genotypes.

genotypes reaction to *C. chinensis* infestation was further highlighted by variation among genotypes in percent weight loss (Table 4). The resistant genotypes were not immune to *C. chinensis,* but suffered considerably less weight loss compared to the susceptible genotypes. Weight loss is an economic loss indicator and an economic loss of 10.47% within three months of storage implies that if soybean is to be stored for long periods, some form of protective measure has to be used to avoid enormous yield losses. The percent seed weight loss observed in this study was higher than the reported loss by Sharma and Thakur (2014a), which was 4.93%. Reports on losses due to bruchid damage vary from crop to crop (Sharma and Thakur, 2014b; Ebinu *et al.,* 2016) and genotype to genotype (Sharma and Thakur, 2014c; Gevina *et al.,* 2016). For example, common beans in Malawi have been reported to incur losses of up to 38%, Uganda reported up to 8%; while Kenya and Tanzania reported as high as 78% within six months of storage (Kananji, 2007).

Bruchid population dynamics

Oviposition. Lack of significant difference among genotypes for number of eggs laid, implied that the genotypes did not influence oviposition by female bruchids. The other finding was that no genotype was totally

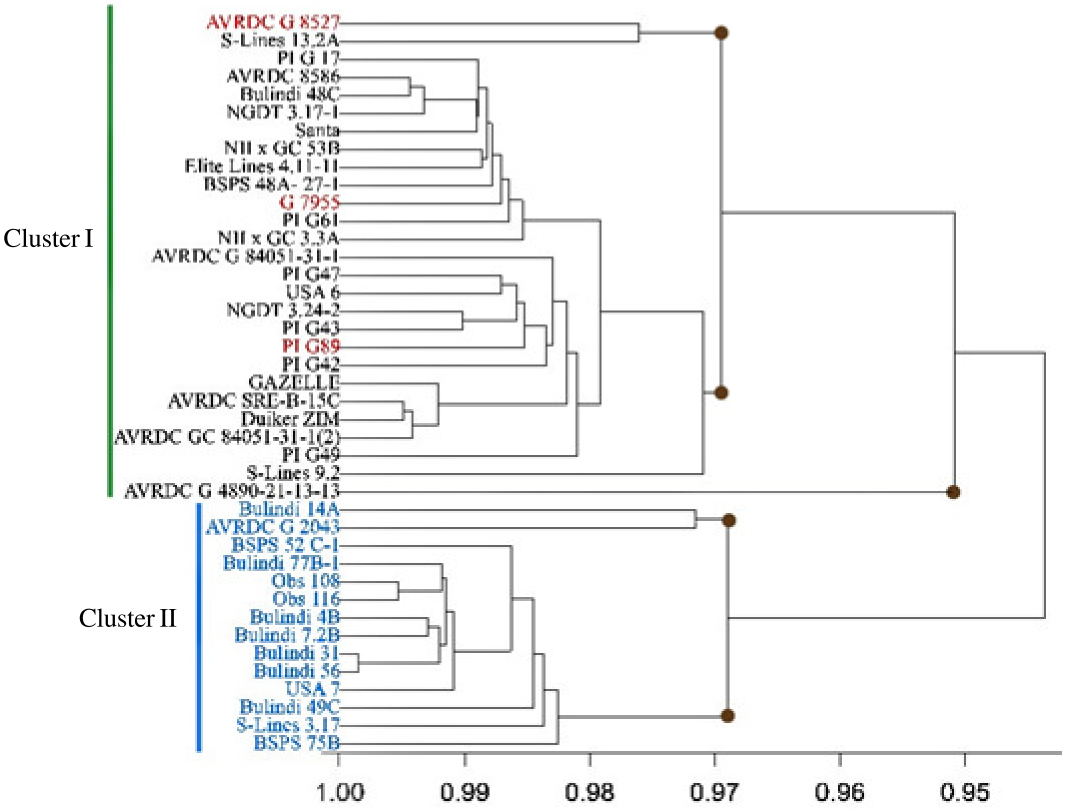


Figure 5. Dendrogram showing relationships amongst 27 most resistant and 15 most susceptible genotypes.

rejected for oviposition. Results of this study demonstrated that oviposition trait alone failed to distinguish genotype suitability for *C. chinensis* development. This finding was in agreement with the theory that where the insects have no choice, females oviposit on hosts in which the chances for larval survival are low or absent (König *et al.*, 2016). This behaviour is basically associated with unpredictability of the environmental resources. Furthermore, results indicate that resistance to *C. chinensis* in soybean might be in the seed or seed coat and that the nature of resistance might be antibiosis and not antixenosis. Somta *et al.* (2006) and Swella and Mushobozy (2009) reported that *Callosobruchus spp* could oviposit on any seed, even though the seed might not be suitable for development of insects. Amusa *et al.* (2014) reported that the number of eggs laid by insects was less important than the rate of oviposition in its influence of the rate of multiplication. However, the number of eggs laid helped to know whether there was a likelihood of getting adults since no adults would emerge from zero laid eggs (Azeez and Pitan, 2014).

Magnitude of infestation. Number of adult emergence indicates the magnitude of infestation and the loss of market value of the crop (Emeka, 2010). The resistant genotypes like AVRDC G8527 were characterised by delayed and low adult emergence; while in susceptible genotypes, the adult emergence was relatively early, extremely rapid and in high numbers. Each adult emerging leaves a hole on the seed, which implies loss of appeal in the market (Kananji, 2007), and could lead to loss of seed viability (Kumar and Kalita, 2017).

It can be speculated that genotype variations in magnitude of infestation by bruchids observed in this study could be due to differences in antinutritional factors (Amusa *et al.,* 2014).

Insect median development period. Results of median development periods indicated variability in the genotypes, with genotypes PI G89 and AVRDC G84051-31-2 (2) having the longest development periods (Table 4) indicating that such genotypes probably were either hard-textured (Soumia, 2015) and difficult to ingest or digest for the larvae; partially toxic (Gevina *et al.,* 2016); and/or nutritionally inadequate to support optimal development rates of the pest (Hiiesaar *et al.,* 2009). Kananji (2007) and Hiiesaar *et al.* (2009) suggested that extended development period was due to antibiosis or anti-feedant activity which could be the actual resistance mechanism. The results also indicated shorter development periods compared to results of Sharma and Thakur (2014a), who reported it to be 40-50 days, implying that soybean can no longer be stored safely for more than a month without some form of protection in Uganda. This finding has a negative impact on the farmers who would be forced to sell their produce as soon as they harvest even when the market prices are still low to avoid incurring losses.

Insect growth index. The results on insect growth index (Table 4) which is an indicator of genotype suitability to *Callosobruchus chinensis* development, showed that the insect had the capacity to infest and develop on all soybean genotypes tested but with significant differences. Larval development within the seed depends on chemical composition of the grain (Sharma and Thakur, 2014c). The inability of *C. chinensis* to develop at the same rate in the genotypes would be an indication that genotypes had varying contents of saponin (Swella and Mushobozy, 2009), fat content (Tripathi *et al.,* 2013) and protein-carbohydrates ratio (Srinivasan and Durairaj, 2007). Maximum growth of *C. chinensis* was on genotype USA 7, implying that this genotype had the least anti-nutritional factors and, therefore, was the most suitable genotype for development of the bruchid; while AVRDC G8527 had the most anti-nutritional factors which made it the least suitable.

Correlations between variables. Absence of significant correlation between 100 seed weight and DSI in this study (Table 5) implied that the association between these two variables was curvilinear or non-linear. This explanation was true for all other non- significant associations, with correlation coefficients closer to zero. Furthermore, these results suggested that resistance in soybean did not really depend on the nutritional factors nor space but presence of anti-nutritional factors which may not depend on the seed size or seed density (Sharma and Thakur, 2014c). The weak correlation between 100 seed weight with eggs and adults suggested that oviposition and adult emergence did not depend on 100 seed weight which was similar to what Dent (2000) reported that seed weight was a very complex variable in legumes and as such it does not have linear relationships with other variables (Acquaah, 2007).

The correlation coefficients (Table 5) suggested that the number of adults' emergence could be used for predicting resistance in soybean because it had an almost perfect positive correlation with growth index and strong correlation with DSI. Similar findings were reported by Kananji (2007), who worked on beans and Hiruy and Getu (2018) on maize. Results on strong positive correlation between GI with percent weight loss indicated that rapid insect growth and development could lead to high percent seed weight losses. On the other hand, resistant genotypes reduced the number of adult insects emergence thereby minimising the post harvest losses. If the resistance in the lines with low GI could be enhanced it would be an environmental friendly way of reducing losses from *C. chinensis.*

Regression analysis of percent seed weight loss against adult insect emergence (Fig. 3) implied that post harvest losses due *C. chinensis* increased with increase in adult emergence which eventually would lead to increased economic losses. Further more, insect adult emergence explained 62% of the variability in percent seed weight loss. The information generated is important for determining economic injury levels for *C. chinensis* in soybean in Uganda. Tefera *et al.,* (2011) and Musa *et al.* (2015) reported that increased adult emergence produces a corresponding increase in percent weight loss in grains until there is no more food for larva development in the grains. Results on regression of DSI against seed coat colour for 498 genotypes (Fig. 4) indicated that seed coat colour explained 74% of the variability in seed resistance therefore seed coat colour could be one of traits used to predict resistance of soybean to *C. chinensis*. The results were in agreement with El-Hamid *et al.* (2008).

Results from cluster analysis of 27 most resistant and 15 most susceptible selected genotypes (Fig. 5) implied that geographical distances between sources of accessions were not associated with genetic distances among genotypes. However, the genetic gap between resistant and susceptible genotypes was evident suggesting that the variability was an important trait for classification of germplasm. The similarity of genotype AVRDC G8527 to S-line 13.2A suggest that these lines would be equally used as sources of resistance genes to *C. chinensis*.

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